Hepatology


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Future Meetings

AASLD/AGA Academic Skills Workshop
January 25 – 26, 2008
Westin Riverwalk
San Antonio, Texas

EASL/AASLD/APASL Hepatitis B and C Virus Resistance to Antiviral Therapies
February 14 – 16, 2008
Paris, France

APASL/AASLD Patient Oriented Research in Liver Disease
March 25, 2008
COEX Convention Center
Seoul, Korea

Digestive Disease Week® (DDW)
May 17 – 22, 2008
San Diego Convention Center
San Diego, California

Hepatology SEP Learning Session
May 21, 2008
During DDW

The Henry M. and Lillian Stratton Basic Research Single Topic Conference
Pathobiology of Biliary Epithelia and Cholangiocarcinoma
June 6 – 8, 2008
Emory Conference Center Hotel
Atlanta, Georgia

Hepatitis Single Topic Conference
HIV and Viral Hepatitis: Lessons Learned
June 19 – 21, 2008
Westin Chicago River North
Chicago, Illinois

Clinical Research Single Topic Conference
Circulatory and Renal Failure in Cirrhosis: Mechanisms and Emerging Therapies
September 5 – 7, 2008
Emory Conference Center Hotel
Atlanta, Georgia

ALEH/AASLD/EASL Postgraduate Course
From Fibrosis to Liver Cancer: Advances in Prevention, Diagnosis, and Treatment
September 15, 2008
Margarita Island, Venezuela

Transplant Hepatology Certification Review Course
September 2008
Westin O’Hare
Chicago, Illinois

59th AASLD Annual Meeting
The Liver Meeting®
October 31 – November 4, 2008
Moscone West Convention Center
San Francisco, California

Endpoints Single Topic Conference
Endpoints in Clinical Trials for Primary Biliary Cirrhosis
December 6, 2008
Emory Conference Center Hotel
Atlanta, Georgia

Call for Nominations

GOVERNANCE

Nominations for the 2009 Governing Board (Councilor, Councilor-at-Large), and the 2009 Nominating Committee will be accepted from regular AASLD members through Thursday, January 31, 2008. Nominations must include a letter of recommendation outlining the nominee’s professional credentials, commitment to the profession, leadership experience, and service to AASLD. Please direct all correspondence to Gregory J. Gores, MD, Chair, 2008 Nominating Committee, AASLD Central Office, 1001 N. Fairfax Street, Suite 400, Alexandria, VA 22314.
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THE 58th ANNUAL MEETING OF THE AMERICAN ASSOCIATION FOR THE STUDY OF LIVER DISEASES

THE LIVER MEETING®

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Meeting-at-a-Glance

Described for physicians, surgeons, scientists, educators, nurses, physician assistants, and all other hepatology health professionals, The Liver Meeting® is the premier event in the science and practice of hepatology where the cutting edge in the study and treatment of liver and biliary diseases is defined.

The Liver Meeting® continues its tradition of providing a forum for the exchange of groundbreaking research and clinical information in a variety of formats, including workshops, plenary and parallel sessions, poster sessions, scientific exhibits, and State-of-the-Art Lectures.

The majority of events during The Liver Meeting® will take place in the John B. Hynes Convention Center, 900 Boylston Street, Boston, Massachusetts.

Friday, November 2, 2007

8:00 AM - 3:30 PM AASLD/ITRS Transplant Course Hynes, Ballroom A,B,C
10:00 AM - 4:00 PM AASLD/ASGE Endoscopy Course Hynes, 304/306
Noon - 3:00 PM AASLD/PGSHPN Pediatric Symposium Hynes, 302
Noon - 4:00 PM Career Development Workshop Hynes, 210
4:00 - 8:00 PM AASLD Postgraduate Course Hynes, Auditorium

Saturday, November 3, 2007

8:00 AM - 5:15 PM AASLD Postgraduate Course Hynes, Auditorium
2:00 - 8:00 PM Poster Session I Hynes, Hall C
5:30 - 8:00 PM Opening Reception Hynes, Hall D
8:00 PM Industry-supported Satellite Symposium See page 28A for rooms
Midnight Daylight Savings Time ends: turn back your clock one hour

Sunday, November 4, 2007

6:30 - 7:45 AM Networking Breakfast for Women in Hepatology Sheraton, Back Bay B,C
6:45 - 7:45 AM Early Morning Workshops Rooms TBA
8:00 - 9:30 AM Liver Transplant Plenary I Hynes, Auditorium
8:00 AM - Noon Basic Research Workshop Hynes, Ballroom B
8:00 AM - 2:45 PM Hepatology Associates Course Sheraton, Grand Ballroom
8:00 AM - 5:30 PM Poster Session II Hynes, Hall C
9:30 AM - 3:00 PM Exhibits Open Hynes, Hall D
9:30 - 10:00 AM Thomas E. Starzl Transplant Surgery State-of-the-Art Lecture Hynes, Auditorium
10:30 AM - Noon Liver Transplantation II Hynes, Auditorium
Noon - 12:30 PM Hans Popper Basic Science State-of-the-Art Lecture Hynes, Auditorium
1:00 - 2:00 PM 50 Years of Interferons Hynes, Auditorium
1:00 - 4:00 PM NIH Corner Hynes, Ballroom B
2:00 - 3:30 PM Therapeutic Advances in HCC Hynes, Auditorium
3:00 - 4:30 PM General Hepatology Update Hynes, Auditorium
3:00 - 4:30 PM Parallel Sessions
HCV: Pathogenesis Hynes, 312
Hepatobiliary Neoplasia: Clinical and Translational Aspects Hynes, 302
Liver Transplantation Hynes, Ballroom A
Mechanisms of Cell Function and Injury Hynes, 309
Portal Hypertension: Clinical Hynes, 304/306

Monday, November 5, 2007

6:45 - 7:45 AM Early Morning Workshops Rooms TBA
8:00 - 9:30 AM Presidential Plenary I Hynes, Auditorium
8:00 AM - 5:30 PM Poster Session I Hynes, Hall C
9:30 - 10:00 AM Award Presentations Hynes, Auditorium
9:30 AM - 3:00 PM Exhibits Open Hynes, Hall D
10:00 - 10:30 AM Hyman J. Zimmerman Hepatotoxicity State-of-the-Art Lecture Hynes, Auditorium
11:00 AM - 12:30 PM Advances for Practitioners Hynes, Ballroom B
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HCV: Virology Hynes, 312
Hepatic Stellate Cell Biology Hynes, 304/306
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Late-breaking Abstracts Hynes, Auditorium

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Innate and Adaptive Immunity in Liver Disease Hynes, 311
Signal Transduction and Nuclear Receptors Hynes, 309
Steatosis: Experimental Hynes, Ballroom B
3:00 - 4:30 PM Focused Study Groups (FSG)
Endpoints and Study Design in Clinical Trials in Pediatric Hepatology Hynes, 302
Portopulmonary Hypertension in the Era of Liver Transplantation Hynes, 304/306
7:00 - 9:00 PM AASLD Special Interest Group (SIG) Meeting [Members Only] TBA

Tuesday, November 6, 2007

6:45 - 7:45 AM Early Morning Workshops Rooms TBA
8:00 - 9:30 AM Presidential Plenary III Hynes, Auditorium
8:00 AM - 12:30 PM Poster Session IV Hynes, Hall C
9:30 - 10:00 AM Leon Schiff State-of-the-Art Lecture Hynes, Auditorium
10:00 - 11:00 AM AASLD Business Meeting (Members Only)
11:15 AM - 12:45 PM Parallel Sessions
Advances in the Molecular and Cellular Biology of Bile Formation Hynes, 309
HCV: Clinical Development Strategies II Hynes, Auditorium Noninvasive Assessment of Liver Fibrosis Hynes, Ballroom B
Stem Cell Biology and Differentiation Hynes, 302
All attendees must register to attend the meeting. There are separate registrations and fees for all events held during The Liver Meeting®. You are required to wear your badge to enter the Hynes Convention Center. If you did not receive your badge or pre-register, you are permitted to proceed directly to the registration area to obtain your meeting materials before moving around the center.

If you pre-registered and have your badge and tickets for the meeting, it is not necessary to go to the registration counter. You may go directly to the area marked “Program Distribution” to pick up your meeting bag and materials.

Program Distribution gets very busy just prior to the courses starting. By being early, you will avoid the long lines and not risk missing the start of your course.

All course handouts will be available at Program Distribution.

Course Handout Sales
Course handouts from the Postgraduate Course, Transplant Course, Pediatric Symposium, Research Workshop, Hepatology Associates Course, and the Endoscopy Course are available for sale in the Registration Area beginning Sunday, November 4.

Press Room • Hynes, 107

All course handouts will be available at Program Distribution.

Abstract Downloads
Abstracts selected for presentation at The Liver Meeting® are available for download to a USB drive. The download service is supported by Schering-Plough and is available to all attendees at Schering-Plough's booth # 301 during exhibit hall hours.

Daylight Saving Time Ends
Please be advised that Sunday, November 4, 2007, will start Standard Time. Turn your clocks back one hour Saturday night, November 3.
Shuttle Buses
Shuttle bus service is available from the Park Plaza Hotel and Towers, Radisson Hotel, Hyatt Regency Boston, Hampton Inn, Doubletree, Langham Hotel, Marriott Courtyard Tremont, and Omni Parker House, to the Hynes Convention Center daily beginning at 6:15 AM. Please check for confirmed shuttle times in the hotel and Hynes lobbies.

Cash Luncheons
- Hynes, Main Lobby
  Friday, November 2 – Monday, November 5
- Hynes, Halls C and D
  Saturday, November 3 – Monday, November 5

Boston Information and Restaurant Reservations  •  Hynes, Main Lobby
Attendees may obtain valuable discounts for tours and information about Boston area attractions. You can also make reservations for Boston restaurants here. This desk is open Friday, November 2, through Monday, November 5, from 10:00 AM - 6:00 PM.

Internet Center and Message Center  •  Hynes, Hall A
Keep in touch with home and the office as well as leave messages for other meeting attendees. The Internet Center will be open during regular registration hours. Free wireless service is available throughout the Hynes. Remember, you can also create your meeting itinerary, complete your Liver Meeting® overall evaluation, and print your CME certificate at these workstations.

Business Center  •  Hynes, Main Lobby
FedEx/Kinko’s business center is open from 7:00 AM - 6:00 PM, Friday, November 2 – Monday, November 5, and from 7:00 AM - 1:00 PM on Tuesday, November 6.

Coat Check  •  Hynes, Main Lobby
Cost to check items is $2.00 per piece.

HOURS:
- Friday, November 2 7:00 AM - 8:00 PM
- Saturday, November 3 7:00 AM - 8:00 PM
- Sunday, November 4 6:30 AM - 6:30 PM
- Monday, November 5 6:30 AM - 6:30 PM
- Tuesday, November 6 6:30 AM - 1:00 PM

Local Currency and Banking
The local currency is the U.S. dollar. All major credit cards are accepted at all hotels, shops and restaurants. There are two automatic teller machines (ATM) easily accessible to the Hynes, one at the entrance off Boylston Street (lower level) and one directly outside the entrance to the Prudential Center Mall.

Tips and Gratuities
It is common practice in the U.S. to tip restaurant servers, bellmen, and taxi drivers. The standard tip in restaurants is 18-20 percent. For bellmen and airport baggage handlers, the standard is $1.00 per bag; for taxis 15 percent is acceptable.

First Aid  •  Hynes, Main Lobby
First Aid is located in the Hynes main lobby to the left of Hall A. A registered nurse is available during meeting hours.

Lost and Found  •  Hynes, Main Lobby
Lost items left in meeting rooms may be found in the Hynes Security Office.

Smoking Policy
Smoking is prohibited in the Hynes.

ADA Compliance
The Hynes Convention Center and all participating hotels in The Liver Meeting® are fully accessible to the physically challenged. Anyone who has a need for special assistance should notify the appropriate hotel or the Hynes and indicate the type of assistance needed. AASLD cannot ensure the availability of appropriate assistance without prior notice.
Abstract Embargo
Accepted abstracts are made available to the public on the AASLD Web site and are published in a special supplement of HEPATOLOGY in advance of the meeting. Information contained in those abstracts may not be released until the abstracts appear on the AASLD Web site.

Academic institutions, private organizations, and companies with products whose value may be influenced by information contained in an abstract may issue a press release to coincide with the availability of the abstract on the AASLD Web site. However, AASLD requires information that goes beyond that contained in the abstract (e.g., discussion of the abstract done as part of a scientific presentation or presentation of additional or new information that will be available at the time of the meeting) be under embargo from release to the general public until November 2, 2007, the first day of the meeting. Information released prior to this day is a violation of the AASLD Abstract Embargo Policy and the abstract is subject to withdrawal from The Liver Meeting® program. Authors are responsible for notifying financial and other sponsors about this policy.

Late-breaking Abstracts
AASLD will present late-breaking abstracts
Oral session: 3:00 - 4:30 PM, Hynes, Auditorium
(Monday, November 5)
Poster session: 8:00 AM - 12:30 PM, Hynes, Hall C,
for viewing (Tuesday, November 6); presentation time is
10:00 - 11:30 AM.

Use of AASLD
Scientific Program Content
Information presented during the 58th Annual Meeting is the property of AASLD and the presenter. Information may not be recorded, photographed, copied, photocopied, transferred to electronic format, reproduced, or distributed without the written permission of AASLD and the presenter. Any use of the program content, which includes, but is not limited to oral presentations, audiovisual materials used by speakers, and program handouts, without the written consent of AASLD is prohibited.

Cameras and Cell Phones
Cameras and/or video and audio taping of sessions and posters are strictly prohibited during The Liver Meeting®. Continued use of any type of camera will result in your removal from the meeting venue.

As a courtesy to fellow attendees, please turn off cell phones during education sessions.

Speaker Ready Room • Hynes, 207
All speakers must check in to the Speaker Ready Room a minimum of two hours prior to the start of the speaker’s session. If possible, check in the day before your presentation to ensure a seamless presentation.

HOURS:
Thursday, November 1 ............. 1:00 - 6:00 PM
Friday, November 2 ............... 6:30 AM - 8:00 PM
Saturday, November 3 .......... 6:30 AM - 8:00 PM
Sunday, November 4 ............ 6:30 AM - 6:30 PM
Monday, November 5 ........... 6:30 AM - 6:30 PM
Tuesday, November 6 .......... 6:30 AM - 12:30 PM

View the Best of The Liver Meeting® Online
Enhance your meeting experience or catch a session you may have missed. View the Best of The Liver Meeting® 2007 Online each week starting mid-November. State-of-the-Art Lectures will be available for viewing first, followed by the General Hepatology Update, and the Plenary Sessions. The 2007 Postgraduate Course and Hepatology Associates Course will be available in the spring of 2008. Stop by The Liver Meeting® Online booth located in the main lobby of the Hynes, and sign up for this free service.
Poster Sessions • Hynes, Hall C

The Poster Sessions are important educational events during The Liver Meeting®. The top 10% of posters accepted are designated in the program as a “Presidential Poster of Distinction”. Posters must be dismantled immediately following the viewing time. Posters left on the boards will be removed and discarded.

Presenting authors must be available during presentation times noted below.

Posters should be set-up and viewed during the following times:

**Poster Session I**
Saturday, November 3

- **Set-up** . . . . . . . . . . . . . . . 8:00 AM - 2:00 PM
- **Viewing Time** . . . . . . . . . . . . 2:00 - 8:00 PM
  *Posters must be displayed until 8:00 PM.
- **Presentation Time** . . . . . . . . . 5:30 - 7:00 PM
  *Presenting authors are available to discuss their posters.
- **Dismantle Time** . . . . . . . . . . . 8:00 - 8:30 PM

**Poster Session II**
Sunday, November 4

- **Set-up** . . . . . . . . . . . . . . . 6:30 - 8:00 AM
- **Viewing Time** . . . . . . . . . . . . 8:00 AM - 5:30 PM
  *Posters must be displayed until 5:30 PM.
- **Presentation Time** . . . . . . . . . 1:00 - 2:30 PM
  *Presenting authors are available to discuss their posters.
- **Dismantle Time** . . . . . . . . . . . 5:30 - 6:00 PM

**Poster Session III**
Monday, November 5

- **Set-up** . . . . . . . . . . . . . . . 6:30 - 8:00 AM
- **Viewing Time** . . . . . . . . . . . . 8:00 AM - 5:30 PM
  *Posters must be displayed until 5:30 PM.
- **Presentation Time** . . . . . . . . . 1:00 - 2:30 PM
  *Presenting authors are available to discuss their posters.
- **Dismantle Time** . . . . . . . . . . . 5:30 - 6:00 PM

**Poster Session IV**
Tuesday, November 6

- **Set-up** . . . . . . . . . . . . . . . 6:30 - 8:00 AM
- **Viewing Time** . . . . . . . . . . . . 8:00 AM - 12:30 PM
  *Posters must be displayed until 12:30 PM.
- **Presentation Time** . . . . . . . . . 10:00 - 11:30 AM
  *Presenting authors are available to discuss their posters.
- **Dismantle Time** . . . . . . . . . . . 12:30 - 1:00 PM
Presentation of the Distinguished Service Award to William F. Balistreri, MD

The 2007 Distinguished Service Award will be presented to Dr. Balistreri during Presidential Plenary I at The Liver Meeting® on November 5, 2007. The AASLD Distinguished Service Award is presented annually to an individual in honor of his or her sustained service to AASLD and/or liver disease research. It recognizes the recipient’s lifelong commitment to the field of hepatology, and contribution to AASLD through service on numerous governance and scientific committees over the years.

William F. Balistreri, MD, is director of the Pediatric Liver Care Center and medical director of the Liver Transplantation Program at Cincinnati Children’s Hospital. Dr. Balistreri is professor of pediatrics and medicine at the University of Cincinnati College of Medicine; he has served as director of the Division of Pediatric Gastroenterology, Hepatology, and Nutrition for 25 years. Dr. Balistreri has authored more than 450 publications, including original articles, editorials, reviews, and chapters. He has edited or co-edited several books, including the first multi-authored text on pediatric hepatology and liver disease in children. He has helped to clarify the understanding of many aspects of pediatric hepatology, particularly in the areas of bile acid metabolism, neonatal cholestasis, hepatitis, and liver transplantation. Of significant importance, Dr. Balistreri has served as editor of the Journal of Pediatrics (1995 - present), as well as editor-in-chief of the Journal of Pediatric Gastroenterology and Nutrition (1991-1995).

In addition to being the first pediatrician to serve as president of AASLD, Dr. Balistreri is or has been a member of numerous prestigious, scholarly societies, including the North American Society for Pediatric Gastroenterology, Hepatology, and Nutrition (NASPGHAN) (President 1982–1984), Children’s Digestive Health and Nutrition Foundation (CDHNF) (President 2005 - present), American Board of Pediatrics (Sub-board Chair 1991 - 1993, and Board of Directors 1991 - 1997 and 2000 - 2003), and the American Liver Foundation (Board of Directors 1980 - 1983). He has also served on various committees and study groups in review of grants with the United States Department of Agriculture, the National Institutes of Health, the Department of Health and Human Services, and others.

Dr. Balistreri has received awards from various prestigious societies, including the Distinguished Leadership Award from the Crohn’s & Colitis Foundation of America (1995), the Andrew Sass-Kortsak Memorial Award from the Canadian Liver Foundation and the Canadian Association for the Study of the Liver (1998), the Murray Davidson Award from the American Academy of Pediatrics (AAP) Section on Gastroenterology & Nutrition (1999), the Shwachman Award (1999) from NASPGHAN, the Distinguished Alumnus Award from the University of Buffalo, the Founders Award from the Cincinnati Pediatric Society (1999), the Outstanding Pediatrician of the Year 2001 from the Ohio Chapter of the AAP, and the Daniel Drake Medal from the University of Cincinnati.

DISTINGUISHED SERVICE AWARDEES

1981 . . . . . . . . . . . . . . . . . . . Leon Schiff, MD
1983 . . . . . . . . . . . . . . . . . . . Hans Popper, MD
1984 . . . . . . . . . . . . . . . . . . . Burton Combes, MD
1988 . . . . . . . . . . . . . . . . . . . Dame Sheila Sherlock, MD
1989 . . . . . . . . . . . . . . . . . . . Herbert Falk, MD
1990 . . . . . . . . . . . . . . . . . . . Kunio Okuda, MD
1991 . . . . . . . . . . . . . . . . . . . Carroll M. Leevy, MD
1993 . . . . . . . . . . . . . . . . . . . Sarah C. Kalser, PhD
1997 . . . . . . . . . . . . . . . . . . . Steven Schenker, MD
1998 . . . . . . . . . . . . . . . . . . . Kamal G. Ishak, MD
1999 . . . . . . . . . . . . . . . . . . . Michael F. Sorrell, MD
2000 . . . . . . . . . . . . . . . . . . . Willis C. Maddrey, MD
2001 . . . . . . . . . . . . . . . . . . . Anthony S. Tavill, MD
2002 . . . . . . . . . . . . . . . . . . . Esteban Mezey, MD
2003 . . . . . . . . . . . . . . . . . . . Paul D. Berk, MD
2004 . . . . . . . . . . . . . . . . . . . Eugene R. Schiff, MD
2005 . . . . . . . . . . . . . . . . . . . Leonard Seeff, MD
2006 . . . . . . . . . . . . . . . . . . . Allan W. Wolkoff, MD
2007 . . . . . . . . . . . . . . . . . . . William F. Balistreri, MD
Presentation of the Distinguished Achievement Award to Juan Rodés, MD

The 2007 Distinguished Achievement Award will be presented to Dr. Rodés, during Presidential Plenary I at the The Liver Meeting® on November 5, 2007. The AASLD Distinguished Achievement Award is given to an individual in honor of his or her sustained scientific contributions to the field of liver disease and the scientific foundations of hepatology. The award honors a sustained contribution rather than a single discovery or major achievement.

Juan Rodés graduated from the University of Barcelona, School of Medicine in 1967. Hospital Clinic, run by the University of Barcelona Department of Medicine, has been his professional base where he has held a variety of different posts: medical director (1984 - 1986), director of research (1997 - 2003), and, since 2003, the hospital’s director general.

In 1972 he created and led the Liver Unit, which has gained international recognition for its work and research. He has published more than 470 original articles in the top ranking journals dedicated to the speciality, contributed more than 100 book chapters, and has been editor of 29 books.

In 1993 he founded and became the director of the Institute of Biomedical Investigation August Pi y Sunyer (IDIBAPS). He has been awarded various different honours, one of the most important being the National Award for Medical Investigation ‘Gregorio Marañon’ in 2006.

DISTINGUISHED ACHIEVEMENT Awardees

<table>
<thead>
<tr>
<th>Year</th>
<th>Awardee</th>
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<tbody>
<tr>
<td>1986</td>
<td>Hyman J. Zimmerman, MD</td>
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<td>1989</td>
<td>Aron Rappaport, MD</td>
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<td>1990</td>
<td>Rudi Schmid, MD</td>
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<td>1991</td>
<td>Thomas E. Starzl, MD</td>
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<td>1993</td>
<td>Stanley E. Bradley, MD and Carl A. Goresky, MD</td>
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<td>1994</td>
<td>Irwin M. Arias, MD</td>
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<td>1995</td>
<td>Telfer B. Reynolds, MD</td>
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<td>1996</td>
<td>Irmin Sternlieb, MD</td>
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<td>1997</td>
<td>Alan F. Hofmann, MD</td>
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<td>1998</td>
<td>James L. Boyer, MD</td>
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<td>1999</td>
<td>Francis V. Chisari, MD</td>
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<td>2000</td>
<td>Robert H. Purcell, MD</td>
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<td>2001</td>
<td>Jay H. Hoofnagle, MD</td>
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<td>2002</td>
<td>Roberto J. Groszmann, MD</td>
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<td>2003</td>
<td>Nicholas F. LaRusso, MD</td>
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<td>2004</td>
<td>D. Montgomery Bissell, MD</td>
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<td>E. Jenny Heathcote, MD</td>
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<td>2006</td>
<td>Neil Kaplowitz, MD</td>
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<td>2007</td>
<td>Juan Rodés, MD</td>
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PSC Partners Seeking a Cure Award for the Most Promising PSC Research

The 2007 award recipient is Tom H. Karlsen, MD.

PSC Partners Seeking a Cure is a non-profit foundation whose three-fold mission is to:

- Raise funds with which to research the causes and cures of primary sclerosing cholangitis (PSC),
- Promote PSC and organ donation awareness, and
- Provide education and support to PSC patients and their families.

Towards the goal of encouraging PSC research, the foundation offered an award to be presented at The Liver Meeting®. The annual award was given to the investigator presenting the most promising PSC research at the meeting.

Support of the PSC Partners Seeking a Cure Award for the Most Promising PSC Research by PSC Partners is gratefully acknowledged.

AASLD Student Research Award

This award is given to students who made a major contribution to the research study in the abstract submitted to the Annual Meeting. The 2007 award recipients are Montserrat Moreno and Michael Passeri. Dr. Moreno will present his abstract during the Hepatic Fibrogenesis Parallel Session. Dr. Passeri will present his abstract at the Alcohol: Clinical and Experimental Poster Session.

AASLD Young Investigator Travel Awards

These awards enable young researchers submitting abstracts for the AASLD Research Workshop to receive travel support. The 2007 award recipients are:

- Malte Heeg – Dr. Heeg will make a presentation during the Innate and Adaptive Immunity in Liver Diseases Parallel Session.
- Gadi Lalazar – Dr. Lalazar will make a presentation during the Innate and Adaptive Immunity in Liver Diseases Parallel Session.
- Ichiyo Hose – Dr. Hose will make a presentation during the Viral Hepatitis-Pathobiology Poster Session.
- Hyun Young Woo – Dr. Woo will make a presentation during the Clinical Liver Transplantation and Liver Surgery Poster Session.
- Mark Claassen – Dr. Claassen will make a presentation during the Inflammation and Immunobiology Poster Session.
- Maria Longhi – Dr. Longhi will make a presentation during the Cholestatic and Autoimmune Liver Disease Poster Session.
- Masashi Sasaki – Dr. Sasaki will make a presentation during the Inflammation and Immunobiology Poster Session.

AASLD Pediatric Fellow Research Award

The purpose of this award is to foster excellence in pediatric liver disease research. The award is given to a physician/scientist with a primary appointment affiliation in a department of pediatrics. The 2007 recipient is Ursula Matte. Dr. Matte will make a presentation during the Advances in Pediatric Hepatology Parallel Session.

AASLD Fellow Research Award

This prize is awarded for the best abstract submitted by a fellow. The 2007 recipient is Puneet Puri. Dr. Puri will make a presentation during the Presidential Plenary III Session.

AASLD/ALF Liver Scholar Award

The AASLD/ALF Liver Scholar Award is a three-year, basic science award of $75,000 per year that endeavors to encourage young investigators to pursue a career in liver-related research. The goal of the program is to permit scientists to bridge the gap between completion of research training and attainment of status as an independent research scientist. This additional research experience should enable them to compete for research grants from national sources, particularly the NIH. Recipients of these awards include:

2007 - 2009

- Rongze Yang, MD, PhD • University of Maryland, Baltimore
  PROJECT TITLE: Isoform-specific ALT Assay and its Application in Liver Disease

- Carlo Spirli, PhD • Yale University
  PROJECT TITLE: Epithelial Angiogenic Signaling in Polycystic Diseases of the Liver

2006 – 2008

- Lynette A. Gillis, MD • Vanderbilt University
  PROJECT TITLE: Mitochondrial Short-chain 3-Hydroxyacyl-CoA Dehydrogenase in Hyperinsulinism and Hepatic Steatosis

- Li Wang, PhD • The University of Kansas Medical Center
  PROJECT TITLE: Nuclear Receptor SHP – Mediated Regulation of Hepatic Lipid Homeostasis

2005 – 2007

- Antonia Follenzi, MD, PhD • Albert Einstein College of Medicine
  PROJECT TITLE: Mechanisms of Lentiviral Gene Transfer in Liver Sinusoidal Endothelial Cells

- Arumugam Jayakumar, MD • University of Miami
  PROJECT TITLE: Intracellular Signaling in Ammonia-induced Astrocyte Swelling: Potential Role in Brain Edema in Acute Liver Failure
**AASLD Jan Albrecht Commitment to Clinical Research in Liver Diseases Award**

Janice K. Albrecht, PhD, is vice president, hepatology clinical research for Schering-Plough Research Institute, the pharmaceutical research arm of Schering-Plough Corporation. Dr. Albrecht joined Schering-Plough Research Institute in 1981, was appointed to her present position in 1997, and is responsible for the planning, management, and oversight of Schering-Plough’s research and development program across the hepatology and gastroenterology therapeutic areas, including the treatment of viral hepatitis. Dr. Albrecht earned a BS degree from Iowa State University, an MS degree from Roosevelt University, Chicago, Illinois, and her PhD in pharmaceutics from Temple University, Philadelphia, Pennsylvania. Her work has been published in leading scientific journals.

The purpose of the AASLD Jan Albrecht Commitment to Clinical Research in Liver Diseases Award is to foster career development for individuals performing clinical research and/or translational research in a liver-related area and who have shown commitment to excellence at an early stage of their research study. Recipients of this two-year award include:

**2007 - 2009**

Patrick G. Northup, MD • University of Virginia
PROJECT TITLE: *Clinical, Inflammatory, and Economic Impact of Dextran-70 in Treating SBP*
MENTOR: Carl L. Berg, MD

**2006 - 2008**

George N. Ioannou, BMBCh, MS • University of Washington
PROJECT TITLE: *Liver Transplantation for Hepatocellular Carcinoma in the MELD Allocation Era*
MENTOR: Robert L. Carithers, Jr, MD

**2005 - 2007**

Scott W. Biggins, MD • University of California, San Francisco
PROJECT TITLE: *Selection Bias in Re-transplantation Survival Models: The Impact of Listing Criteria and Hepatitis C*
MENTOR: Norah Terrault, MD, MPH

Support of the AASLD Jan Albrecht Commitment to Clinical Research in Liver Diseases Award by Schering-Plough is gratefully acknowledged.

**AASLD Sheila Sherlock Clinical and Translational Awards in Liver Diseases**

Dame Sheila Sherlock began her study in medicine in 1936 at Edinburgh University. She graduated MB, BCh (summa cum laude) in 1941, and at the top of her class, won the Ettles Scholarship. As a woman, she was unable to receive an internship at the Royal Infirmary and became clinical assistant to the Professor of Surgery where she was introduced to medical research. In 1942, she began her pioneering career in hepatology at Hammersmith Hospital and the Postgraduate Medical School. In 1943, she co-published a paper on the pathology of acute hepatitis. This work was the basis of her thesis for an Edinburgh MD (and gold medal) in 1945. She proceeded to study the biochemistry of the liver and its disorders. In 1947, she was awarded a Rockefeller traveling fellowship and went to the Department of Physiological Chemistry at Yale. A year later, at the age of only thirty, she returned to Hammersmith as Lecturer and Honorary Consultant Physician. Within a few short years, she and her liver unit were famous, attracting research fellows from around the world. In 1959, she was the first woman appointed to the Chair of a British Department of Medicine at the Royal Free Hospital School of Medicine.

Some of the numerous contributions Sheila and her co-workers at the Royal Free made to hepatology include:

- Linked the hepatitis B virus (when it was still the “Australia antigen”) to the development of cirrhosis and hepatocellular carcinoma, and studied the role of sexual transmission and male homosexuality.
- Introduced the antimitochondrial antibody as a test for primary biliary cirrhosis.
- Recognized the benefit of immunosuppression with prednisolone for autoimmune hepatitis.

Numerous papers were also produced including classics on Wilson’s disease, hemochromatosis, Budd-Chiari syndrome, extrahepatic portal vein block, partial nodular transformation of the liver, and primary sclerosing cholangitis.

Dame Sheila Sherlock wrote, edited, and contributed to many works, most notably *Diseases of the Liver and Biliary System*, which, in its eleventh edition, remains a classic and is translated into several languages.

The purpose of the AASLD Sheila Sherlock Clinical and Translational Awards in Liver Diseases is to foster career development for individuals performing clinical research and/or translational research in a liver-related area and who have shown commitment to excellence at an early stage of their research study. Recipients of these two-year awards include:

**2007 - 2009**

Matthew Cave, MD • University of Louisville
PROJECT TITLE: *Environmental Toxins and Non-alcoholic Fatty Liver Disease*
MENTOR: Craig McClain, MD

Support of the AASLD Sheila Sherlock Clinical and Translational Awards in Liver Diseases by Schering-Plough is gratefully acknowledged.
**AASLD Awards (continued)**

**Tamar H. Taddei, MD, PhD • Yale University School of Medicine**

**PROJECT TITLE:** Phenotypic and Pathophysiologic Determinants of the Hepatobiliary Manifestations of Gaucher Disease  
MENTOR: Pramod K. Mistry, MD, PhD  
2006 – 2008

**Ningling Kang-Decker, PhD • Mayo Foundation**

**PROJECT TITLE:** Mechanism of Nitric Oxide Inhibition of Hepatic Stellate Cell Migration in Liver Metastasis of Pancreatic Cancer  
MENTOR: Vijay Shah, MD

**Ravi R. Jhaveri, MD • Duke University Medical Center**

**PROJECT TITLE:** Investigating the Pathogenesis of Steatosis in Hepatitis C Genotype 3 Infection  
MENTOR: Anna Mae Diehl, MD  
2005 - 2007

**Kathleen M. Campbell, MD • Cincinnati Children’s Hospital Medical Center**

**PROJECT TITLE:** Pharmacogenetics of Posttransplant Renal Dysfunction  
MENTOR: William F. Balisteri, MD

**Aaron Robert Turkish, MD • Columbia University**

**PROJECT TITLE:** DGAT1 and the DGAT2 Gene Family in Nonalcoholic Fatty Liver Disease  
MENTOR: Stephen L. Sturley, PhD

Support of the AASLD Sheila Sherlock Clinical and Translational Awards in Liver Diseases by Roche is gratefully acknowledged.

**AASLD Career Development Award in Liver Transplantation**

The purpose of this award is to foster career development for a junior faculty member performing clinical and/or translational research in the field of liver transplantation and who has shown commitment to excellence in the field at an early stage of his or her career. The 2007 recipient will be announced at the meeting.  
Support of the AASLD Career Development Award in Liver Transplantation by Astellas USA Foundation is gratefully acknowledged.

**AASLD Advanced/Transplant Hepatology Fellowship Program**

This one-year award of $60,000 provides salary and benefit support for gastroenterology fellows pursuing an additional full year of training focused on patient care in advanced/transplant hepatology. The intent of the program is to prepare the trainee to be eligible for certification in transplant hepatology by the American Board of Internal Medicine (ABIM). The 2007 recipients are:

- **Karin L. Anderson • Massachusetts General Hospital**  
  MENTOR: Raymond T. Chung, MD
- **Ottar M. Bergman • University of Iowa Hospitals and Clinics**  
  MENTORS: Kyle E. Brown, MD and Michael D. Voight, MD
- **Virginia C. Clark • University of Florida**  
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- **Linda A. DiTeodoro • Mayo Clinic Jacksonville**  
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- **Amy C. McClune • Mount Sinai School of Medicine**  
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- **Kerry N. Whitt • Mount Sinai Hospital**  
  MENTOR: Thomas D. Schiano, MD

Support of the 2007 Advanced/Transplant Hepatology Fellowship Program by Bristol-Myers Squibb, Roche, and Schering-Plough is gratefully acknowledged.

**AASLD NP/PA Clinical Hepatology Fellowship Program**

Designed to increase the number of mid-level practitioners in clinical hepatology, the purpose of this program is to provide salary and benefit support for certified and licensed physician assistants (PA) or nurse practitioners (NP) pursuing a full year of training focused on clinical care in hepatology. The 2007 recipients are:

- **Foong-chee (Joann) Cheah • The Liver Institute at Methodist Dallas**  
  MENTOR: Reem H Ghalib, MD
- **Joseph P. Colagreco • Beth Israel Deaconess Medical Center**  
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Support of the 2007 AASLD NP/PA Clinical Hepatology Fellowship Program by Roche and Schering-Plough is gratefully acknowledged.
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Tushar Patel, MD
Jean-Michel Pawlotsky, MD, PhD
Steven J. Polyak, PhD
John J. Peterucha, MD
Jorge L. Rakela, MD
Charles M. Rice, PhD
Lewis R. Roberts, MD, PhD
Simon C. Robson, MD, PhD
Marianne J. Rosati, MSN, NP
Hugo R. Rosen, MD
Michael L. Schilsky, MD
Leonard B. Seeff, MD
Stewart Selig, MD
Vijay Shah, MD
Kenneth E. Sherman, MD, PhD
Kirti Shetty, MD
Mitchell L. Shiffman, MD
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Richard K. Sterling, MD
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American Liver Foundation

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Public policy issues having a direct impact on the discipline are actively monitored and communicated by AASLD’s Public Policy Committee, which makes recommendations to influence issues as they are raised in the nation’s capital. The AASLD 2007 Public Policy Agenda developed by the Public Policy Committee and approved by the Governing Board follows.

Global
AASLD will continue to implement a policy and public relations program focused on liver health. Specifically, AASLD will (a) further develop and implement a strategy for making ALT levels analogous to cholesterol levels with the public, (b) finalize a white paper that summarizes the state of the science, and (c) begin to work with the NCQA on the creation of a HEDIS standard related to ALT levels.

Research Issues
AASLD strongly supports funding for scientific research and training throughout the federal government. Specifically:

- AASLD supports the highest attainable funding levels to rapidly advance progress throughout the NIH as envisioned by the Trans-NIH Action Plan for Liver Disease Research (TAPLDR), adopted by NIDDK in 2004.
- AASLD supports legislation to reauthorize NIH and non-legislative revisions in structures and processes at NIH only to the extent they enhance the implementation of the TAPLDR. AASLD opposes changes in the structure of NIH that, in its judgment, have the potential to siphon off research funds from liver-related research.
- AASLD supports the highest attainable funding levels for liver research through the Department of Veterans Affairs and other agencies.
- AASLD supports keeping all functions of the Armed Forces Institute of Pathology (AFIP) (its consultive services, education and training, and research) consolidated and available to researchers both within and without government.

Patient Care Issues
AASLD supports federal policies that will improve and enhance medical treatment for patients with liver disease, and promote liver wellness. Specifically:

- AASLD supports the provision of liver health care to all Americans, regardless of ability to pay.
- AASLD supports the highest attainable level of federal funding for states’ efforts to prevent the spread of infectious liver diseases (particularly viral hepatitis), and to diagnose and treat those who are already infected.

- AASLD supports developing legislation to create a chronic liver disease program at the Centers for Disease Control and Prevention (CDC).
- AASLD strongly encourages the FDA to address aggressively adverse events caused by inadvertent overdoses of acetaminophen, particularly related to compound drugs.
- AASLD supports an aggressive effort to diagnose and treat liver disease for veterans under the jurisdiction of the Veterans Health Administration.

Transplantation-related Issues
AASLD supports aggressive federal action to increase the number of cadaveric and living donor organs, including livers, available to be transplanted into patients who are eligible pursuant to current transplantation standards.

- AASLD supports increasing funding to promote organ donation programs directly by the federal government, as well as through the states, to the highest attainable levels.
- AASLD supports continuing research at the NIH designed to reduce the need for organs to be transplanted and designed to improve outcomes from transplantation.

Key Tactics
To fulfill its agenda, AASLD will increase its visibility as the premiere scientific organization dedicated to the prevention and cure of liver disease and promotion of liver wellness. AASLD will:

- Meet regularly with key leaders in NIH, CDC, FDA, and other agencies, as well as in Congress. A list of these leaders will be updated annually.
- Inform decision makers via letters, emails, and meetings concerning its accomplishments in liver education and important research breakthroughs made by AASLD members.
- Provide contact information sheets to ensure that the expert testimony of its members will be at the disposal of the press, state and national officials.
- Support the Governing Board in its efforts to obtain greater public recognition of liver disease and of AASLD’s effort to fight it. Use this as a basis for press releases, press conferences, high visibility meetings, awards, etc., as appropriate.
- Continue to refine policy statements on issues facing liver disease research and patient care. When approved, these statements will be posted on AASLD’s Web site and distributed to all potential ad hoc partners.
- Work in partnership with government, industry, and other nonprofit patient and professional associations to achieve these aims.
AASLD gratefully acknowledges and thanks its corporate supporters whose generosity and educational grants make many features of The Liver Meeting® possible. As of July 1, 2007, the following commitments have been received in support of The Liver Meeting®:

**Bristol-Myers Squibb Company**
- Online Abstract Viewer and Itinerary Planner
- Meeting Planner Notebook

**GILEAD**
- Boston City Guide & Map
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- The Liver Meeting® Abstracts USB Download
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The Exhibit Hall, an informative and important component of The Liver Meeting®, provides you with a valuable opportunity to meet with representatives from various organizations that are committed to the field of hepatology. A complete listing of Exhibitors and their product descriptions begins below.

### EXHIBIT HOURS

<table>
<thead>
<tr>
<th>Day</th>
<th>Time</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Saturday, November 3</em></td>
<td>5:30 - 8:00 PM</td>
</tr>
<tr>
<td><em>Sunday, November 4</em></td>
<td>9:30 AM - 3:00 PM</td>
</tr>
<tr>
<td><em>Monday, November 5</em></td>
<td>9:30 AM - 3:00 PM</td>
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</tbody>
</table>

*Please join your colleagues on Saturday, November 3, for the Exhibit Hall Opening Reception.

### Exhibitors

<table>
<thead>
<tr>
<th>Company</th>
<th>Booth #</th>
</tr>
</thead>
<tbody>
<tr>
<td>AGA Institute</td>
<td>1</td>
</tr>
<tr>
<td>4930 Del Ray Avenue</td>
<td></td>
</tr>
<tr>
<td>Bethesda, MD 20814</td>
<td></td>
</tr>
<tr>
<td>Phone: 301-654-2055</td>
<td></td>
</tr>
<tr>
<td>Website: <a href="http://www.gastro.org">www.gastro.org</a></td>
<td></td>
</tr>
<tr>
<td>AGA's 16,000-plus members include physicians and scientists who research, diagnose, and treat disorders of the gastrointestinal tract and liver. Stop by our booth to learn about membership as well as programs and products offered by the AGA Institute, which runs the organization’s practice, research and educational initiatives. View demonstrations of popular AGA Institute educational offerings.</td>
<td></td>
</tr>
</tbody>
</table>

| American Porphyria Foundation | 13        |
| 4900 Woodway                 |          |
| Suite 780                    |          |
| Houston, TX 77056            |          |
| Phone: 866-APF:3635          | Fax: 713-840-9552 |
| Website: www.porphyriafoundation.com |  |
| The American Porphyria Foundation is dedicated to improving the health and well-being of individuals and families affected by porphyria. Our mission is to: enhance public awareness about porphyria, develop education programs, distribute educational material for patients and physicians, and support research to improve treatment and discover a cure. |  |

| American Liver Foundation   | 9, 10    |
| 75 Maiden Lane             |          |
| Suite 603                  |          |
| New York, NY 10038         |          |
| Phone: 212-668-1000        | Fax: 212-483-8179 |
| Website: www.liverfoundation.org |  |
| The American Liver Foundation is the nation’s leading non-profit organization promoting liver health and disease prevention. ALF provides research, education, and advocacy for those affected by liver-related diseases, including hepatitis. |  |

| American Transplant Congress | 11        |
| 15000 Commerce Parkway      |          |
| Suite C                     |          |
| Mt. Laurel, NJ 08054        |          |
| Phone: 856-439-0500         | Fax: 856-439-0525 |
| Website: www.atcmeeting.org |  |
| The American Society of Transplant Surgeons and American Society of Transplantation will hold their annual American Transplant Congress (ATC), May 31– June 4, 2008 at Metro Toronto Convention Centre in Toronto, Ontario, Canada. ATC focuses on all clinical and research aspects of organ transplantation. Stop by the booth to pick up information about the meeting and Toronto. |  |

| Apotex Corporation          | 630, 632 |
| 2400 N. Commerce Parkway    |          |
| Suite 400                   |          |
| Weston, FL 33326            |          |
| Phone: 800-706-5575         | Fax: 800-706-5576 |
| Apotex produces more than 260 generic pharmaceuticals distributed in over 115 countries worldwide. Apotex is active in biotechnology through publicly traded Cangene Corporation. (TSE). In April, 2007 HepaGam B™ was the first and only HGIg product granted FDA approval to prevent hepatitis B recurrence following liver transplantation in Hepatitis B surface antigen (“HBsAg”)-positive liver transplant patients. For more information, please visit www.hepagamb.com or call 1-877-HEPAGAMB (437-2426.) |  |
Arbios Systems, Inc.  
Booth # 101  
8797 Beverly Blvd.  
Suite 304  
Los Angeles, CA 90048  
Phone: 310-657-4898  
Fax: 310-657-4879  
Website: www.arbios.com  

Arbios Systems, Inc. is a medical device and cell-therapy company focused on the development of products for the treatment of liver failure. Our lead product currently in clinical trials is the SEPET™ Liver Assist Device, a novel extracorporeal blood purification therapy using a specially designed hollow fiber cartridge.

Asklepion Pharmaceuticals, LLC  
Booth # 125  
5200 Maryland Way  
Suite 204  
Brentwood, TN 37027  
Phone: 615-377-4617  
Fax: 615-467-3083  
Website: www.asklepionpharm.com  

The corporate vision is that of a company focused on researching, developing, and marketing biopharmaceutical products for the unmet or underserved needs of patients afflicted with liver-based diseases. Asklepion is developing products which range from small, orphan products to blockbuster products.

Astellas Pharma US, Inc.  
Booth # 535  
3 Parkway North  
Deerfield, IL 60015  
Phone: 800-888-7704  
Fax: 847-317-5953  
Website: www.astellas.com  

Astellas Pharma, Inc. manufactures and markets proprietary pharmaceutical products in specialty therapeutic areas where there is an unmet medical need. Astellas Pharma’s global mission to explore the frontiers of human health underscores the company’s commitment to the field of immunology, where aggressive research and development initiatives have helped to put Astellas at the forefront of transplantation.

Bayer Healthcare Corporation & Onyx Pharmaceuticals, Inc.  
Booth # 107, 109  
400 Morgan Lane  
West Haven, CT 06516  
Phone: 203-812-2000  
Website: www.bayer.com  
www.onyx-pharm.com  

Bayer Oncology is committed to combining our passion for science, discovery and innovation to help improve the lives of cancer patients. We invite you to learn more about Bayer Oncology and NEXAVAR (sorafenib) tablets, for the treatment of patients with advanced renal cell carcinoma.

Axcan Pharma  
Booth # 104, 205, 207  
597 Laurier Blvd.  
Mont-Saint-Hilaire, QC J3H 6C4  
Canada  
Phone: 450-467-5138  
Fax: 450-467-3067  
Website: www.axcan.com  

Axcan is a leading multinational specialty pharmaceutical company focused on gastroenterology. The company develops and markets a broad line of prescription products to treat a range of gastrointestinal diseases and disorders such as inflammatory bowel disease, irritable bowel syndrome, cholestatic liver diseases, and complications related to pancreatic insufficiency.

Bristol-Myers Squibb  
Booth # 128, 325  
PO Box 4500  
Princeton, NJ 08543-4500  
Phone: 609-897-2000  
Fax: 609-897-6722  
Website: www.bms.com  

Bristol-Myers Squibb welcomes you to Boston! We invite you to visit our exhibit and welcome the opportunity to meet our representatives to discuss the products and services we have to offer.

Centers for Disease Control & Prevention  
Booth # 7  
1600 Clifton Road, NE  
MS: G37, DVH CDC  
Atlanta, GA 30329  
Phone: 404-718-8525  
Fax: 404-718-8595  
Website: www.cdc.gov/hepatitis  

CDC’s Division of Viral Hepatitis offers expertise on preventing and controlling viral hepatitis. Recommendations, guidelines, surveillance forms, disease burden data, and other publications on the epidemiology and prevention of viral hepatitis are available at www.cdc.gov/hepatitis. Training curricula, courses, posters, brochures, slide sets, and fact sheets are also available.

Chronic Liver Disease Foundation  
Booth # 637  
1199 Raritan Road  
Clark, NJ 07066  
Phone: 973-644-9777  
Fax: 973-939-8516  
Website: www.chronicliverdisease.org  

The Chronic Liver Disease Foundation is a nonprofit 501(c)(3) educational organization dedicated to increasing awareness of the effect of chronic liver disease (CLD) in the United States. The foundation’s goal is to provide health professionals with the most current education and information on CLD.
Clinical Care Options

1894 Preston White Drive
Suite 110
Reston, VA 20191
Phone: 970-513-9174  Fax: 866-691-8321
Website: www.clinicaloptions.com

Clinical Care Options (CCO) is the leader in the development of innovative interactive medical education programs for hepatitis healthcare professionals. CCO creates and publishes original continuing medical education and information resources that translate the latest developments in science and medicine into treatment options. Available online at: http://clinicaloptions.com/hepatitis.

The Cochrane Hepato-Biliary Group

Rigshospitalet
Dept. 3344
Copenhagen, DK-2100
Denmark
Phone: 45-3545-7169  Fax: 45-3545-7101
Website: http://ctu.rh.dk/chbg

The CHBG is a non-profit, international clinical research group with 1200+ members. Cochrane systematic reviews of interventions for hepatic and biliary diseases are our main product. 163 peer-reviewed protocols for systematic reviews and 80 systematic reviews are published in Issue 3, 2007. A CHBG Register with 11,000 references on randomised or controlled clinical trials is maintained in CENTRAL database in The Cochrane Library. Cochrane reviews are independent of industry interests and funding.

Corgenix, Inc.

11575 Main Street
Suite 400
Broomfield, CO 80020
Phone: 303-457-4345  Fax: 303-457-4519
Website: www.corgenixonline.com

Corgenix, Inc. develops, manufactures, and markets clinical diagnostic products for autoimmune and vascular (thrombosis) disorders, as well as the Hyaluronic Acid Test Kit for the early detection of liver fibrosis and cirrhosis. Corgenix is based in metropolitan Denver, Colorado with its international office near Cambridge, England.

CuraScript Inc.

6272 Lee Vista Blvd.
Orlando, FL 32811
Phone: 888-773-7376  Fax: 888-773-7386
Website: www.curascript.com

CuraScript is a leading specialty pharmacy provider of healthcare products and services to individuals with chronic health conditions such as asthma, growth hormone deficiencies, hepatitis C, hemophilia, HIV/AIDS, oncology, multiple sclerosis, and rheumatoid arthritis. CuraScript’s patient management services include clinical case management, counseling, education, outcomes measurement, social services, and reimbursement services.

Cylex, Inc.

8980-I Old Annapolis Road
Columbia, MD 21045
Phone: 410-964-0236  Fax: 410-964-0367
Website: www.cylex.net

Cylex® manufactures the only FDA-cleared assay measuring the vitality of patients’ immune systems from a single drop of blood. ImmuKnow® enables physicians to better manage and individualize treatments for life-threatening diseases, such as HIV, HCV, cancer, and autoimmune disorders, as well as solid organ and bone marrow transplantation.

Digestive Care, Inc.

1120 Win Drive
Bethlehem, PA 18017
Phone: 610-882-5950  Fax: 610-882-0349
Website: www.digestivecare.com

Digestive Care, Inc. (DCI) is dedicated to developing unique pharmaceutical products to alleviate complications and symptoms of gastrointestinal disorders. DCI’s research into the controlled delivery of gastric acid resistant digestive enzymes and buffered bile acids through micro encapsulation led to the development of the highly successful drug product, PANCRECARB® (pancrelipase).

Duke Clinical Research Institute

2424 Erwin Road
Suite 402
Durham, NC 27705
Phone: 919-668-8700  Fax: 919-668-7114
Website: www.dcri.org

The Duke Clinical Research Institute (DCRI) offers a unique combination of clinical expertise, academic leadership, operational capabilities, and business acumen that translates into targeted and sound research results. With thought-leaders who stay at the cutting edge of scientific discovery and industry trends, our efforts consistently help sponsors to be not only first to market, but also first to peak sales. The DCRI... more than a CRO.
EchoSens
153 Avenue D’Italie
Paris, 75013
France
Phone: 33-1-44-82-78-50 Fax: 33-1-44-82-78-60
Website: www.echosens.com

Created in 2001, EchoSens is a French company specializing in innovative medical devices based on its own discovery, the transient elastography. Its first device, the FibroScan® and its dedicated probe, has quickly become a reference and is supported by worldwide medical practitioners, hepatologists and professionals in other pathologies. Totally non-invasive and painless, our device enables users to quantify liver fibrosis.

Elsevier
PO Box 390
Humarock, MA 02047
Phone: 781-837-0797 Fax: 781-837-1437

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European Association for the Study of the Liver (EASL)
PO Box 1726 – 17, rue du Cendrier CH-1211, Geneva Switzerland
Phone: 41-22-906-9151 Fax: 41-22-732-2607
Website: www.easl.ch

The essential mission of the EASL is to promote liver research and to improve the treatment of liver disease throughout the world. As an active advisor to the European/National Health authorities, EASL aims to raise public awareness in liver disease and attempts to fulfill its mission by forging friendships and linkages amongst hepatologists around the world. This is mainly done through its annual meeting and educational programs.

Evivar Medical
501/100 Victoria Parade
E. Melbourne, VIC 3002 Australia
Phone: 613-9662-4900 Fax: 613-9662-4933

Evivar Medical is a joint venture company established to develop and commercialise SeqHepB, a web-based program incorporating Hepatitis B virus mutations associated with antiviral drug resistance. Evivar and its consortium partners have built an extensive patent portfolio that includes recent discoveries associated with multiple pathways of evolution of HBV drug-resistance as well as cross-resistance and multi-drug resistance.

Gambro AB
Scheelevagen 34
PO Box 10101
Lundia, SE-22010
Sweden
Phone: 46-70-8589212 Website: www.gambro.com

Over the last 40 years, Gambro has been a world leading company in renal therapy, with sales, service, and support in more than 100 countries. Through extensive investment in research and development, and in cooperation with leading clinicians and scientists worldwide, we are able to provide high quality, innovative, therapy-enhancing products and services. With the MARS Therapy, Gambro proudly introduces the first unified approach to hepatic & renal support therapy for severe liver failure. Please visit our booth and we will explain further to you about our offerings.

Gastroenterology & Hepatology
611 Broadway
Suite 310
New York, NY 10012
Phone: 212-995-5522 Fax: 212-995-5572
Website: www.clinicaladvances.com

Gastroenterology & Hepatology is a monthly, independent peer reviewed journal circulated to all U.S. gastroenterologists, pediatric gastroenterologists, colon/rectal surgeons, hepatologists, and 2,800 primary care physicians (total circulation = 16,200+). Our mission is to contribute to the advancement of these fields by providing indispensable editorial content. Gastroenterology & Hepatology fulfills the need of practicing physicians for a journal containing the most up-to-date clinical information in gastrointestinal disorders, including diseases of the liver and biliary tract.

Gilead Sciences, Inc.
333 Lakeside Drive
Foster City, CA 94404
Phone: 650-574-3000 Fax: 650-578-9264
Website: www.gilead.com

Gilead Sciences is a biopharmaceutical company that discovers, develops, and commercializes therapeutics to advance the care of patients suffering from life-threatening disease worldwide. For more information, please visit www.gilead.com.
GlaxoSmithKline is one of the world’s leading research-based pharmaceutical companies with a powerful combination of skills to discover and deliver innovative medicines. In support of the appropriate use of our products, GlaxoSmithKline offers a number of programs to support our mutual goals to achieve effective health management strategies and improve patient care. Please visit our exhibit to learn more about our products and programs.

HepaHope, Inc. is dedicated to the development and commercialization of technologies that aid in the treatment of diseased livers. HepaHope presents the HepaPheresis Instrument™, a Bio-Artificial Liver (BAL) system that leverages HepaHope’s platform organ slice technology to replicate normal liver functionality, acting as a treatment bridge to liver transplant.

The Hepatitis Foundation International (HFI) conducts Foundation for Decision Making Training sessions promoting healthy lifestyle behaviors and prevention of viral hepatitis. HFI also conducts education programs for health professionals, teachers, nurses, community leaders, substance abuse counselors, and the public.

Cheng Si Yuan (China – International) Hepatitis Research Foundation is an independent charitable organization founded by the Vice-Chairman of the National People’s Congress, PR China, The Hon Cheng Si-yuan and The Hon Wu Jieping as well as the late Mr James Lau in 1998. Aimed at improving the health standard of Chinese people, the Foundation will recruit the most reputable and top hepatologists from Mainland China, Hong Kong, Taiwan and overseas together to exchange views and knowledge on hepatology research, and to improve the standard of prevention, diagnosis and treatment of liver diseases.

Hepatitis Research Foundation Cheng Si Yuan Booth # 14
Room 102-105, School of General Nursing
Queen Mary Hospital
Hong Kong
China
Phone: 852-2818-4300 Fax: 852-2818-4030
Website: www.csfoundation.org

Cheng Si Yuan (China – International) Hepatitis Research Foundation is an independent charitable organization founded by the Vice-Chairman of the National People’s Congress, PR China, The Hon Cheng Si-yuan and The Hon Wu Jieping as well as the late Mr James Lau in 1998. Aimed at improving the health standard of Chinese people, the Foundation will recruit the most reputable and top hepatologists from Mainland China, Hong Kong, Taiwan and overseas together to exchange views and knowledge on hepatology research, and to improve the standard of prevention, diagnosis and treatment of liver diseases.

Honso USA offers standardized herbal medicine to physicians including Sho-saiko-to (SST), which has been used by millions of patients with hepatitis B and C in Asia and thousands in the U.S. SST is now offered in capsule form under the name Hepzone SST. Backed by extensive scientific research and promising results from two U.S. phase II clinical trials (IND #62,929 and #65,793), SST is receiving acclaim from doctors and patients as an effective, evidence-based herbal medicine supporting healthy liver function.

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Room 102-105, School of General Nursing
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China
Phone: 852-2818-4300 Fax: 852-2818-4030
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Honso USA, Inc. Booth # 706
4602 E. Elwood Street
Suite 6
Phoenix, AZ 85040
Phone: 888-461-5808 Fax: 888-408-5808
Website: www.honsousa.com

Honso USA offers standardized herbal medicine to physicians including Sho-saiko-to (SST), which has been used by millions of patients with hepatitis B and C in Asia and thousands in the U.S. SST is now offered in capsule form under the name Hepzone SST. Backed by extensive scientific research and promising results from two U.S. phase II clinical trials (IND #62,929 and #65,793), SST is receiving acclaim from doctors and patients as an effective, evidence-based herbal medicine supporting healthy liver function.

Humana Press is a worldwide publisher of books and journals in molecular biology, neuroscience, cancer research, and medicine. Humana publishes approximately 150 book titles per year as well as a number of successful non-series titles and has a backlist of approximately 1,700 titles and a portfolio of 25 journals. Humana’s clinical series include Contemporary Cardiology™ and Current Clinical Practice™, Contemporary Endocrinology™, Contemporary Diabetes™, Current Clinical Urology™s, Current Clinical Oncology™, Forensic Science and Medicine™, and Nutrition and Health™.

Idenix Pharmaceuticals and Novartis Pharma AG have created an alliance that seeks to bring the world new therapeutic options for the treatment of hepatitis. This alliance is committed to shaping the future of hepatitis therapy by investigating and introducing innovative medicines that may offer physicians additional tools for disease management and, ultimately, improved outcomes for patients.

Idenix Pharmaceuticals Booth # 313
One Kendall Square
Bldg. 1400
Cambridge, MA 02139
Phone: 617-995-9230 Fax: 617-577-1715
Website: www.idenix.com

Idenix Pharmaceuticals and Novartis Pharma AG have created an alliance that seeks to bring the world new therapeutic options for the treatment of hepatitis. This alliance is committed to shaping the future of hepatitis therapy by investigating and introducing innovative medicines that may offer physicians additional tools for disease management and, ultimately, improved outcomes for patients.
Innogenetics, Inc.  
2580 Westside Parkway  
Suite 400  
Alpharetta, GA 30004  
Phone: 678-393-1672  
Fax: 678-393-1673  
Website: www.innogenetics.com

Innogenetics is an international biopharmaceutical company building parallel businesses in specialty diagnostics and therapeutic vaccines. Its Diagnostics Division develops a number of specialty products, especially for infectious diseases (hepatitis C, hepatitis B, HIV, and HPV). Within the field of hepatitis, Innogenetics has developed leading assays for genotyping of hepatitis C virus (a prototype drug resistance assay is under development), as well as genotyping, drug resistance, and pre-core mutants of hepatitis B virus.

INOVA Diagnostics, Inc.  
9900 Old Grove Road  
San Diego, CA 92131  
Phone: 858-586-9900  
Fax: 858-586-9911  
Website: www.inovadx.com

INOVA Diagnostics, Inc. develops, manufacturers and sells a complete menu of autoimmune disease diagnostic kits and components for screening and specific autoantibody determinations. Product groups include kits and components for rheumatoid arthritis, connective tissue disease, coagulation, gastrointestinal, Vasculitis, endocrine, and autoimmune liver disease.

InScope  
4545 Creek Road  
Cincinnati, OH 45242  
Phone: 513-337-7000  
Fax: 513-337-1225  
Website: www.ethiconendo.com

InScope, a division of Ethicon Endo-Surgery, Inc., is committed to the development of Endoscopic devices designed to make a difference in clinical practice. By creating practical, innovative, and procedure-enabling devices, InScope is dedicated to enhancing the future of everyday Endoscopy.

International Association for the Study of the Liver (IASL)  
StMUGV-ref: 31  
P.O.B. 810140  
Munich, 81901  
Germany  
Phone: 49-89-9214-2141  
Fax: 49-89-9214-2384  
Website: www.iaslonline.com

The International Association for the Study of the Liver (IASL) is a non-profit organization composed of AASLD, AFASLD, APASL, EASL and LAASL. Its mission is: 1) to promote international education of hepatologists; 2) to facilitate research, and 3) to organize meetings on hepatobiliary disease in conjunction with the Constituent Associations.

International Liver Transplantation Society  
15000 Commerce Parkway  
Suite C  
Mt. Laurel, NJ 08054  
Phone: 856-642-4215  
Fax: 856-439-0525  
Website: www.ilts.org

The ILTS mission is to promote and disseminate multidisciplinary advances in liver transplantation worldwide. ILTS has over 850 members from 60 countries whose primary focus is to advance the practice of liver transplantation through education. Join us in Paris, France July 9 - 12, 2008 for our International Congress on Liver Transplantation. Abstracts are now being accepted. See www.ilts.org for more information.

iQur Ltd  
Mailpoint 811, Level D, South Block  
Southampton General Hospital, Tremona Road  
Southampton, Hants SO16 6YD  
United Kingdom  
Phone: 44-23-8079-8945  
Fax: 44-23-8079-4145  
Website: www.iqur.com

iQur is a biotechnology company specialising in the diagnosis, monitoring and treatment of liver disease. We provide a rapid turnaround, comprehensive diagnostic testing service (quantification, qualification, and genotyping) for hepatitis, HIV, and other diseases affecting the liver. Our menu also includes a patient-friendly repeatable test to measure the extent of liver fibrosis in a number of chronic liver diseases using serum markers (the ELF™ Test).

Lab 21  
184, Cambridge Science Park  
Cambridge, CB4 OGA  
United Kingdom  
Phone: 44-1489-898-600  
Fax: 44-1489-582-327  
Website: www.lab-21.com

Lab21 is a clinical reference laboratory based in Europe offering a full spectrum of molecular diagnostic services for clinical trials and patient management. The company is especially strong in liver disease with a comprehensive portfolio of gold-standard and proprietary assays including drug resistance, fibrosis assessment, and interferon response analysis.

LSU AgCenter  
805 Saint Louis Street  
Baton Rouge, LA 70802  
Phone: 225-389-3055  
Fax: 225-389-7634  
Website: www.lsuagcenter.com

Preventing deadly Vibrio vulnificus infection (from eating raw oysters or exposing wounds to seawater) in patients with liver diseases/disorders is emphasized. Free publications and other educational resources for both physicians and patients are highlighted. The website SafeOysters.org provides additional information and online resources for physicians and patients.
MDS Nordion
447 March Road
Ottawa, ON K2K 1X8
Canada
Phone: 613-592-2790  Fax: 613-592-6815
Website: www.therasphere.com
MDS Nordion is a world-leading supplier of radiation technologies. Its liver cancer treatment TheraSphere®, (Yttrium-90 glass microspheres) is used in radiation treatment or as a bridge to surgery or transplantation. It is an intra-arterial, minimally embolic therapy providing an approved treatment option of portal vein thrombosis patients, improved quality of life and re-treatment potential.

MedPage Today, LLC
150 Clove Road
Little Falls, NJ 07424
Phone: 973-890-0985  Fax: 973-890-1327
Website: www.medpagetoday.com
MedPage Today is "News + CME." We provide clinicians with real time medical news coverage that also offers CME/CE credit. The result is daily engagement with a highly loyal audience. Our news coverage is reviewed and approved by the University of Pennsylvania School of Medicine, which provides CME accreditation.

Nabi Biopharmaceuticals
5800 Park of Commerce Blvd., NW
Boca Raton, FL 33487
Phone: 561-989-5800  Fax: 561-989-5899
Website: www.nabi.com
Our experience and knowledge in powering the immune system drives our continued development of products that address the needs of the liver transplant community. In addition to a rich pipeline of immunologically derived products, we presently have one marketed product, Nabi-HB® [Hepatitis B Immune Globulin (Human)].

Novartis AG
Lichtstrasse 35
Basel, 4056
Switzerland
Phone: 41-61-324-1111
Website: www.novartis.com
Novartis AG is a world leader in developing and offering medicines to patients and physicians worldwide to cure diseases and thus further improve health and overall well being. Aiming to advance the standard of care for chronic liver disease, Novartis is building a portfolio of agents for hepatitis B and C with complementary mechanisms of action, designed to prevent or postpone end stage liver disease and liver transplantation.

Option Care, Inc.
485 Half Day Road
Suite 300
Buffalo Grove, IL 60089
Phone: 888-282-5166
Website: www.optioncare.com
For over 25 years, Option Care, Inc. has made patients' lives easier with a range of healthcare services outside the hospital setting, working with more than 400 payor organizations representing over 75 million Americans. With the largest home infusion and specialty pharmacy footprint in the industry, OptionCare offers treatment nationwide to patients in their homes, physician offices or other alternate sites, including ambulatory treatment centers. Services are provided by highly skilled, clinical professionals from 110+ pharmacy locations.

Orphan Therapeutics, LLC
3 Werner Way
Suite 210
Lebanon, NJ 08833
Phone: 908-849-4805  Fax: 908-849-4806
Website: www.orphantherapeutics.com
Orphan Therapeutics, LLC, specializes in developing and commercializing treatments for rare but serious diseases. We will submit our first NDA for terlipressin in the 2nd half of 2007. Pre-launch activities will focus on hepatorenal syndrome medical education.

Ovation Pharmaceuticals, Inc.
4 Parkway North
Suite 200
Deerfield, IL 60015
Phone: 847-282-1000  Fax: 847-282-1001
Website: www.ovationpharma.com
Ovation Pharmaceuticals, Inc. is a leading biopharmaceutical company that develops, manufactures, and commercializes innovative medicines for severely ill patients with unmet medical needs. Our mission is to provide an uninterrupted supply of potentially lifesaving products and ensure access to these products for your patients.

PharmaCare Specialty Pharmacy
600 Penn Center Blvd.
Pittsburgh, PA 15235
Phone: 800-238-7828  Fax: 412-717-9090
Website: www.pharmacare.com
PharmaCare Specialty Pharmacy is a retail pharmacy that provides reimbursement and clinical services to individuals living with hepatitis and other complex conditions. Choose PharmaCare Specialty and know that your patient will receive the most comprehensive care available for injectable medications. To learn more, call 1-800-238-7828.
PharmXpand LLC  
Booth # 117  
PharmXpand is a healthcare promotion company that markets innovative products such as noninvasive Biomarkers for liver disease diagnosis (fibrosis/cirrhosis): FibroTest, FibroMax to healthcare providers and patients through an exclusive partnership with Biopredictive, a biotech specialized in Hepatology and world leader in liver disease diagnosis. Visit us at www.pharmxpand.com and www.biopredictive.com.

Projects in Knowledge, Inc.  
Booth # 602  
Projects in Knowledge, Inc. is a CME provider that has served the physician community since 1980 by providing cutting-edge, innovative educational activities, specifically in hepatology. Its mission is to improve the quality of healthcare by delivering highest quality CME, with demonstrated results, to physicians throughout the U.S. and worldwide using creative, effective, easily accessible instructional modalities. Projects In Knowledge has been awarded the highest rating achievable by a CME provider.

Quest Diagnostics  
Booth # 705  
Quest Diagnostics is the leading provider of diagnostic testing, information and services that patients and doctors need to make better healthcare decisions. We offer the broadest access to health testing services. Each day, over half a million people rely on us to provide their doctors with medical information to help them assess whether their patients are healthy or ill.

Rare Disease Therapeutics, Inc.  
Booth # 114  
Rare Disease Therapeutics, Inc. is a biopharmaceutical company committed to developing and marketing drugs for rare disorders. RDT’s focus is on inborn errors of metabolism and has two products available: Orfadin® (nitisone), oral treatment for Hereditary Tyrosinemia Type I; and Cystadone® (betaine anhydrous for oral solution), for the treatment of homocystinuria.

Roche  
Booth # 501, 502  
Roche is a worldwide leading innovator of pharmaceuticals and diagnostics. Our people are engaged in the discovery, development, manufacturing, and marketing of prescription medicines in a wide variety of therapeutic areas, including cancer, HIV/AIDS, hepatitis C, transplantation, influenza, and osteoporosis. We invite you to our booth to learn more about Pegasis and CellCept.

Salix Pharmaceuticals, Inc.  
Booth # 604, 606  
Salix Pharmaceuticals, Inc. follows a competitive strategy of in-licensing late-stage pharmaceutical products to treat GI diseases. The Salix portfolio includes COLAZAL®, XIFAXAN®, OsmoPrep™, MOVIPREP®, AZASAN®, ANUSOL-HC®, PROCTOCORT®, PEPCID® Oral Suspension, and DIURIL® Oral Suspension. Exceptional customer service, a dedicated specialty sales force, and quality products underscore Salix’s commitment to the gastroenterology community.

SC Liver Research Consortium  
Booth # 316  
SC Liver Research Consortium is a clinical trial network made up of 90+ hepatologists at 47 sites throughout the United States. In 2005, SCLRC continued its successful HCV CME series, “Hepatitis & Infectious Disease Training Programs,” for clinicians throughout the United States and added a brand new HBV training program.

Schering-Plough  
Booth # 301  
Schering-Plough is a global science-based health care company with leading prescription, consumer, and animal health products. Through internal research and collaborations with partners, Schering-Plough discovers, develops, manufactures, and markets advanced drug therapies to meet important medical needs. Schering-Plough’s vision is to earn the trust of the physicians, patients and customers served by its more than 32,000 people around the world. The company is based in Kenilworth, N.J., and its Website is www.schering-plough.com.
SciClone Pharmaceuticals Booth # 318, 320
901 Mariners Island Blvd.
Suite #205
San Mateo, CA 94404
Phone: 650-358-3456 Fax: 650-358-3469
Website: www.sciclone.com
SciClone Pharmaceuticals is a global biopharmaceutical company developing medicines treating the world’s most serious diseases, including cancer, hepatitis B and hepatitis C. Our lead drug ZADAXIN™ is approved in 34 countries worldwide and is currently in E.U. and U.S. phase 2 & 3 trials for hepatitis C and malignant melanoma.

Sirtex Medical, Inc. Booth # 635
1401 N. Western Avenue
Lake Forest, IL 60045
Phone: 847-482-9023 Fax: 847-482-9103
Website: www.sirtex.com
Sirtex Medical provides SIR-Spheres microspheres for the treatment of unresectable metastatic liver tumors from primary colorectal cancer. SIR-Spheres microspheres are biocompatible resin microspheres loaded with Yttrium 90 that are infused via the hepatic artery using a femoral catheter.

Specialty Scripts Pharmacy Booth # 419
187 Plymouth Avenue
Bldg. 8, 1st Floor
Fall River, MA 02721
Phone: 800-218-5688 Fax: 800-830-5292
Website: www.specialtyscripts.com
SpecialtyScripts Pharmacy, a Cardinal Health Company, is a Massachusetts-based specialty pharmacy that takes a patient-centric approach to specialty pharmacy. The leadership team at SpecialtyScripts™ Pharmacy assists individuals with the clinical, emotional, and practical challenges of taking customized and highly intensive therapies through compassion, knowledge, and advocacy. Our clinical and service excellence is consistently proven through measurable outcomes.

Synovate Healthcare Booth # 716
The Embankment Putney
London SW15 1LB
UK
Phone: 44-208-246-6200 Fax: 44-208-246-6300
Website: www.synovate.com/healthcare
Synovate Healthcare is an independent market research agency specialising 100% in pharmaceuticals and healthcare. Our specialist conference research team is present at the conference to conduct research among delegates. We look forward to speaking to you and listening to your opinions and experiences.

Three Rivers Pharmaceuticals, LLC Booth # 622
301 Commerce Park Drive
Cranberry Township, PA 16066
Phone: 724-778-6100 Fax: 724-778-6101
Website: www.3riverspharma.com
Three Rivers Pharmaceuticals devotes its efforts and resources to developing, manufacturing, and marketing pharmaceutical therapies which are indicated for diseases/medical conditions requiring specialized treatment. Currently, Three Rivers Pharmaceuticals markets prescription drugs in both the U.S. and internationally in the therapeutic categories of antiviral and antifungal agents. Three Rivers Pharmaceuticals has broadened its presence in the hepatitis C market with the December 2005 launch of Ribasphere® Tablets and RibaPak™.

University of Washington Booth # 5
Dept. of Microbiology
Box 358070
Seattle, WA 98195-8070
Phone: 206-732-6134 Fax: 206-732-6056
Website: http://nida.viromics.washington.edu
The NIDA Center for Functional Genomics and HCV-Associated Liver Disease brings a multidisciplinary approach to the study of hepatitis C virus infection and its impact on liver function. Our goal is to provide a comprehensive molecular blueprint of the cellular response to hepatitis C virus infection and a detailed picture of the cellular events characteristic of hepatitis C virus-associated liver disease.

US Bioservices Booth # 200, 201
16750 Westgrove Drive
Suite 100
Addison, TX 75001
Phone: 888-518-7246 Fax: 469-461-0277
Website: www.usbioservices.com
US Bioservices, a Specialty Pharmacy, removes the burden of managing drug compliance by bridging the gap between therapy, nursing, and reimbursement. We guide your patients through treatment — from insurance verification and therapy education to convenient medication delivery and ongoing clinical support. In essence, we work as an extension of your office to help improve your patients’ lives — and your practice’s operations.

Valeant Pharmaceuticals Booth # 524
One Enterprise
Aliso Viejo, CA 92656
Phone: 949-461-6000 Fax: 949-461-6627
Website: www.valeant.com
Valeant Pharmaceuticals International is a global specialty pharmaceutical company that develops, manufactures, and markets a broad range of pharmaceutical products primarily in the areas of neurology, infectious disease, and dermatology. More information about Valeant can be found at www.valeant.com.
Vertex Pharmaceuticals, Inc. Booth # 413
130 Waverly Street
Cambridge, MA 02139
Phone: 617-444-6100 Fax: 617-444-7180
Website: www.vrtx.com

Vertex Pharmaceuticals is a global biotechnology company committed to the discovery and development of small molecule drugs for serious diseases. Telaprevir (VX-950), Vertex’s investigational hepatitis C protease inhibitor, is being evaluated in three Phase 2 clinical trials that have enrolled over 1,000 patients with chronic genotype 1 hepatitis C virus infection.

ViraCor Laboratories Booth # 112
1210 NE Windsor Drive
Lee’s Summit, MO 64086
Phone: 816-347-0113 Fax: 816-347-0143
Website: www.viracor.com

ViraCor Laboratories, the leader in Molecular Diagnostics, provides cutting edge molecular testing. ViraCor qPCR (quantitative) assays are continuously monitored for mutations. The growing list of 24 hr TAT assays includes BK, Adenovirus, HHV-6, and Toxoplasma. Get the updated information on all validated assays from our expanding menu at the booth.

Wako Diagnostics Booth # 608
1600 Bellwood Road
Richmond, VA 23237
Phone: 804-714-1924 Fax: 804-271-0449
Website: www.wakodiagnostics.com

Wako Diagnostics is a comprehensive manufacturer of clinical diagnostic reagents. Wako Diagnostics offers the newly-approved des-γ-carboxy prothrombin (DCP, also called PIVKA-II), plus AFP-L3% and the test system, LiBASys. DCP and AFP-L3% are complementary liver biomarkers for HCC risk. Both tests are FDA cleared for the assessment of risk of developing HCC in patients with chronic liver diseases. Now our FDA-approved, CMS-reimbursed AFP-L3% test is available at major reference laboratories.

Walgreens Specialty Pharmacy Booth # 704
1411 Lake Cook Road
MSL220
Deerfield, IL 60015-5238
Website: www.walgreensspecialtyrx.com

Personalized. Supportive. Reliable. That’s Walgreens Specialty Pharmacy. We are a single-source provider of specialty therapies: oral, injectable, and infused. Our experienced Care Team of pharmacists and nurses provides outstanding patient-focused services: side-effect management, compliance monitoring, insurance coordination, and express delivery or pickup at over 5,000 Walgreens pharmacies nationwide.

Wiley-Blackwell Publishing Booth # 127, 129
350 Main Street
Malden, MA 02148
Phone: 800-759-6102
Website: www.blackwellhepatology.com

Blackwell Publishing merged with John Wiley & Sons, Inc.’s scientific, technical, and medical business in February 2007 to become Wiley-Blackwell, the world’s #1 publisher in hepatology and gastroenterology. We are the official partner of AASLD, publishing HEPATOLOGY and Liver Transplantation. Pick up your journal today and save 15% on all books!

Yasoo Health Booth # 616
2109 W. Market Street
Suite 164
Johnson City, TN 37604
Phone: 423-926-2798 Fax: 423-926-3586
Website: www.yasoo.com

Yasoo Health is a research based company focusing on vitamin malabsorption. Its products include AquAdekSTM, a supplement designed to advance the standard of nutritional care for cystic fibrosis patients and other medical conditions that cause malabsorption, and Aqua-E®, a clinically tested water-soluble form of complete vitamin E.
Open to all meeting registrants

These programs are not affiliated with AASLD.

AASLD provides an opportunity for registrants at The Liver Meeting® to attend independent symposia financially supported by the pharmaceutical industry. These symposia will take place following The Liver Meeting®’s scheduled educational events on Saturday, November 3 after 8 PM, and after 6:30 PM on Sunday, November 4 and Monday, November 5.

Each of the symposia organizers has made a financial contribution in support of the educational mission of AASLD. This support allows symposia organizers access to The Liver Meeting®’s attendees before the meeting by way of mail promotions, and on specific evenings during the meeting following official AASLD functions. They also make many features of The Liver Meeting® possible and help maintain reasonable registration fees. This support is gratefully acknowledged. This acknowledgement, however, does not constitute an endorsement of any product, nor AASLD oversight or endorsement of the content of the program. These programs are not affiliated with AASLD.

Please visit the satellite symposia desk in the Main Lobby for more information and to register for any of the following events.

Saturday, November 3, 2007

Case Debates with the Experts: Applying the Updated Practice Guidelines to the Management of Hepatitis B

Supported by Bristol-Myers Squibb Company
Organized by Clinical Care Options, LLC
CME provided by Postgraduate Institute for Medicine
Constitution A&B, Sheraton Boston Hotel

On completion of this activity, participants should be able to:

- Describe the key factors used in making decisions regarding first-line and rescue therapy in patients with chronic hepatitis B as described by the AASLD Practice Guidelines.
- Review data regarding strategies for preventing and identifying resistance in hepatitis B patients.
- Discuss differences in management strategies for HBeAg-positive and HBeAg-negative patients.
- Summarize data regarding the use of monotherapy vs. combination therapy in hepatitis B treatment.

For more information, contact Amy Goldman at 585-533-1874 or agoldman@clinicaloptions.com.

Decision Points in the Lifelong Management of Chronic Hepatitis B

Supported by F. Hoffmann-La Roche Ltd
Organized by Elements Communications Ltd
CME provided by Centre for Bio-Medical Communications, Inc
Grand & Liberty, Sheraton Boston Hotel

At the conclusion of this program, attendees should be able to understand:

- The natural history of CHB and the different phases (immune tolerant, immune clearance, inactive, and reactivation) as well as the clinical differences between HBeAg-positive and HBeAg-negative hepatitis B.
- How the natural course of infection can lead to spontaneous resolution via the development of HBsAg seroconversion.
- The relevance of the HBsAg as a clinical marker – being the closest outcome to cure of this chronic disease – and other markers which may be used to assess clinical response.
- Which patients need to be treated, the treatment strategies available, and appropriate determinants of treatment success.
- The rationale and modes of action of interferon-based and antiviral therapies and the patterns of response, both on- and off-therapy.
- Why one particular treatment approach may be more appropriate for a given patient scenario.
- The incidence of, and the clinical problems associated with the emergence of resistance to nucleos(t)ide analog therapy.
- The problems associated with cross-resistance between nucleos(t)ide analogs and present treatment options for patients in whom drug-resistant variants have arisen.
- The potential serious consequences of disease progression and the development of hepatocellular carcinoma associated with unresolved CHB infection.
- The management issues around the prevention of serious clinical complications of CHB, also for patients who are co-infected with HCV or HIV.

For more information, contact Wendy Burgess at 44-1959-568-150 or wendyb Burgess@elementscommunications.com.
The Potential Role of Novel Thrombopoietic Growth Factors in Chronic Liver Diseases – Contemporary Considerations of Thrombocytopenia
Supported by GlaxoSmithKline
Organized by Continuing Edge a division of Global Edge, Inc.
CME provided by Educational Review Systems
Republic A&B, Sheraton Boston Hotel
At the conclusion of this program, participants should be able to:
• Identify the major types and respective potential causes of thrombocytopenia in chronic liver disease.
• Describe the incidence, epidemiology, and etiology of thrombocytopenia in HCV infection and other chronic liver diseases.
• Explain the role and clinical consequences of thrombocytopenia in HCV infection and other chronic liver diseases.
• Evaluate recent data investigating the potential to stimulate platelet production in helping to improve treatment for HCV infection.
• Assess current and evolving management strategies specifically for thrombocytopenia in patients with HCV infection and other chronic liver diseases.

For more information, contact Barbara Lovit at 212-792-6628 or blovitt@globaledge-us.com.

Harnessing Scientific Innovations to Improve HCV Outcomes
Supported by Vertex Pharmaceuticals, Inc.
Organized and CME provided by Projects In Knowledge, Inc.
Back Bay A-D, Sheraton Boston Hotel
After participating in this activity, the clinicians should be able to:
• Integrate knowledge of viral kinetics of HCV, molecular interactions with HCV at the cellular level, and mechanisms of action of protease and polymerase inhibitors to determine the potential role of these therapies in HCV patients.
• Utilize knowledge of HCV replication, potential reservoirs of infection, and disease burden to better diagnose patients with HCV and determine strategies for treatment.
• Contrast potential efficacy and safety considerations of protease and polymerase inhibitors, based on preliminary data, as therapeutic options in the future treatment of patients with HCV infection.
• Formulate potential therapeutic strategies involving protease and polymerase inhibitors in combination therapy and their clinical implications based on preliminary data.

For more information, contact Michele Ingram at 973-890-8988 or m.ingram@projectsinknowledge.com.

Sunday, November 4, 2007
Hepatitis B Prevention Following Liver Transplant: A New Era in Viral Prophylaxis
Supported by Apotex Corporation
Organized and CME provided by i3 CME
Republic A&B, Sheraton Boston Hotel
The learning objectives for participants include the ability to:
• Identify factors that affect the rate of HBV recurrence after liver transplantation.
• Give case vignettes, describe the role of hepatitis B immune globulin immunoprophylaxis in the management of HBV post-liver transplantation.
• Assess knowledge of clinical trial evidence supporting the role of nucleos(t)ide analogues in the management of HBV pre- and post-liver transplantation.
• Select new treatment approaches to preempt or prevent HBV reinfection post-transplantation.

For more information, contact Kelly Hanley at 973-579-2268 or kelly.hanley@i3cme.com.

Experts Forecast HCV Treatment Revolution: Live Coverage from the HCV PIPELINE
Supported by Idenix Pharmaceutical Inc/Novartis Pharmaceuticals Corp/Human Genome Sciences, Inc.
Organized and CME provided by AdvancMed, LLC
Constitution A&B, Sheraton Boston Hotel
Upon completion of this activity, participants will be able to:
• Identify markers of treatment success and parameters that contribute to treatment response.
• Differentiate the targets of specifically targeted antiviral therapies (STAT C) that are currently under investigation.
• Analyze the relevance of emerging data of STAT C therapies and the potential utility for the treatment of HCV infection.
• Explore the role of interferon therapy in conjunction with STAT C therapy.
• Outline strategies for the proactive management of treatment resistance.

For more information, contact Rhonda Parrott at 859-543-4117 or rparrott@advancmed.org.
Chronic HBV Infection: Navigating the Road to Successful Patient Outcomes  
**Supported by Novartis Pharmaceuticals**  
**Organized by MDG Development Group, LLC**  
**CME provided by University of Wisconsin School of Medicine and Public Health**  
**Back Bay A-D, Sheraton Boston Hotel**

At the conclusion of this activity, physicians should be able to:

- Discuss the association between HBV DNA levels and 1) patient candidacy for therapy, 2) emergence of treatment resistance, and 3) disease progression, and the implications of this information on HBV treatment strategies.
- Describe how the use of HBV DNA measurement as an early predictor of response to antiviral therapy may lead to improvements in disease management.
- Evaluate new antiviral therapies for CHB in terms of their clinical profiles and role in individualized patient treatment decisions.
- Discuss key clinical considerations with respect to when to initiate, when to stop, when to change, and when to combine antiviral therapy in patients with CHB.
- Describe recommendations for managing challenging patient populations with CHB, including suboptimal responders, women of childbearing age, and persons with HIV/HBV coinfection.

For more information, contact Ellen Donaldson at 908-605-4380 or edonaldson@mktdevgrp.com.

Targeting the Ras/Raf Signaling Pathway: Implications in the Management of Hepatocellular Carcinoma  
**Supported by Onyx Pharmaceuticals/Bayer HealthCare Pharmaceuticals**  
**Organized and CME provided by Educational Concepts Group**  
**Independence East & West, Sheraton Boston Hotel**

Upon completion of this educational activity, participants should be better able to:

- Describe the basic tumor biology and rationale for the development and use of new therapeutic approaches to hepatocellular carcinoma.
- Discuss the clinical implications of the results of pivotal clinical trials, including early phase trials, that have potential to impact the use of novel multikinase-targeting therapies.
- Analyze potential treatment strategies and ongoing clinical trial designs for incorporating new multikinase-targeting therapies in the treatment of hepatocellular carcinoma, including novel combinations.

For more information, contact Tina Stacy at 770-933-1684 or tstacy@educationalconcepts.net.

Medical Crossfire: Chronic Hepatitis C Treatment Challenges in an Era of Advances  
**Supported by Roche Laboratories**  
**Organized by Liberty Communications Network/Medical Crossfire**  
**CME provided by The American Academy of CME, Inc**  
**Grand & Liberty, Sheraton Boston Hotel**

As a result of this activity, participants will be able to:

- Evaluate the clinical significance of response time and the potential influence on treatment decisions.
- Categorize patients that fail to respond to treatment and review treatment options in patients who have failed to respond to prior treatment.
- Describe the issues surrounding liver biopsies and the use of non-invasive tests for fibrosis.
- Examine the potential role of small molecule agents in the treatment of patients with hepatitis C.

For more information, contact Melissa Hefner at 609-918-2087 or hefner@medicalcrossfire.com.

**Monday, November 5, 2007**

**Hepatitis B Makeover: A Case Based Approach for Optimal Patient Care**  
**Supported by Gilead Sciences**  
**Organized and CME provided by Summit Institute of Knowledge, Inc.**  
**Back Bay A-D, Sheraton Boston Hotel**

At the completion of this educational activity, participants should be able to:

- Understand the disease mutations and determine the most appropriate course of therapy.
- Review diagnostic and virological tests which are necessary for staging disease and making treatment decisions.
- Formulate HBV treatment strategies based upon the safety and efficacy of current and emerging therapies to improve patient outcomes.

For more information, contact Jeff Laufer at 732-933-2671 or jlaufer@summit-institute.org.
Current Perspectives on Preventing Recurrence of Hepatitis B in Liver Transplant Recipients
Supported by Nabi Biopharmaceuticals
Organized by The MedEd Group, Inc.
CME provided by Medical Education Resources
Constitution A&B, Sheraton Boston Hotel
At the conclusion of this program, participants will:
• Gain a greater knowledge of the historical perspective regarding liver transplantation and the use of HBIG to prevent recurrence of HBV in liver transplant recipients.
• Gain a greater knowledge of the most recent findings (including outcomes data) from the published literature with regard to the appropriate regimens to prevent recurrence of HBV in liver transplant recipients. This includes current practices in managing the viral replicator versus non-replicator transplant recipient.
• Gain a greater knowledge on current perspectives regarding indefinite versus limited use of HBIG, as well as high-dose versus low-dose HBIG as immunoprophylaxis in the liver transplant recipient.

For more information, contact Cecy Martinez at 561-626-3950 or cmartinez@themededgroup.com.

Emerging Concepts in Diagnosis and Management of Hepatic Encephalopathy: Past, Present and Future
Supported by Salix Pharmaceuticals, Inc
Organized by MDG Development Group, LLC
CME provided by University of Wisconsin School of Medicine and Public Health
Republic A&B, Sheraton Boston Hotel
At the conclusion of this program, participants should be able to:
• Distinguish the differences between minimal and overt hepatic encephalopathy and the importance of early diagnosis.
• Evaluate the various burdens that need to be overcome to adequately distinguish minimal from overt hepatic encephalopathy.
• Discuss issues with current methods for diagnosis and grading of hepatic encephalopathy, and review data on emerging methods that are currently under clinical evaluation.
• Review the clinical experience with currently available and emerging treatment options for hepatic encephalopathy.

For more information, contact Elena Dattolo at 908-605-4375 or edattolo@mktdevgrp.com.
Continuing Medical Education and Disclosures

The American Association for the Study of Liver Diseases (AASLD) is accredited by the Accreditation Council for Continuing Medical Education (ACCME) to provide continuing medical education for physicians.

AASLD designates these educational activities for AMA PRA Category 1 Credits™. Physicians should only claim credit commensurate with the extent of their participation in the activity.

<table>
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<th>Maximum Credits Available</th>
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<td><strong>Annual Meeting</strong></td>
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<td>(State-of-the-Art Lectures; General Hepatology Update; Advances for Practitioners; 50 Years of Interferons; Plenaries, Parallel, and Poster Sessions; NIH Corner; and Early Morning Workshops)</td>
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<td><strong>Hepatology Associates Course</strong></td>
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<td><strong>AASLD/ILTS Transplant Course</strong></td>
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<td><strong>AASLD/NASPGHAN</strong></td>
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<td>Pediatric Symposium**</td>
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<td><strong>AASLD/ASGE Endoscopy Course</strong></td>
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<td><strong>Early Morning Workshops</strong></td>
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*This activity has been planned and implemented in accordance with the Essential Areas and Policies of the Accreditation Council for Continuing Medical Education through the joint sponsorship of the American Association for the Study of Liver Diseases (AASLD), and the International Liver Transplantation Society (ILTS). AASLD is accredited by the ACCME to provide continuing medical education for physicians.

**Co-sponsored activity: The American Association for the Study of Liver Diseases (AASLD) is accredited by the Accreditation Council for Continuing Medical Education to provide continuing medical education for physicians.

***Amedco, St. Paul, MN, is an approved provider of nursing education by the Wisconsin Nurses Association Continuing Education Approval Program Committee, an accredited approver by the American Nurses Credentialing Center’s Commission on Accreditation.

For nurses, these courses are co-provided by Amedco and AASLD. Maximum contact hours are as follows: Hepatology Associates Course – 4.5 contact hours; Postgraduate Course – 10.0 contact hours; IITS Transplant Course – 6.0 contact hours; NASPGHAN Pediatric Symposium – 2.75 contact hours.

General Learning Objectives and Goals

- Provide a forum for exchange of groundbreaking basic and clinical research in liver diseases.
- Create an arena for the presentation and interchange of opinions on state-of-the-art care and management of the full spectrum of patients with liver diseases.

Disclaimer Notice

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Program Planning

As a sponsor accredited by the Accreditation Council for Continuing Medical Education (ACCME), the American Association for the Study of Liver Diseases (AASLD) must ensure balance, independence, objectivity, and scientific rigor in all its sponsored, co-sponsored, and jointly-sponsored activities. All faculty participating in AASLD CME activities are expected to disclose to the program audience relationships with a commercial interest if both (a) the relationship is financial and occurred within the past 12 months and (b) the individual has the opportunity to effect the content of CME about the products or services of that commercial interest. It is the responsibility of the program planners (educational committee members) to ensure that content is valid and aligned with the interest of the public. All conflicts are resolved prior to execution of educational programs.

CME Instructions

Complete the overall Annual Meeting Evaluation and CME Certificate, and do it all online. You may access the online system at the Internet Center in the Hynes Convention Center, as well as from your personal or office computer via the AASLD Web site, www.aasld.org, after the meeting concludes. You may print your CME Certificate or a Certificate of Attendance before leaving The Liver Meeting® or at your leisure when you return home. Please complete the overall evaluation and print out your Certificate by the end of March 2008. Stop by the CME desk in the Registration Area for assistance.
Disclosures

AASLD is committed to ensuring balance, independence, objectivity, and scientific rigor in its sponsored and jointly sponsored educational activities. Individuals in a position to control the content of an AASLD-sponsored activity (program planners, course directors, speakers, etc.) are expected to disclose to the audience all relevant financial relationships during the past 12 months if both (a) the relationship is financial and occurred within the past 12 months and (b) the individual has the opportunity to affect the content of CME about the products or services of that commercial interest.

When an unlabeled use of a commercial product, or an investigational use not yet approved for any purpose is discussed during an educational activity, the speaker shall disclose to the audience that the product is not labeled for the use under discussion or that the product is still investigational.

AASLD will identify and resolve all conflicts of interest prior to program implementation.

Invited speakers, course directors, moderators and program planners have provided the following disclosures:

**Adams, Paul C., MD**
(Postgraduate Course, Meet-the-Professor Luncheon)
Nothing to disclose
Content of presentation does not include discussion of off-label/investigative use of medicine(s), medical devices or procedure(s)

**Afdhal, Nezam H., MD**
(Photographic Workshop, Schering-Plough, Valeant Pharmaceuticals, Isis Pharmaceuticals, Idenix, Novartis, Idun Pharmaceuticals, Bristol-Myers Squibb, United Therapeutics, Cooley Pharmaceuticals, Prometheus, InterMune, Glaxo Smith Kline, EchoSens, Vertex, Gilead, Salk, Quest
Consultant/Advisor: Ortho Biotech, Valeant Pharmaceuticals, Isis Pharmaceuticals, Idenix, Gilead, EchoSens, Prometheus, Biogen, XTL Pharmaceuticals, Glaxo Smith Kline, EchoSens, Vertex, InterMune, Novartis, Wyeth/ViroPharma, Salk, Schering-Plough, Idera Pharmaceuticals, Stomedia, Sirtris, BioCryst, Arrow Pharmaceuticals, Atlas Pharmaceuticals
Speaker’s Bureau: Schering-Plough, Gilead, Bristol-Myers Squibb, Idenix/Novartis
Content of presentation does include discussion of off-label/investigative use of medicine(s), medical devices or procedure(s)

**Anania, Frank A., MD**
(Photographic Workshop, General Hepatology Update, Education Committee)
Nothing to disclose
Content of presentation does not include discussion of off-label/investigative use of medicine(s), medical devices or procedure(s)

**Angulo, Paul, MD**
(Postgraduate Course, Meet-the-Professor Luncheon)
Nothing to disclose
Content of presentation does not include discussion of off-label/investigative use of medicine(s), medical devices or procedure(s)

**Aranda-Michel, Jaime, MD**
(Photographic Workshop)
Nothing to disclose
Content of presentation does not include discussion of off-label/investigative use of medicine(s), medical devices or procedure(s)

**Arteel, Gavin E., PhD**
(Photographic Workshop)
Grant/Research Support: NIH/NIAAA
Content of presentation does not include discussion of off-label/investigative use of medicine(s), medical devices or procedure(s)

**Bacon, Bruce R., MD**
(Career Development Workshop)
Nothing to disclose
Content of presentation does not include discussion of off-label/investigative use of medicine(s), medical devices or procedure(s)

**Balistreri, William F., MD**
(Postgraduate Course, Meet-the-Professor Luncheon)
Nothing to disclose
Content of presentation does not include discussion of off-label/investigative use of medicine(s), medical devices or procedure(s)

**Bansal, Meena, MD**
(Career Development Workshop, Training and Clinical Policy Committee)
Nothing to disclose
Content of presentation does not include discussion of off-label/investigative use of medicine(s), medical devices or procedure(s)

**Berenguer, Marina, MD**
(Surgery and Liver Transplant Committee)
Nothing to disclose

**Beresford, Thomas P., MD**
(Transplant Course)
Nothing to disclose
Content of presentation does not include discussion of off-label/investigative use of medicine(s), medical devices or procedure(s)

**Bezerra, Jorge A., MD**
(Photographic Workshop)
Nothing to disclose
Content of presentation does not include discussion of off-label/investigative use of medicine(s), medical devices or procedure(s)

**Bini, Edmund, MD**
(Clinical Research Committee)
Grant/Research Support: Schering-Plough, Roche, InterMune, Valeant, SciClone, GlaxoSmithKline, Bristol-Myers Squibb, Wyeth, Idenix
Consultant/Advisor: InScope

**Boyle, Clifford W., MD**
(Pediatric Symposium)
Nothing to disclose
Content of presentation does not include discussion of off-label/investigative use of medicine(s), medical devices or procedure(s)

**Boyce, Thomas D., MD**
(Postgraduate Course, Advances for Practitioners)
Nothing to disclose
Content of presentation does not include discussion of off-label/investigative use of medicine(s), medical devices or procedure(s)

**Brenner, David A., MD**
(Photographic Workshop)
Nothing to disclose
Content of presentation does not include discussion of off-label/investigative use of medicine(s), medical devices or procedure(s)
Brown, Kimberly Ann, MD  
(Surgery and Liver Transplant Committee)  
Speakers’ Bureau: Schering-Plough, Roche, Gilead  
Consultant/Advisor: BlueCross Quality Centers for Transplantation  

Brown, Robert, MD  
(Training and Clinical Policy Committee)  
Grant/Research Support: Schering-Plough, Roche, Vertex  
Consultant/Advisor: Schering-Plough, Vertex  

Brugge, William R., MD  
(Endoscopy Course)  
Grant/Research Support: Pentax  
Consultant/Advisor: Boston Scientific  
Content of presentation does not include discussion of off-label/investigative use of medicine(s), medical devices or procedure(s)  

Bruix, Jordi, MD  
(Therapeutic Advances in HCC: Sorafenib)  
Grant/Research Support: Schering-Plough, Biocompatibles, Bayer  
Consultant/Advisor: Bayer, Bristol-Myers Squibb  

Brunt, Elizabeth M., MD  
(Postgraduate Course, Meet-the-Professor Luncheon)  
Nothing to disclose  
Content of presentation does not include discussion of off-label/investigative use of medicine(s), medical devices or procedure(s)  

Burroughs, Andrew K., FRCP  
(Transplant Course)  
Nothing to disclose  
Content of presentation does not include discussion of off-label/investigative use of medicine(s), medical devices or procedure(s)  

Caldwell, Stephen H., MD  
(Early Morning Workshop, Postgraduate Course)  
Grant/Research Support: Nordic Natural  
Speakers’ Bureau: Axcan  
Content of presentation does include discussion of off-label/investigative use of medicine(s), medical devices or procedure(s)  

Casey, Carol, PhD  
(Basic Research Committee)  
Nothing to disclose  

Chalasani, Naga P., MD  
(Early Morning Workshop, Clinical Research Committee)  
Speakers’ Bureau: Advanced Life Science, Ortho Neil, Metabasis, Abbott, Lilly  
Content of presentation does include discussion of off-label/investigative use of medicine(s), medical devices or procedure(s)  

Chang, Charissa, MD  
(Career Development Workshop)  
Nothing to disclose  
Content of presentation does not include discussion of off-label/investigative use of medicine(s), medical devices or procedure(s)  

Chang, Kyong-Mi, MD  
(Basic Research Workshop)  
Nothing to disclose  
Content of presentation does not include discussion of off-label/investigative use of medicine(s), medical devices or procedure(s)  

Charlton, Michael R., MD  
(Career Development Workshop, Early Morning Workshop, Surgery and Liver Transplant Committee)  
Consultant/Advisor: Roche, NABI, Bristol-Myers Squibb  

Chen, Yang K., MD  
(Endoscopy Course)  
Nothing to disclose  
Content of presentation does not include discussion of off-label/investigative use of medicine(s), medical devices or procedure(s)  

Chiang, John, PhD  
(Early Morning Workshop)  
Nothing to disclose  
Content of presentation does not include discussion of off-label/investigative use of medicine(s), medical devices or procedure(s)  

Chojker, Mario, MD  
(Early Morning Workshop)  
Nothing to disclose  
Content of presentation does not include discussion of off-label/investigative use of medicine(s), medical devices or procedure(s)  

Chung, Raymond, MD  
(Clinical Research Committee)  
Grant/Research Support: Schering-Plough, Roche  

Clarke-Platt, Janet, RN  
(Education Committee)  
Nothing to disclose  

Clouston, Andrew D., MBBS, PhD  
(Postgraduate Course)  
Disclosure will be provided prior to presentation  

Cohen, David, MD, PhD  
(Basic Research Committee)  
Consultant: Aecerion, Inc.  

Conjevaram, Hari S., MD  
(Early Morning Workshop, Training and Clinical Policy Committee)  
Nothing to disclose  

Corbett, Ruth J., MSN, ARNP  
(Hepatology Associates Course, Education Committee, Hepatology Associates Committee)  
Speakers’ Bureau: Schering-Plough  
Consultant/Advisor: Vertex  

Crispe, T. Nicholas, PhD  
(Early Morning Workshop)  
Nothing to disclose  
Content of presentation does not include discussion of off-label/investigative use of medicine(s), medical devices or procedure(s)  

Czaja, Mark J., MD  
(Early Morning Workshop)  
Nothing to disclose  
Content of presentation does not include discussion of off-label/investigative use of medicine(s), medical devices or procedure(s)  

Darling, Jama, MD  
(Basic Research Committee)  
Nothing to disclose  

Dasarathy, Srinivasan, MD  
(Early Morning Workshop)  
Disclosure will be provided prior to presentation  

Davis, Gary, MD  
(Meet-the-Professor Luncheon)  
Grant/Research Support: Schering-Plough, Roche, HGS, Vertex  
Content of presentation does not include discussion of off-label/investigative use of medicine(s), medical devices or procedure(s)
Davis, Richard H., Jr, PA-C
(Hepatology Associates Course)
Nothing to disclose
Content of presentation does not include discussion of off-label/ investigative use of medicine(s), medical devices or procedure(s)

Day, Christopher P, MD, PhD
(Transplant Course)
Nothing to disclose
Content of presentation does include discussion of off-label/ investigative use of medicine(s), medical devices or procedure(s)

DeLeve, Laurie, MD, PhD
(Governing Board, Postgraduate Course, Meet-the-Professor Luncheon)
Consultant: Johnson and Johnson, Ono Pharma USA, Wyeth-Ayerst
Content of presentation does not include discussion of off-label/investi- gative use of medicine(s), medical devices or procedure(s)

Di Bisceglie, Adrian M., MD
(Early Morning Workshop, Postgraduate Course)
Nothing to disclose
Content of presentation does not include discussion of off-label/ investigative use of medicine(s), medical devices or procedure(s)

Diehl, Anna Mae, MD
(Postgraduate Course, Basic Research Committee, Early Morning Workshop)
Grant/Research Support: GlaxoSmithKline, Axcan Pharma
Content of presentation does not include discussion of off-label/ investigative use of medicine(s), medical devices or procedure(s)

Dienstag, Jules L., MD
(Early Morning Workshop)
Grant/Research Support: Vertex - Consultant/Advisor: GlaxoSmithKline, Vertex, Bristol-Myers Squibb, Gilead Sciences, Idenix, Achillion, Metabasis, Scicloone, Nucleonics, Valeant, Pharmasset, Genzyme, CombiatorRx
Content of presentation does not include discussion of off-label/ investigative use of medicine(s), medical devices or procedure(s)

Dieterich, Douglas, MD
(Meet-the-Professor Luncheon)
Grant/Research Support: Schering-Plough, Roche, Gilead, Bristol-Myers Squibb
Consultant/Advisor: Schering-Plough, Roche, Gilead, Bristol-Myers Squibb
Speakers’ Bureau: Schering-Plough, Roche, Gilead, Bristol-Myers Squibb
Content of presentation does not include discussion of off-label/ investigative use of medicine(s), medical devices or procedure(s)

DiMartini, Andrea, MD
(Transplant Course)
Grant/Research Support: NIAAA, NIDDK
Content of presentation does not include discussion of off-label/ investigative use of medicine(s), medical devices or procedure(s)

Doo, Edward, MD
(NIH Corner, Clinical Research Committee)
Nothing to disclose
Content of presentation does not include discussion of off-label/ investigative use of medicine(s), medical devices or procedure(s)

Durand, Francois, MD
(Transplant Course)
Nothing to disclose
Content of presentation does not include discussion of off-label/ investigative use of medicine(s), medical devices or procedure(s)

Durfee, Janet, MSN, ARNP
(Hepatology Associates Course, Hepatology Associates Committee)
Nothing to disclose

El-Serag, Hashem, MD
(Clinical Research Committee)
Grant/Research Support: Schering-Plough
Consultant/Advisor: Roche

Evers, Gregory, MD
(Postgraduate Course, Transplant Course)
Grant/Research Support: Schering-Plough, Roche, Valeant, Vertex, Source, Gilead Johnson, Gilead
Consultant/Advisor: Schering-Plough, Roche, Valeant, Source, Gilead Johnson, Gilead
Content of presentation does not include discussion of off-label/ investigative use of medicine(s), medical devices or procedure(s)

Everson, Gregory, MD
(Postgraduate Course, Endoscopy Course, Surgery and Liver Transplant Committee)
Grant/Research Support: Schering-Plough, Roche, Valeant, Vertex, Source, Gilead Johnson, Gilead
Consultant/Advisor: Schering-Plough, Roche, Valeant, Source, Gilead Johnson, Gilead
Content of presentation does include discussion of off-label/ investigative use of medicine(s), medical devices or procedure(s)

Faigel, Douglas O., MD
(Endoscopy Course)
Consultant/Advisor: Olympus America, Inc.
Content of presentation does not include discussion of off-label/ investigative use of medicine(s), medical devices or procedure(s)

Fallon, Michael B., MD
(Postgraduate Course, Meet-the-Professor Luncheon)
Nothing to disclose
Content of presentation does not include discussion of off-label/ investigative use of medicine(s), medical devices or procedure(s)

Federle, Michael P., MD
(Postgraduate Course)
Nothing to disclose
Content of presentation does not include discussion of off-label/ investigative use of medicine(s), medical devices or procedure(s)

Feldstein, Ariel E., MD
(Early Morning Workshop, Postgraduate Course)
Nothing to disclose
Content of presentation does not include discussion of off-label/ investigative use of medicine(s), medical devices or procedure(s)

Feng, Sandy, MD, PhD
(Early Morning Workshop, Transplant Course, Scientific Program Committee, Surgery and Liver Transplant Committee)
Grant/Research Support: Genzyme
Consultant/Advisor: Pfizer, Genzyme
Content of presentation does not include discussion of off-label/ investigative use of medicine(s), medical devices or procedure(s)

Ferenci, Peter, MD
(Meet-the-Professors Luncheons)
Nothing to disclose
Content of presentation does not include discussion of off-label/ investigative use of medicine(s), medical devices or procedure(s)

Flamm, Steve L., MD
(Early Morning Workshop)
Nothing to disclose
Content of presentation does include discussion of off-label/ investigative use of medicine(s), medical devices or procedure(s)
<table>
<thead>
<tr>
<th>Name</th>
<th>MD/PhD/MD, MD/PhD</th>
<th>Committee/Role</th>
<th>Nothing to disclose</th>
<th>Off-label Discussion</th>
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<tbody>
<tr>
<td>Fleckenstein, Jacquelyn, MD</td>
<td>(Training and Clinical Policy Committee)</td>
<td>Nothing to disclose</td>
<td>Investigative use of medicine(s), medical devices or procedure(s)</td>
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<tr>
<td>Fong, Tse-Ling, MD</td>
<td>(Education Committee)</td>
<td>Speakers’ Bureau: Roche, Bristol-Myers Squibb Consultant/Advisor: Bristol-Myers Squibb</td>
<td>Nothing to disclose</td>
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<tr>
<td>Fontana, Robert J., MD</td>
<td>(Early Morning Workshop) Consultant/Advisor: Hepatolope Content of presentation does include discussion of off-label/investigative use of medicine(s), medical devices or procedure(s)</td>
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<tr>
<td>Frenette, Catherine, MD</td>
<td>(Clinical Research Committee)</td>
<td>Nothing to disclose</td>
<td>Investigative use of medicine(s), medical devices or procedure(s)</td>
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<tr>
<td>Fried, Michael W., MD</td>
<td>(Early Morning Workshop, Postgraduate Course Grant/Research Support: Roche, Valeant, Idenix, GlaxoSmithKline, Vertex Consultant/Advisor: Roche, Idenix, Valeant Content of presentation does include discussion of off-label/investigative use of medicine(s), medical devices or procedure(s)</td>
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<tr>
<td>Friedman, Scott L., MD</td>
<td>(Governing Board, Career Development Workshop, Early Morning Workshop, Scientific Program Committee) Grant/Research Support: Propharmaceuticals, Intercept Pharmaceuticals Consultant/Advisor: Biogen-Idec, Genzyme, Gilead, GlaxoSmithKline, BreathID, Propharmaceuticals, XTL Corps Major Stockholder: BreathID, Intercept Pharmaceuticals, Conatus Content of presentation does not include discussion of off-label/investigative use of medicine(s), medical devices or procedure(s)</td>
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<tr>
<td>Galle, Peter, MD</td>
<td>(Basic Research Committee, Early Morning Workshop) Consultant/Advisor: Pfizer Content of presentation does not include discussion of off-label/investigative use of medicine(s), medical devices or procedure(s)</td>
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<tr>
<td>Garcia-Bao, Guadalupe, MD</td>
<td>(Postgraduate Course, Meet-the-Professor Luncheon, Early Morning Workshop) Nothing to disclose Content of presentation does not include discussion of off-label/investigative use of medicine(s), medical devices or procedure(s)</td>
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<td>Garcia-Valdecasas, Juan Carlos, MD</td>
<td>(Transplant Course) Nothing to disclose Content of presentation does not include discussion of off-label/investigative use of medicine(s), medical devices or procedure(s)</td>
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<td>Gershowin, Eric M., MD</td>
<td>(Basic Research Workshop, Meet-the-Professors Luncheon) Nothing to disclose Content of presentation does not include discussion of off-label/investigative use of medicine(s), medical devices or procedure(s)</td>
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<tr>
<td>Gerson, Lauren, MD</td>
<td>(Endoscopy Course Grant/Research Support: Fujinon Consultant/Advisor: Xenonart, TAP, Given Imaging Speakers Bureau: Santarus Content of presentation does not include discussion of off-label/investigative use of medicine(s), medical devices or procedure(s)</td>
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<tr>
<td>Gervais, Debra A., MD</td>
<td>(Endoscopy Course) Nothing to disclose Content of presentation does not include discussion of off-label/investigative use of medicine(s), medical devices or procedure(s)</td>
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<td>Ghany, Marc, MD</td>
<td>(NIH Corner) Nothing to disclose Content of presentation does include discussion of off-label/investigative use of medicine(s), medical devices or procedure(s)</td>
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<td>Gholam, Pierre, MD</td>
<td>(Early Morning Workshop) Nothing to disclose Content of presentation does not include discussion of off-label/investigative use of medicine(s), medical devices or procedure(s)</td>
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<td>Gines, Pere, MD</td>
<td>(Postgraduate Course, Meet-the-Professor Luncheon) Nothing to disclose Content of presentation does not include discussion of off-label/investigative use of medicine(s), medical devices or procedure(s)</td>
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<tr>
<td>Goodwin, Zachary D., MD, PhD</td>
<td>(Postgraduate Course) Nothing to disclose Content of presentation does not include discussion of off-label/investigative use of medicine(s), medical devices or procedure(s)</td>
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<tr>
<td>Gores, Gregory J., MD</td>
<td>(Governing Board, Endoscopy Course, Scientific Program Committee, Postgraduate Course Consultant/Advisor: Amgen, Bayer, Centocor Research and Development, Conatus, Hoffman-LaRoche, Intercept Pharmaceuticals, MDS Nordion, NPS Pharmaceuticals, Pfizer, Schering-Plough, Stromedix, VioBay Content of presentation does not include discussion of off-label/investigative use of medicine(s), medical devices or procedure(s)</td>
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<tr>
<td>Gorham, James, MD, PhD</td>
<td>(Basic Research Committee) Nothing to disclose Content of presentation does not include discussion of off-label/investigative use of medicine(s), medical devices or procedure(s)</td>
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<tr>
<td>Gostout, Christopher J., MD</td>
<td>(Endoscopy Course) Nothing to disclose Content of presentation does not include discussion of off-label/investigative use of medicine(s), medical devices or procedure(s)</td>
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<tr>
<td>Green, Douglass, MD</td>
<td>(Hans Popper State-of-the-Art Lecture Consultant/Advisor: Keystone Symposia Content of presentation does not include discussion of off-label/investigative use of medicine(s), medical devices or procedure(s)</td>
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<tr>
<td>Green, Richard, MD</td>
<td>(Basic Research Committee, Early Morning Workshop) Nothing to disclose Content of presentation does not include discussion of off-label/investigative use of medicine(s), medical devices or procedure(s)</td>
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Continuing Medical Education and Disclosures (continued)

Greenbaum, Linda, MD  
(Scientific Program Committee)  
Nothing to disclose

Guerrero, Heather, MD  
(Clinical Research Committee)  
Nothing to disclose

Hancock, Wayne W., MBBS, PhD  
(Basic Research Workshop)  
Nothing to disclose  
Content of presentation does not include discussion of off-label/investigative use of medicine(s), medical devices or procedure(s)

Hanto, Douglas, MD, PhD  
(Governing Board)  
Consultant/Advisor: INO Therapeutics

Harris, Peter, MD  
(Pediatric Symposium)  
Nothing to disclose  
Content of presentation does not include discussion of off-label/investigative use of medicine(s), medical devices or procedure(s)

Harrison, Stephen A., MD  
(Early Morning Workshop, Hepatology Associates Course, Training and Clinical Policy Committee, Postgraduate Course)  
Grant/Research Support: Roche, Schering-Plough, Valeant, Bristol-Myers Squibb  
Consultant/Advisor: Roche, Schering-Plough, Valeant, Bristol-Myers Squibb  
Speakers’ Bureau: Roche, Schering-Plough  
Content of presentation does not include discussion of off-label/investigative use of medicine(s), medical devices or procedure(s)

Haussinger, Dieter, MD  
(Postgraduate Course)  
Disclosure will be provided prior to presentation

Hay, J. Eileen, MD  
(Meet-the-Professors Luncheon)  
Nothing to disclose  
Content of presentation does not include discussion of off-label/investigative use of medicine(s), medical devices or procedure(s)

Heathcote, E. Jenny, MD  
(Meet-the-Professors Luncheon)  
Nothing to disclose  
Content of presentation does not include discussion of off-label/investigative use of medicine(s), medical devices or procedure(s)

Heller, Theo, MD  
(Pediatric Symposium)  
Nothing to disclose  
Content of presentation does not include discussion of off-label/investigative use of medicine(s), medical devices or procedure(s)

Henderson, J. Michael, MD  
(Postgraduate Course)  
Disclosure will be provided prior to presentation

Herrine, Steven K., MD  
(Career Development Workshop, Training and Clinical Policy Committee)  
Grant/Research Support: Idenix, Roche, Sanofi-Aventis, Schering-Plough  
Speakers’ Bureau: Roche, Schering-Plough  
Content of presentation does not include discussion of off-label/investigative use of medicine(s), medical devices or procedure(s)

Hoofnagle, Jay, MD  
(50 Years of Interferon, NIH Corner)  
Nothing to disclose  
Content of presentation does not include discussion of off-label/investigative use of medicine(s), medical devices or procedure(s)

Hu, Ke-Qin, MD  
(Education Committee)  
Grant/Research Support: Valeant, Schering-Plough, Roche, Gilead, Bristol-Myers Squibb  
Speakers’ Bureau: Valeant, Schering-Plough, Roche, Gilead, Bristol-Myers Squibb

Hubbard, Sarah, PA-C  
(Hepatology Associates Course)  
Speakers’ Bureau: Schering-Plough  
Content of presentation does not include discussion of off-label/investigative use of medicine(s), medical devices or procedure(s)

Jacobson, Ira M., MD  
(Early Morning Workshop)  
Grant/Research Support: Intermune, Schering-Plough, Valeant, Coley, Gilead, Vertex, GlobImmune, Idenix  
Consultant/Advisor: Idenix, Bristol-Myers Squibb, Novartis, Gilead, Coley, Valeant, Schering-Plough, Intermune, Pfizer, GlaxoSmithKline, Vertex, GlobImmune, Human Genome Sciences  
Speakers’ Bureau: Schering-Plough, Gilead, Bristol-Myers Squibb

Jalan, Rajiv, MD  
(Meet-the-Professors Luncheon)  
Nothing to disclose  
Content of presentation does include discussion of off-label/investigative use of medicine(s), medical devices or procedure(s)

Johnson, Lynt, MD  
(Education Committee)  
Speakers’ Bureau: Schering-Plough

Jonas, Maureen M., MD  
(Postgraduate Course)  
Grant/Research Support: Gilead Sciences, Inc., Schering-Plough  
Consultant/Advisor: Bristol-Myers Squibb, BSC  
Speakers’ Bureau: AstraZeneca, BSC

Jude, Carl, MD  
(Basic Research Workshop)  
Disclosure will be provided prior to presentation

Kalhan, Satish C., MBBS, FRCP  
(Early Morning Workshop)  
Disclosure will be provided prior to presentation

Kamath, Patrick, MD  
(Pediatric Symposium)  
Nothing to disclose  
Content of presentation does not include discussion of off-label/investigative use of medicine(s), medical devices or procedure(s)
Continuing Medical Education and Disclosures (continued)

Kaplowitz, Neil, MD  
(Meet-the-Professor Luncheon)  
Consultant/Advisor: Abbott, Adams Respiratory Therapy, Allergan, Amgen, AstraZeneca, Avera, BG Medicine, Biogen, Boehringer/Ingelheim, Cadence, Daiichi Sankyo, DOV, Elan, Enanta, Encysive, GSK, GTX, Incyte, ISIS, Jansen, Johnson & Johnson, Maxygen, Merck, Millennium, Ono, Pfizer, Rigol, Roche, Sankyo, TAP, Threshold, Teva, Wyeth

Karpen, Saul, MD, PhD  
(Meet-the-Professors Luncheon, Surgery and Liver Transplant Committee)  
Nothing to disclose

Content of presentation does not include discussion of off-label/investigative use of medicine(s), medical devices or procedure(s)

Keeffe, Emmet B., MD  
(General Hepatology Update)  
Grant/Research Support: Roche, Romark

Consultant/Advisor: Bristol-Myers Squibb, Gilead, GlaxoSmithKline, Idenix, Novartis, Roche

Speakers’ Bureau: Bristol-Myers Squibb, Gilead, GlaxoSmithKline, Idenix, Novartis, Roche

Content of presentation does not include discussion of off-label/investigative use of medicine(s), medical devices or procedure(s)

Kerlinski, Mariann, RN, BSN  
(Hepatology Associates Course, Hepatology Associates Committee)  
Consultant/Advisor: Roche

Kleiner, David, MD  
(Pediatric Symposium)  
Nothing to disclose

Content of presentation does not include discussion of off-label/investigative use of medicine(s), medical devices or procedure(s)

Kashy, Rajen, PhD  
(Early Morning Workshop, NIH Corner)  
Nothing to disclose

Content of presentation does not include discussion of off-label/investigative use of medicine(s), medical devices or procedure(s)

Kowdlely, Kris V., MD  
(Career Development Workshop, Postgraduate Course)  
Nothing to disclose

Content of presentation does not include discussion of off-label/investigative use of medicine(s), medical devices or procedure(s)

Koziel, Margaret, MD  
(Basic Research Committee, Basic Research Workshop)  
Grant/Research Support: Valeant, Bristol-Myers Squibb, Idera

Krowka, Michael J., MD  
(Meet-the-Professors Luncheon)  
Consultant/Advisor: CoTherix, Gilead

Content of presentation does not include discussion of off-label/investigative use of medicine(s), medical devices or procedure(s)

Kuiken, Carla, PhD  
(NIH Corner)  
Nothing to disclose

Content of presentation does not include discussion of off-label/investigative use of medicine(s), medical devices or procedure(s)

Kunos, George, MD, PhD  
(Hyman Zimmerman State-of-the-Art Lecture)  
Nothing to disclose

Content of presentation does include discussion of off-label/investigative use of medicine(s), medical devices or procedure(s)

Lake, John R., MD  
(Hepatology Associates Course, Transplant Course)  
Grant/Research Support: Roche, Wyeth, Sandofi, Gilead

Consultant/Advisor: Astellas, Genzyme, Vital Therapie, Bristol-Myers Squibb, Prosanos

Content of presentation does not include discussion of off-label/investigative use of medicine(s), medical devices or procedure(s)

Lavine, Joel, MD  
(Meet-the-Professor Luncheon)  
Grant/Research Support: NIDDK

Content of presentation does include discussion of off-label/investigative use of medicine(s), medical devices or procedure(s)

Lee, Fred, MD  
(Transplant Course)  
Disclosure will be provided prior to presentation

Lee, William M., MD  
(Early Morning Workshop)  
Grant/Research Support: Roche, Gilead, Bristol-Myers Squibb, Schering-Plough, Vertex

Consultant/Advisor: Eli Lilly, Astra Zeneca

Content of presentation does not include discussion of off-label/investigative use of medicine(s), medical devices or procedure(s)

Lemasters, John J., MD, PhD  
(Early Morning Workshop)  
Nothing to disclose

Content of presentation does not include discussion of off-label/investigative use of medicine(s), medical devices or procedure(s)

Levy, Cynthia, MD  
(Early Morning Workshop)  
Nothing to disclose

Content of presentation does include discussion of off-label/investigative use of medicine(s), medical devices or procedure(s)

Liang, T. Jake, MD  
(Governing Board, Early Morning Workshop, NIH Corner)  
Nothing to disclose

Content of presentation does not include discussion of off-label/investigative use of medicine(s), medical devices or procedure(s)

Liow, Yun-Fan, MD  
(NIH Corner)  
Grant/Research Support: BMS, Idenix, Novartis, Roche, SciClone, Gilead

Consultant/Advisor: BMS, GSK, Novartis, Roche, Schering-Plough, SciClone

Content of presentation does not include discussion of off-label/investigative use of medicine(s), medical devices or procedure(s)

Lindor, Keith D., MD  
(Early Morning Workshop)  
Grant/Research Support: Axcen

Consultant/Advisor: BMS, GSK, Novartis, Roche, Schering-Plough, SciClone

Content of presentation does not include discussion of off-label/investigative use of medicine(s), medical devices or procedure(s)

Liu, Chen, MD  
(Basic Research Committee)  
Nothing to disclose

Llovet, Josep M., MD  
(Early Morning Workshop, Transplant Course, Postgraduate Course)  
Consultant/Advisor: Bayer Pharmaceuticals, Biocompatibles

Content of presentation does not include discussion of off-label/investigative use of medicine(s), medical devices or procedure(s)
Locarnini, Stephen, MD, PhD
[NIH Corner]
Grant/Research Support: Evivar Pty Ltd, Gilead
Consultant/Advisor: Evivar Pty Ltd, Gilead, Pharmasset, BMS
Major Stockholder: Pharmasset
Content of presentation does not include discussion of off-label/investigative use of medicine(s), medical devices or procedure(s)

Lok, Anna SF, MD
[Early Morning Workshop, NIH Corner]
Grant/Research: Bristol-Myers Squibb, Gilead, GlaxoSmithKline, Idenix, Roche, Schering-Plough, Innogenetics, Valeant
Consultant/Advisor: Bristol-Myers Squibb, Gilead, GlaxoSmithKline, Idenix, Roche, Anadys, Innogenetics, Pharmasset
Stockholder: Anadys
Content of presentation does include discussion of off-label/investigative use of medicine(s), medical devices or procedure(s)

Lu, Shelly, MD
[Basic Research Committee, Early Morning Workshop]
Nothing to disclose
Content of presentation does not include discussion of off-label/investigative use of medicine(s), medical devices or procedure(s)

Lucy, Michael R., MD
[Early Morning Workshop, Transplant Course]
Grant/Research Support: BMS, Roche, Schering-Plough, SAUX
Content of presentation does not include discussion of off-label/investigative use of medicine(s), medical devices or procedure(s)

Lukin, Karen, MS, ARNP
[Hepatology Associates Course, Hepatology Associates Committee]
Speakers’ Bureau: Roche

Luxon, Bruce A., MD, PhD
[Clinical Research Committee]
Grant/Research Support: Bristol-Myers Squibb, Gilead, GlaxoSmithKline, Idenix, Roche, Schering-Plough, Innogenetics, Valeant
Consultant/Advisor: Bristol-Myers Squibb, Gilead, GlaxoSmithKline, Idenix, Roche, Anadys, Innogenetics, Pharmasset
Stockholder: Anadys
Content of presentation does not include discussion of off-label/investigative use of medicine(s), medical devices or procedure(s)

Ma, Mang M., MD
[Early Morning Workshop]
Grant/Research Support: Vertex, Novartis
Consultant/Advisor: Novartis, Roche
Content of presentation does not include discussion of off-label/investigative use of medicine(s), medical devices or procedure(s)

Macik, Gail, MD
[Postgraduate Course]
Nothing to disclose
Content of presentation does not include discussion of off-label/investigative use of medicine(s), medical devices or procedure(s)

Maher, Jacqueylin J., MD
[Governing Board, Career Development Workshop, Early Morning Workshop, Scientific Program Committee]
Consultant/Advisor: Pfizer
Content of presentation does not include discussion of off-label/investigative use of medicine(s), medical devices or procedure(s)

Majno, Pietro, MD
[Transplant Course]
Nothing to disclose
Content of presentation does not include discussion of off-label/investigative use of medicine(s), medical devices or procedure(s)

Malhi, Harmeet, MD
[Basic Research Committee]
Nothing to disclose
Content of presentation does not include discussion of off-label/investigative use of medicine(s), medical devices or procedure(s)

Manns, Michael, MD
[Meet-the-Professors Luncheon]
Grant/Research Support: Falk Pharma GmbH
Consultant/Advisor: Falk Pharma GmbH

Marrero, Jorge A., MD
[Postgraduate Course]
Nothing to disclose
Content of presentation does not include discussion of off-label/investigative use of medicine(s), medical devices or procedure(s)

Martin, Paul, MD
[Early Morning Workshop]
Grant/Research Support: Roche, Schering-Plough
Consultant/Advisor: Roche
Speakers’ Bureau: Roche, Schering-Plough
Content of presentation does include discussion of off-label/investigative use of medicine(s), medical devices or procedure(s)

Mathurin, Philippe, MD, PhD
[Transplant Course]
Nothing to disclose
Content of presentation does not include discussion of off-label/investigative use of medicine(s), medical devices or procedure(s)

Mayo, Marilyn, MD
[Meet-the-Professor Luncheon]
Nothing to disclose
Content of presentation does not include discussion of off-label/investigative use of medicine(s), medical devices or procedure(s)

Mazzariegos, George, MD
[Meet-the-Professor Luncheon]
Disclosure will be provided prior to presentation

Mazzaferro, Vincenzo, MD
[Transplant Course]
Nothing to disclose
Content of presentation does not include discussion of off-label/investigative use of medicine(s), medical devices or procedure(s)

McCashland, Timothy M., MD
[Hepatology Associates Course]
No relationship to disclose
Content of presentation does not include discussion of off-label/investigative use of medicine(s), medical devices or procedure(s)

McCaughan, Geoffrey, MD, PhD
[Clinical Research Committee]
Grant/Research Support: Roche
Consultant/Advisor: Roche, Astellas, Schering-Plough

McClain, Craig J., MD
[Early Morning Workshop]
Grant/Research Support: Astra Zeneca, Schering-Plough, Roche
Consultant/Advisor: Roche, Ross Laboratories, nestle, IDUN, Elan Pharmaceuticals, Pharmavite
Speakers’ Bureau: Wyeth Ayerst, Roche, Axcan
Content of presentation does not include discussion of off-label/investigative use of medicine(s), medical devices or procedure(s)
McCullough, Arthur J., MD
(Governing Board, Scientific Program Committee,
Postgraduate Course)
Consultant/Advisor: Intercept Pharmaceuticals, Pfizer, Wyeth
Content of presentation does not include discussion of off-label/
investigative use of medicine(s), medical devices or procedure(s)

McDonald, George, MD
(Meet-the-Professor Luncheon)
Disclosure will be provided prior to presentation

McHutchison, John G., MD
(Early Morning Workshop, NIH Corner, Clinical Research
Committee, Scientific Program Committee)
Grant/Research Support: Anadys, Coley Pharmaceuticals, First
Circle Medical, GlaxoSmithKline, Globe Immune, Human Genome
Sciences, Idenix, Intarcia, Novartis, Pfizer, Salix Pharmaceuticals,
Sanofi-Aventis, Schering-Plough, Valeant, Vertex, Wyeth
Consultant/Advisor: Anadys, Biolex, Coley Pharmaceuticals,
Epiphany Biosciences, First Circle Medical, GlaxoSmithKline,
Human Genome Sciences, Idenix, Idera, Intarcia, InterMune
Pharmaceuticals, National Genetics, Novartis, Peregrine, Pfizer,
Schering-Plough, United Therapeutics, Valeant, Vertex, Wyeth
Content of presentation does not include discussion of off-label/
investigative use of medicine(s), medical devices or procedure(s)

Merion, Robert, MD
(Thomas Starzl State-of-the-Art Lecture)
Nothing to disclose

Merriman, Raphael B., MD
(Postgraduate Course)
Nothing to disclose

Merrigan, Giorgina
(Meet-the-Professor Luncheon)
Nothing to disclose

Miller, Charles M., MD
(Early Morning Workshop, Surgery and Liver Transplant Committee)
Nothing to disclose

Mindikoglu, Ayse, MD
(Education Committee)
Nothing to disclose

Mishra, Lopa, MD
(Basic Research Committee)
Nothing to disclose

Mistry, Pramod, K., MD
(Pediatric Symposium)
Disclosure will be provided prior to presentation

Mizokami, Masashi, MD
(NIH Corner)
Disclosure will be provided prior to presentation

Monto, Alexander, MD
(Clinical Research Committee)
Nothing to disclose

Moon, Scott D., PA-C
(Hepatology Associates Course, Hepatology Associates Committee)
Speakers’ Bureau: Schering-Plough, Roche, Axcan, Gilead,
Valeant

Morgan, Timothy R., MD
(Early Morning Workshop)
Nothing to disclose

Muir, Andrew, MD
(Early Morning Workshop)
Grant/Research Support: Human Genome Sciences, Idenix,
Intarcia, Pfizer, Roche, Schering-Plough, Valeant, Wyeth
Consultant/Advisor: Zymogenetics
Speakers’ Bureau: Schering-Plough
Content of presentation does not include discussion of off-label/
investigative use of medicine(s), medical devices or procedure(s)

Murray, Karen F., MD
(Pediatric Symposium)
Grant/Research Support: Gilead, Schering-Plough, Roche
Content of presentation does not include discussion of off-label/
investigative use of medicine(s), medical devices or procedure(s)

Myers, Jaime, RN
(Hepatology Associates Course)
Nothing to disclose

Nagy, Laura, E., PhD
(Early Morning Workshop)
Nothing to disclose

Nathanson, Michael H., MD, PhD
(Early Morning Workshop)
Nothing to disclose

Nelson, David R., MD
(Early Morning Workshop)
Grant/Research Support: Roche, Schering-Plough, Vertex, Astellas,
Pharmasset, HGS, Novartis, DeBioTechPharm, Romark, Medarex
Consultant/Advisor: Roche, HGS, Novartis, Romark
Speakers’ Bureau: Roche, Schering-Plough, BMS
Content of presentation does not include discussion of off-label/
investigative use of medicine(s), medical devices or procedure(s)

Neuberger, James, MD
(Transplant Course)
Speakers Bureau: Roche, Astellas, Novartis
Content of presentation does not include discussion of off-label/
investigative use of medicine(s), medical devices or procedure(s)

Norris, Suzanne, MRCP, PhD
(Education Committee)
Nothing to disclose

Opperman, Suzanne, RN, MSN
(Hepatology Associates Course)
Grant/Research Support: Roche,
Content of presentation does not include discussion of off-label/
investigative use of medicine(s), medical devices or procedure(s)
<table>
<thead>
<tr>
<th>Name</th>
<th>Workshop/Committee</th>
<th>Disclosures</th>
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<tr>
<td>Pardoll, Drew M., MD, PhD</td>
<td>(Basic Research Workshop)</td>
<td>Nothing to disclose. Content of presentation does not include discussion of off-label/investigative use of medicine(s), medical devices or procedure(s)</td>
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<tr>
<td>Pawlotsky, Jean-Michel, MD</td>
<td>(Early Morning Workshop, NIH Corner)</td>
<td>Nothing to disclose. Content of presentation does not include discussion of off-label/investigative use of medicine(s), medical devices or procedure(s)</td>
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<tr>
<td>Peters, Marion G., MD</td>
<td>(Hepatology Associates Course)</td>
<td>Grant/Research Support: Achillion Pharmaceuticals. Consultant/Advisor: CCO</td>
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<tr>
<td>Petersen, Bryon, PhD</td>
<td>(Basic Research Committee)</td>
<td>Consultant: ReGenMed. Stock: ReGenMed.</td>
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<tr>
<td>Petricoin, Emanuel, MD</td>
<td>(Meet-the-Professor Luncheon)</td>
<td>Nothing to disclose. Content of presentation does not include discussion of off-label/investigative use of medicine(s), medical devices or procedure(s)</td>
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<tr>
<td>Pietrangelo, Antonello, MD</td>
<td>(Postgraduate Course)</td>
<td>Nothing to disclose. Content of presentation does not include discussion of off-label/investigative use of medicine(s), medical devices or procedure(s)</td>
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<tr>
<td>Pinzani, Massimo, MD</td>
<td>(Advances for Practitioners)</td>
<td>Nothing to disclose. Content of presentation does not include discussion of off-label/investigative use of medicine(s), medical devices or procedure(s)</td>
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<tr>
<td>Podskalny, Judith, PhD</td>
<td>(Career Development Workshop)</td>
<td>Nothing to disclose. Content of presentation does not include discussion of off-label/investigative use of medicine(s), medical devices or procedure(s)</td>
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<tr>
<td>Poterucha, John, MD</td>
<td>(Training and Clinical Policy Committee)</td>
<td>Nothing to disclose.</td>
</tr>
<tr>
<td>Poupon, Raoul, MD</td>
<td>(Leon Schiff State-of-the-Art Lecture)</td>
<td>Nothing to disclose. Content of presentation does not include discussion of off-label/investigative use of medicine(s), medical devices or procedure(s)</td>
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<tr>
<td>Rai, Jitha, MD</td>
<td>(Surgery and Liver Transplant Committee)</td>
<td>Nothing to disclose.</td>
</tr>
<tr>
<td>Rakela, Jorge, MD</td>
<td>(Early Morning Workshop)</td>
<td>Disclosure will be provided prior to presentation. Content of presentation does not include discussion of off-label/investigative use of medicine(s), medical devices or procedure(s)</td>
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<tr>
<td>Ray, Stuart C., MD</td>
<td>(Early Morning Workshop)</td>
<td>Disclosure will be provided prior to presentation. Content of presentation does not include discussion of off-label/investigative use of medicine(s), medical devices or procedure(s)</td>
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<tr>
<td>Reddy, K. Rajender, MD</td>
<td>(Training and Clinical Policy Committee)</td>
<td>Nothing to disclose.</td>
</tr>
<tr>
<td>Regev, Arie, MD</td>
<td>(Career Development Workshop, Training and Clinical Policy Committee)</td>
<td>Grant/Research Support: Roche, Gilead, Valeant, Bristol-Myers Squibb. Consultant/Advisor: Roche, Gilead, Valeant. Speakers’ Bureau: Roche, Gilead, Valeant. Employee: Eli Lilly. Content of presentation does not include discussion of off-label/investigative use of medicine(s), medical devices or procedure(s)</td>
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<tr>
<td>Reid, Andrea Ewing, MD</td>
<td>(Education Committee)</td>
<td>Nothing to disclose.</td>
</tr>
<tr>
<td>Reuben, Adrian, MBBS, FRCP</td>
<td>(50 Years of Interferon)</td>
<td>Grant/Research Support: Schering-Plough, Valeant, DSMB for Idenix and Nucleonics. Speakers’ Bureau: Roche, Shering-Plough. Content of presentation does not include discussion of off-label/investigative use of medicine(s), medical devices or procedure(s)</td>
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<tr>
<td>Roberts, Lewis R., MD, PhD</td>
<td>(Early Morning Workshop, Education Committee, Postgraduate Course)</td>
<td>Nothing to disclose. Content of presentation does not include discussion of off-label/investigative use of medicine(s), medical devices or procedure(s)</td>
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<tr>
<td>Rockey, Don C., MD</td>
<td>(Training and Clinical Policy Committee, Meet-the-Professor Luncheon)</td>
<td>Grant/Research Support: NIH, Sucampo. Content of presentation does not include discussion of off-label/investigative use of medicine(s), medical devices or procedure(s)</td>
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<tr>
<td>Rosati, Marianne, MSN, NP</td>
<td>(Basic Research Committee)</td>
<td>Nothing to disclose.</td>
</tr>
<tr>
<td>Roskams, Tania, MD, PhD</td>
<td>(Postgraduate Course)</td>
<td>Nothing to disclose. Content of presentation does not include discussion of off-label/investigative use of medicine(s), medical devices or procedure(s)</td>
</tr>
<tr>
<td>Saab, Sammy, MD</td>
<td>(General Hepatology Update)</td>
<td>Grant/Research Support: Schering-Plough. Consultant/Advisor: Schering-Plough, Roche, InterMune. Stockholder: Schering-Plough, Genetech, Johnson and Johnson. Content of presentation does not include discussion of off-label/investigative use of medicine(s), medical devices or procedure(s)</td>
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</tbody>
</table>
Continuing Medical Education and Disclosures (continued)

Saitz, Richard, MD
(Transplant Course)
Nothing to disclose
Content of presentation does not include discussion of off-label/investigative use of medicine(s), medical devices or procedure(s)

Sandhu, Baljait, MD
(Meet-the-Professor Luncheon)
Nothing to disclose
Content of presentation does include discussion of off-label/investigative use of medicine(s), medical devices or procedure(s)

Sayuk, Stacy, MS, PA-C
(Surgery and Liver Transplant Committee)
Speakers’ Bureau: Santarus Pharm, Valeant

Schiff, Eugene R., MD
(Career Development Workshop, Advances for Practitioners)
Speakers’ Bureau: Gilead, Ortho-Biotech, Schering-Plough

Schilsky, Michael, MD
(Meet-the-Professor Luncheon)
Nothing to disclose
Content of presentation does not include discussion of off-label/investigative use of medicine(s), medical devices or procedure(s)

Schuppan, Detlef, MD, PhD
(Basic Research Committee)
Nothing to disclose

Schwartz, Myron E., MD
(Transplant Course)
Nothing to disclose
Content of presentation does not include discussion of off-label/investigative use of medicine(s), medical devices or procedure(s)

Seef, Leonard, MD
(Meet-the-Professor Luncheon)
Nothing to disclose
Content of presentation does include discussion of off-label/investigative use of medicine(s), medical devices or procedure(s)

Sell, Steward, MD
(Endoscopy Course)
Granted/Research Support: NIH
Content of presentation does not include discussion of off-label/investigative use of medicine(s), medical devices or procedure(s)

Shah, Raj J., MD
(Endoscopy Course)
Nothing to disclose
Content of presentation does not include discussion of off-label/investigative use of medicine(s), medical devices or procedure(s)

Shah, Vijay, MD
(Endoscopy Workshop, Postgraduate Course)
Grant/Research Support: Axcan
Consultant/Advisor: Eli Lilly
Content of presentation does not include discussion of off-label/investigative use of medicine(s), medical devices or procedure(s)

Shakil, A. Obaid, MD
(Clinical Research Committee)
Speakers’ Bureau: Roche, Schering-Plough, Gilead

Shepherd, Ross, MD
(Early Morning Workshop)
Nothing to disclose
Content of presentation does not include discussion of off-label/investigative use of medicine(s), medical devices or procedure(s)

Sherman, Morris, MD, PhD
(Early Morning Workshop)
Consultant/Advisor: Bayer Inc
Content of presentation does not include discussion of off-label/investigative use of medicine(s), medical devices or procedure(s)

Shetty, Kirthi, MD
(Surgery and Liver Transplant Committee)
Grant/Research Support: Idenix, Novartis/Idenix, Ortho-Biotech

Shevach, Ethan M., MD
(Basic Research Workshop)
Nothing to disclose
Content of presentation does not include discussion of off-label/investigative use of medicine(s), medical devices or procedure(s)

Shiffman, Mitchell L., MD
(Endoscopy Workshop, Scientific Program Committee)
Grant/Research Support: Roche, Schering-Plough, Valeant
Consultant/Advisor: Roche, Valeant
Speakers’ Bureau: Roche, Valeant
Content of presentation does not include discussion of off-label/investigative use of medicine(s), medical devices or procedure(s)

Sheiner, Benjamin L., MD
(Early Morning Workshop, Scientific Program Committee)
Nothing to disclose
Content of presentation does not include discussion of off-label/investigative use of medicine(s), medical devices or procedure(s)

Shrestha, Roshan, MD
(Endoscopy Course)
Speakers’ Bureau: Valeant, Gilead, Idenix, Schering-Plough, Axcan, Salix
Content of presentation does not include discussion of off-label/investigative use of medicine(s), medical devices or procedure(s)

Sivka, Adam, MD, PhD
(Endoscopy Course)
Consultant/Advisor: Boston Scientific
Content of presentation does not include discussion of off-label/investigative use of medicine(s), medical devices or procedure(s)

Sokol, Ronald J., MD
(Early Morning Workshop)
License for Patent: Yasoo Health Inc
Content of presentation does not include discussion of off-label/investigative use of medicine(s), medical devices or procedure(s)

Sterling, Richard K., MD
(Meet-the-Professors Luncheon)
Disclosure will be provided prior to presentation

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Stewart, Charmaine, MD  
(Clinical Research Committee)  
Nothing to disclose

Strazzabosco, Mario, MD, PhD  
(Pediatric Symposium)  
Nothing to disclose  
Content of presentation does not include discussion of off-label/ investigative use of medicine(s), medical devices or procedure(s)

Su, Grace, MD  
(Training and Clinical Policy Committee)  
Nothing to disclose

Suchy, Frederick, MD  
(Pediatric Symposium)  
Consultant/Advisor: Novartis  
Content of presentation does not include discussion of off-label/ investigative use of medicine(s), medical devices or procedure(s)

Sulkowski, Mark, MD  
(Postgraduate Course)  
Grant/Research Support: Schering, Roche, Bristol-Myers Squibb  
Consultant/Advisor: Schering, Roche  
Speakers’ Bureau: Roche  
Content of presentation does not include discussion of off-label/ investigative use of medicine(s), medical devices or procedure(s)

Sussman, Norman, MD  
(Clinical Research Committee)  
Grant/Research Support: Roche, Schering-Plough  
Speakers’ Bureau: Roche, Schering-Plough  
Stockholder: Stem Cell Innovations

Szabo, Gyongyi, MD, PhD  
(Scientific Program Committee, Basic Research Committee, Early Morning Workshop)  
Grant/Research Support: GlaxoSmithKline  
Consultant: Maxim, NPS Pharma

Taub, Rebecca A., MD  
(Early Morning Workshop)  
Employee: Roche  
Content of presentation does not include discussion of off-label/ investigative use of medicine(s), medical devices or procedure(s)

Te, Helen, MD  
(Surgery and Liver Transplant Committee)  
Nothing to disclose

Terrault, Norah, MD  
(General Hepatology Update, Education Committee)  
Grant/Research Support: Roche, Gilead, Gengen Corporation, Human Genome Sciences, Vertex, Schering-Plough  
Consultant/Advisor: Siemens Diagnostics, Bristol-Myers Squibb, Idenix, NABI  
Content of presentation does not include discussion of off-label/ investigative use of medicine(s), medical devices or procedure(s)

Thiele, Dwain L., MD  
(Early Morning Workshop, Meet-the-Professor Luncheon)  
No relationship to disclose  
Content of presentation does not include discussion of off-label/ investigative use of medicine(s), medical devices or procedure(s)

Thiele, Neil D., MD  
(Early Morning Workshop)  
Nothing to disclose  
Content of presentation does not include discussion of off-label/ investigative use of medicine(s), medical devices or procedure(s)

Todo, Satoru, MD  
(Transplant Course)  
Nothing to disclose  
Content of presentation does not include discussion of off-label/ investigative use of medicine(s), medical devices or procedure(s)

Trauner, Michael, MD  
(Early Morning Workshop)  
Nothing to disclose  
Content of presentation does not include discussion of off-label/ investigative use of medicine(s), medical devices or procedure(s)

Trautwein, Christian, MD  
(Early Morning Workshop)  
Nothing to disclose  
Content of presentation does not include discussion of off-label/ investigative use of medicine(s), medical devices or procedure(s)

Trotter, James F., MD  
(Early Morning Workshop, Training and Clinical Policy Committee)  
Grant/Research Support: Cellzadized, Roche, Astellas, Novartis, Sanofi-Aventis  
Consultant/Advisor: Roche, Astellas, Novartis, Axcian

Valle, Dominique, MD  
(Postgraduate Course)  
Nothing to disclose  
Content of presentation does include discussion of off-label/ investigative use of medicine(s), medical devices or procedure(s)

Vargas, Hugo E., MD  
(Early Morning Workshop, Hepatology Associates Course, Education Committee, Scientific Program Committee, Postgraduate Course)  
Grant/Research Support: Roche, Novartis, Vertex, SVRI, Debiouision, Pharmasset  
Consultant/Advisor: Johnson and Johnson, NPS Pharma  
Content of presentation does not include discussion of off-label/ investigative use of medicine(s), medical devices or procedure(s)

Vargo, John, MD  
(Endoscopy Course)  
Nothing to disclose  
Content of presentation does not include discussion of off-label/ investigative use of medicine(s), medical devices or procedure(s)

Vierling, John, MD  
(Meet-the-Professor Luncheon)  
Grant/Research Support: Astellas, Breath ID, Bristol-Myers Squibb, Gilead, IdenixNovartis, Roche, ScheringPlough, Valeant, Vertex, Wyeth  
Speakers’ Bureau: Axcian, Bristol-Myers Squibb, Gilead, In Scope, Roche, Schering-Plough  
Consultant/Advisor: Dynavax, Breath ID  
Content of presentation does include discussion of off-label/ investigative use of medicine(s), medical devices or procedure(s)

Volk, Michael, MD  
(Training and Clinical Policy Committee)  
Grant/Research Support: Robert Wall Johnson Foundation

Walker, Christopher, PhD  
(Early Morning Workshop)  
Disclosure will be provided prior to presentation
Continuing Medical Education and Disclosures (continued)

Wanless, Ian R., MD
(Postgraduate Course)
Nothing to disclose
Content of presentation does not include discussion of off-label/investigative use of medicine(s), medical devices or procedure(s)

Weinrieb, Robert, MD
(Transplant Course)
Nothing to disclose
Content of presentation does not include discussion of off-label/investigative use of medicine(s), medical devices or procedure(s)

Wells, Rebecca, MD
(Early Morning Workshop, Education Committee, Hepatology Associates Committee, Meet-the-Professor Luncheon)
Nothing to disclose
Content of presentation does not include discussion of off-label/investigative use of medicine(s), medical devices or procedure(s)

Werner, K. Tuesday, RN, MSN
(Surgery and Liver Transplant Committee)
Nothing to disclose

Whittington, Peter F., MD
(Postgraduate Course)
Nothing to disclose
Content of presentation does not include discussion of off-label/investigative use of medicine(s), medical devices or procedure(s)

Wiesner, Russell H., MD
(Early Morning Workshop)
Grant/Research Support: Roche, Intermune, Human Genome Sciences
Consultant/Advisor: Roche, Intermune, Human Genome Sciences
Content of presentation does not include discussion of off-label/investigative use of medicine(s), medical devices or procedure(s)

Willenbring, Mark, MD
(Hepatology Associates Course)
Nothing to disclose
Content of presentation does include discussion of off-label/investigative use of medicine(s), medical devices or procedure(s)

Wilt, Patricia, MS, MSN
(Training and Clinical Policy Committee)
Nothing to disclose

Wolkoff, Allan W., MD
(Early Morning Workshop)
Nothing to disclose
Content of presentation does not include discussion of off-label/investigative use of medicine(s), medical devices or procedure(s)

Yao, Francis, MD
(Education Committee)
Nothing to disclose

Yee, Hal F., MD, PhD
(Early Morning Workshop)
Nothing to disclose
Content of presentation does not include discussion of off-label/investigative use of medicine(s), medical devices or procedure(s)

Yerian, Lisa, MD
(Postgraduate Course)
Nothing to disclose
Content of presentation does not include discussion of off-label/investigative use of medicine(s), medical devices or procedure(s)

Yim, Colina, RN, MN
(Hepatology Associates Course, Hepatology Associates Committee)
Consultant/Advisor: Roche Canada

Yoshida, Eric M., MD
(Advances for Practitioners)
Grant/Research Support: Hoffman LaRoche Canada, Schering-Plough Canada, GlaxoSmithKline Canada, Ortho Janssen Canada, Pfizer-Canada, Inc., Gilead Sciences-Canada

Younossi, Zobair M., MD
(Early Morning Workshop, Postgraduate Course)
Grant/Research Support: Idenix, Vertex, Wyeth, Axcan, Roche, Amgen, Human Genome Sciences, Novartis
Content of presentation does not include discussion of off-label/investigative use of medicine(s), medical devices or procedure(s)

Zein, Claudia, MD
(Meet-the-Professors Luncheon)
Disclosure will be provided prior to presentation

Zein, Nizar N., MD
(Early Morning Workshop, Clinical Research Committee, Postgraduate Course)
Grant/Research Support: Centercone, Schering-Plough, Roche, Valeant, Coley
Consultant/Advisor: Valeant, Chronic Liver Disease Foundation

Zeuzem, Stefan, MD
(Early Morning Workshop, Postgraduate Course)
Disclosure will be provided prior to presentation

Zoulim, Fabien, MD
(NIH Corner)
Grant/Research Support: Bioalliance, Biomerieux, Gilead
Consultant/Advisor: Gilead, Idenix, Novartis, Innogenetics, Abbott
Speakers’ Bureau: Gilead, BMS, Idenix, Novartis, Abbott
Content of presentation does not include discussion of off-label/investigative use of medicine(s), medical devices or procedure(s)

44A
AASLD/ILTS Transplant Course

Friday, November 2
8:00 AM - 3:30 PM  Hynes, Ballroom A/B/C

Therapeutic Challenges in Liver Transplantation: Hepatocellular Carcinoma, Alcoholic Liver Disease and Other Addictive States

COURSE DIRECTORS: Michael R. Lucey, MD
Juan C. Garcia-Valdecasas, MD

6.5 CME Credits
6 Contact Hours

This course draws on experts from within transplant hepatology and other disciplines, such as hepatobiliary surgery, psychiatry, and radiology, to provide a multidisciplinary review of two of the greatest therapeutic challenges involving liver transplant medicine: multidisciplinary management of hepatocellular carcinoma and integrated approaches to addictions, alcoholism, and alcoholic liver disease.

Goals and Objectives:
• Discuss the diagnosis and presentation of hepatocellular carcinoma, addiction, alcoholism, and alcoholic liver disease.
• Explain how management of the conditions under discussion (HCC and ALD) has changed in the era of transplantation.
• Discuss anticipated future multidisciplinary treatment of HCC, alcoholism, and ALD in potential transplant recipients.

8:00 - 8:10 AM  Introduction: The Problem of HCC and Liver Transplantation
Juan C. Garcia-Valdecasas, MD

8:10 - 8:30 AM  Screening and Diagnostic Work-up
Andrew K. Burroughs, FRCP

8:30 - 8:50 AM  Clinical and Molecular Classification of HCC
Josep M. Llovet, MD

8:50 - 9:10 AM  The Need to Biopsy
Francois Durand, MD

9:10 - 9:30 AM  Bridging Interventional Therapy: The Role of the Interventional Radiology
Fred T. Lee, MD

9:30 - 9:50 AM  The Value of Pre-Transplant Treatment: Time For A Controlled Study?
Pietro Majno, MD

9:50 - 10:00 AM  Discussion

10:00 - 10:20 AM  Break

10:20 - 10:40 AM  Allocation in the MELD Era: Is It Justified?
Sandy Feng, MD, PhD

10:40 - 11:00 AM  Results of Liver Transplantation: With and Without Milan Criteria
Vincenzo Mazzaferro, MD

11:00 - 11:20 AM  Molecular Selection of HCC Patients Beyond Milan
Myron Schwartz, MD

11:20 - 11:40 AM  Extending the Indication: The Role of LDLT
Satoru Todo, MD, PhD

11:40 AM - 12:40 PM  Lunch

12:40 - 1:00 PM  What is Addiction, What is Alcoholism?
Thomas P. Beresford, MD

1:00 - 1:20 PM  Treatment of Addictions and Alcoholism
Richard Saitz, MD

1:20 - 1:30 PM  Public and Professional Attitudes to Transplanting Alcoholic Patients
James M. Neuberger, DM

1:30 - 1:50 PM  Treatment of Alcoholic Liver Disease
Christopher P. Day, MD, PhD

1:50 - 2:00 PM  Discussion

2:00 - 2:10 PM  Break

2:10 - 2:30 PM  Natural History of Alcoholism in Liver Transplant Recipients
Andrea DiMartini, MD

2:30 - 2:50 PM  Treatment of Addictive Behavior in Liver Transplant Patients?
Robert M. Weinrieb, MD

Case Presentations

2:50 - 3:05 PM  The Difficult Patient: Alcoholic Hepatitis
Philippe Mathurin, MD, PhD

3:05 - 3:20 PM  The Difficult Patient: HCV and Alcoholic Liver Disease
John R. Lake, MD

3:20 - 3:30 PM  Discussion
AASLD/ASGE Endoscopy Course

Friday, November 2
10:00 AM - 4:00 PM  Hynes, Room 304/306
Endoscopic Management of End Stage Liver Disease: Challenges and Controversies

COURSE DIRECTORS: Yang K. Chen, MD
Lauren B. Gerson, MD

5 CME Credits

Gastrointestinal endoscopy remains a cornerstone of the management of patients with liver disease. This course will highlight the standard of care and review controversies in the management of bleeding, portal hypertension, biliary problems before and after liver transplantation, and biliary stricture diagnosis and management. New techniques such as capsule endoscopy, double balloon enteroscopy, endosonography/doppler, and gastric variceal injection will be described.

Goals and Objectives:
• Describe the role of endoscopy in screening, prophylaxis, and management of active variceal bleeding.
• Describe the indications for ERCP in primary sclerosing cholangitis and in the management of post-liver transplantation biliary complications.
• Demonstrate familiarity with evolving endoscopic technology for diagnosis and management of hepatobiliary diseases.

10:00 - 10:05 AM  Welcome and Introduction
Yang K. Chen, MD

10:05 - 10:20 AM  Sedation for Endoscopy in Patients with End Stage Liver Disease
John Vargo, MD

10:20 - 10:30 AM  Discussion

10:30 - 10:40 AM  Screening and Grading of Esophageal Varices: Is There a Role for Capsule Endoscopy?
Christopher J. Gostout, MD

10:40 - 10:50 AM  Assessment of Hepatic Arterial and Portal Venous Circulation: Is There a Role for EUS/Doppler?
William R. Brugge, MD

10:50 - 11:05 AM  Discussion

11:05 - 11:15 AM  Obscure GI Bleeding in the Cirrhotic Patient: Is There a Role for Capsule Endoscopy or Double Balloon Enteroscopy?
Lauren B. Gerson, MD

11:15 - 11:25 AM  Symptomatic Gallbladder Diseases: Is There a Role for Gallbladder Stenting?
Yang K. Chen, MD

11:25 - 11:40 AM  Discussion

11:40 - 11:55 AM  Endoscopic or Medical Therapy to Prevent First Variceal Hemorrhage? - Medical Therapy
Arun J. Sanyal, MD

11:55 - 12:10 PM  Endoscopic or Medical Therapy to Prevent First Variceal Hemorrhage? - Endoscopic Therapy
Gregory T. Everson, MD

12:10 - 12:25 PM  Discussion

12:25 - 12:40 PM  Endoscopic Treatment of Actively Bleeding Esophageal and Gastric Varices
Dennis Jensen, MD

12:40 - 12:55 PM  Endoscopic Treatment of Nonvariceal Bleeding: GAVE and Portal Hypertensive Gastropathy
Christopher J. Gostout, MD

12:55 - 1:10 PM  Percutaneous or Endoscopic Approach for Stricture Management in Primary Sclerosing Cholangitis? - Percutaneous Approach
Debra A. Gervais, MD

1:25 - 1:40 PM  Percutaneous or Endoscopic Approach for Stricture Management in Primary Sclerosing Cholangitis? - Endoscopic Approach
Raj J. Shah, MD

1:40 - 1:55 PM  Discussion

2:15 - 2:30 PM  Management of Donor and Recipient Biliary Complications after Liver Transplantation
Roshan Shrestha, MD

2:30 - 2:40 PM  Discussion

2:40 - 2:55 PM  EUS for Diagnosis and Surveillance of Liver and Biliary Cancers
Douglas O. Faigel, MD

2:55 - 3:05 PM  Discussion

3:05 - 3:20 PM  Diagnosis and Staging of Cholangiocarcinoma for Liver Transplantation - Role of Molecular Markers
Gregory J. Gores, MD

3:20 - 3:35 PM  Diagnosis and Staging of Cholangiocarcinoma for Liver Transplantation - Role of ERCP and Cholangioscopy
Adam Slivka, MD, PhD

3:35 - 3:50 PM  Discussion

3:50 - 4:00 PM  Closing Remarks
Lauren B. Gerson, MD
AASLD/NASPGHAN Pediatric Symposium

Friday, November 2
12:00 - 3:00 PM  Hynes, Room 302
Fibrocystic Diseases of the Liver

COURSE DIRECTORS: Karen F. Murray, MD
Pramod K. Mistry, MD, PhD

3 CME Credits
2.5 Contact Hours

This symposium will provide a thorough review of fibrocystic diseases affecting the liver. Intended for both hepatologists and gastroenterologists who care for either pediatric or adult patients with hepatic disease, this symposium will review the embryology, genetics, and molecular biology of these conditions. Additionally, the clinical phenotypes, pathology in, clinical complications of, and treatment for these conditions will be reviewed. Expert speakers in the field will provide the latest information in all aspects of these interesting and complicated conditions.

Goals and Objectives:
- Discuss the variety of, and be able to diagnose, fibrocystic diseases involving the liver.
- Summarize the scientific advances that have occurred in our understanding of these conditions.
- Describe the management of patients with fibrocystic diseases involving the liver.

<table>
<thead>
<tr>
<th>Time</th>
<th>Topic</th>
<th>Speaker</th>
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<tbody>
<tr>
<td>12:00 - 12:10 PM</td>
<td>Introduction</td>
<td>Karen F. Murray, MD</td>
</tr>
<tr>
<td>12:10 - 12:30 PM</td>
<td>Embryology and the Development of the Ductal Plate</td>
<td>Clifford W. Bogue, MD</td>
</tr>
<tr>
<td>12:30 - 12:50 PM</td>
<td>Polycystic Liver Disease and Cholangiocyte Biology</td>
<td>Mario Strazzabosco, MD, PhD</td>
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<tr>
<td>12:50 - 1:10 PM</td>
<td>Genetics of Cystic Diseases of the Liver and Kidney</td>
<td>Peter C. Harris, PhD</td>
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<tr>
<td>1:10 - 1:30 PM</td>
<td>Phenotypic Spectrum and Genetic Correlation in ARPKD/CHF/Caroli’s Disease</td>
<td>Theo Heller, MD</td>
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<tr>
<td>1:30 - 1:40 PM</td>
<td>Pathophysiology and Pathology of the Fibrocystic Disease in the Liver</td>
<td>David Kleiner, MD</td>
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<tr>
<td>1:40 - 1:50 PM</td>
<td>Vascular Complications and Their Treatment in the Fibrocystic Diseases of the Liver</td>
<td>Patrick S. Kamath, MD</td>
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<td>1:50 - 2:10 PM</td>
<td>New Diagnostic Approaches</td>
<td>Frederick J. Suchy, MD</td>
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<td>2:10 - 2:30 PM</td>
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<td>2:30 - 2:50 PM</td>
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<td>2:50 - 3:00 PM</td>
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Career Development Workshop

Friday, November 2
12:00 - 4:00 PM  Hynes, Room 210

Career Development Workshop

COURSE DIRECTORS: Arie Regev, MD
Meena B. Bansal, MD

This course is designed to assist trainees pursuing careers in clinical and academic hepatology. The speakers will cover topics such as how to find/choose a mentor, how to make the most out of the mentor-mentee relationship, the interview and contract negotiation process, and funding opportunities for fellows and junior faculty. In addition, participants will be offered the opportunity to network with leaders in the field.

Goals and Objectives:
- Introduce the principles behind starting a clinical research project while in practice.
- Assist trainees and young faculty in gaining a clearer understanding of the grant writing process and options available for funding research projects.
- Understand the dynamics of the mentor/mentee relationship and how to advance academically as a junior faculty member.
- Learn how to negotiate a top quality academic contract.

12:00 - 12:20 PM  The Past, Present, and Future of Hepatology: Where We Have Been, and Where We Are Going
Eugene R. Schiff, MD

12:20 - 12:40 PM  Basic Research for the Junior Investigator
Michael R. Charlton, MD

12:40 - 1:00 PM  How to Start a Clinical Research Project and Write an Effective Grant
Kris V. Kowdley, MD

1:00 - 1:20 PM  NIH Career Development Awards: Which Grant is Right for You?
Judith Podskalny, PhD

1:20 - 1:35 PM  Break

1:35 - 1:55 PM  Effective Mentorship: The Senior Faculty Perspective
Scott L. Friedman, MD

1:55 - 2:15 PM  How to Make the Most of Your Mentor Relationship: The Mentee Point of View
Charissa Chang, MD

2:15 - 2:35 PM  How to Negotiate a Great Academic Contract
Bruce A. Luxon, MD, PhD

2:35 - 2:55 PM  Academic Advancement: Advice for Junior Faculty
Jacquelyn J. Maher, MD

2:55 - 3:15 PM  Fourth Year Advanced Hepatology Fellowship: Who Needs It and Why?
Bruce R. Bacon, MD

3:15 - 3:30 PM  Closing Remarks
Arie Regev, MD

3:30 - 4:00 PM  Breakout Sessions: Ask the Experts

CD-1: NIDDK Career Development Programs
Room 209  Judith Podskalny, PhD

CD-2: Academic Advancement Pearls
Room 205  Jacquelyn J. Maher, MD

CD-3: Basic Research Pearls
Room 204  Michael R. Charlton, MD

CD-4: Fourth Year Fellowship/Certificate of Added Qualification
Room 210  Bruce R. Bacon, MD
Steven K. Herrine, MD
AASLD Postgraduate Course

Friday, November 2
4:00 - 8:00 PM  Hynes, Auditorium

Liver Disease: Pathophysiologic Basis for the Therapy of Liver Disease

COURSE DIRECTORS: Arthur J. McCullough, MD  Ian R. Wanless, MD  Michael B. Fallon, MD  Lisa M. Yerian, MD

11.5 CME Credits
10 Contact Hours

Over the last few years, advances in our understanding of the pathophysiology of liver disease have led to new therapeutic strategies. This two-day Postgraduate course will emphasize our newly gained understanding of the treatment of major liver diseases, with an emphasis on the cellular mechanisms.

The first day of the course will cover iron overload, cholestasis, polycystic liver disease, and hepatic steatosis, and recent developments in the causes and treatment of the above disorders. The second day of the course will focus on changes and challenges in viral hepatitis and vascular disorders of the liver, followed by evidence to surgical issues in portal vein thrombosis, particularly as it pertains to pediatric patients. These topics will be followed by a segment on the latest developments in hepatocellular carcinoma and the challenges that this disease presents to the pathologist, radiologist and clinician. The course will close with a segment on portal hypertension where the audience will be exposed to mechanisms and changes where portal hypertension has a great impact on the course of liver disease.

Throughout the course, important controversies in each specific area will be debated by thought leaders in each field, bringing our specific issues that will cross all specialties including radiology, surgery, hepatology and pathology.

Goals and Objectives:
• Discuss a broad overview of liver disease with an emphasis on histopathology and emerging pathophysiological mechanisms. An emphasis will also be placed on reviewing developments in our genetic understanding of liver disease and the emergence of new therapies, which may include new antivirals and small molecules, as well as new surgical approaches.
• List controversies in the management, diagnosis and care of important liver disorders. These debates will be presented by thought leaders in each field.
• Summarize an authoritative histopathological perspective on the developments in diagnosis and treatment of liver disorders that will be featured in this course. There will be a significant emphasis on pathology review and assessment of the histological basis of liver disease.
## AASLD Postgraduate Course

**Saturday, November 3**  
8:00 AM - 5:15 PM    Hynes, Auditorium

### Session III: Challenges in Viral Hepatitis

<table>
<thead>
<tr>
<th>Time</th>
<th>Topic</th>
<th>Presenter(s)</th>
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</thead>
<tbody>
<tr>
<td>8:00 - 9:00 AM</td>
<td>Assessment of Fibrosis: Liver Biopsy in HCV</td>
<td>Zachary D. Goodman, MD, PhD</td>
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<tr>
<td>9:00 - 9:20 AM</td>
<td>Viral Hepatitis in the HIV Infected Patient</td>
<td>Mark S. Sulkowski, MD</td>
</tr>
<tr>
<td>9:20 - 10:00 AM</td>
<td>Emerging Therapy in HCV</td>
<td>Stefan Zeuzem, MD</td>
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<tr>
<td>10:00 - 10:30 AM</td>
<td>Controversy: Maintenance Therapy - Pro</td>
<td>Mitchell L. Shiffman, MD</td>
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<tr>
<td>10:30 - 11:00 AM</td>
<td>Controversy: Maintenance Therapy - Con</td>
<td>Michael W. Fried, MD</td>
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<tr>
<td>11:00 AM - 12:00 PM</td>
<td>Meet-the-Professor Luncheons</td>
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### Session IV: Vascular Liver Diseases

<table>
<thead>
<tr>
<th>Time</th>
<th>Topic</th>
<th>Presenter(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10:10 - 10:30 AM</td>
<td>Vascular Mechanisms in Liver Disease</td>
<td>Ian R. Wanless, MD</td>
</tr>
<tr>
<td>10:30 - 11:00 AM</td>
<td>Clotting Disorders and the Liver</td>
<td>Dominique Valla, MD</td>
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<tr>
<td>11:00 - 11:30 AM</td>
<td>Sinusoidal Obstruction Syndrome</td>
<td>Laurie D. DeLeve, MD, PhD</td>
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<tr>
<td>11:30 AM - 12:00 PM</td>
<td>Surgical Approaches in Portal Vein Thrombosis</td>
<td>Peter F. Whittington, MD</td>
</tr>
<tr>
<td>12:00 AM - 1:30 PM</td>
<td>Bleeding Risk in Cirrhosis: What to Know and What to Do</td>
<td>Gail Macik, MD</td>
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### Session V: Cancer

<table>
<thead>
<tr>
<th>Time</th>
<th>Topic</th>
<th>Moderator(s)</th>
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<tbody>
<tr>
<td>1:30 - 1:50 PM</td>
<td>Dysplasia and Neoplasia in the Liver</td>
<td>Tanja Roskams, MD, PhD</td>
</tr>
<tr>
<td>1:50 - 2:10 PM</td>
<td>The Role of Radiology in Diagnosis of Liver Cancer</td>
<td>Michael P. Federle, MD</td>
</tr>
<tr>
<td>2:10 - 2:30 PM</td>
<td>Current Management of HCC: Going for a Cure</td>
<td>Joseph M. Llovet, MD</td>
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<tr>
<td>2:30 - 2:50 PM</td>
<td>Emerging Experimental Therapies for HCC: What if You Can’t Cure?</td>
<td>Lewis R. Roberts, MD, PhD</td>
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<tr>
<td>2:50 - 3:10 PM</td>
<td>Who and How Should a Hepatologist Screen for HCC?</td>
<td>Adrian M. De Bisciglia, MD</td>
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<tr>
<td>3:10 - 3:30 PM</td>
<td>Break</td>
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### Session VI: Portal Hypertension: The Liver and Beyond

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<thead>
<tr>
<th>Time</th>
<th>Topic</th>
<th>Moderator(s)</th>
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<tbody>
<tr>
<td>3:30 - 3:50 PM</td>
<td>Endothelial Dysfunction in Portal Hypertension</td>
<td>Vijay Shah, MD</td>
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<tr>
<td>3:50 - 4:10 PM</td>
<td>Hepatorenal Syndrome</td>
<td>Pere Gines, MD</td>
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<td>4:10 - 4:30 PM</td>
<td>Pulmonary Vascular Complications of Liver Disease</td>
<td>Michael B. Fallon, MD</td>
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<tr>
<td>4:30 - 4:50 PM</td>
<td>Portosystemic Encephalopathy</td>
<td>Dieter Haussinger, PhD</td>
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<tr>
<td>4:50 - 5:00 PM</td>
<td>Controversy: Are HPVG Measurements Warranted in the Management of PHTN? Pro</td>
<td>Guadalupe Garcia-Tsao, MD</td>
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<tr>
<td>5:00 - 5:10 PM</td>
<td>Controversy: Are HPVG Measurements Warranted in the Management of PHTN? Con</td>
<td>Thomas D. Boyer, MD</td>
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<tr>
<td>5:10 - 5:15 PM</td>
<td>Conclusion of Postgraduate Course: Summation and Highlights</td>
<td>Arthur J. McCullough, MD</td>
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</tbody>
</table>

### Poster Session 1

**Saturday, November 3, 2007**  
2:00 – 8:00 PM  
Hynes, Exhibit Hall C  
*Refer to page 95A for Poster Presentations*

### Exhibit Hall Opening and Reception

**Saturday, November 3, 2007**  
5:30 – 8:00 PM  
Hynes, Exhibit Hall D
### Postgraduate Course Meet-the-Professor Luncheons

**Saturday, November 3**  
12:00 - 1:30 PM  Refer to your luncheon ticket for meeting room location.

1.5 CME Credits

**Goals and Objectives:**
The goal of the Meet-the-Professor Luncheons is to provide the attendee with an opportunity to explore in more detail a wide variety of topics in hepatology in a small group setting. Topic moderators are experts in their particular specialty area for a given session.

| MTP-01 | What to Do with a High Ferritin | Paul C. Adams, MD and Kris V. Kowdley, MD |
| MTP-02 | What to Do with a Low Ceruloplasmin | Peter Ferenci, MD and Michael L. Schilsky, MD |
| MTP-03 | Pregnancy in Liver Disease | J. Eileen Hay, MD and E. Jenny Heathcote, MD |
| MTP-04 | Immune Mediated Disease | Eric M. Gershwin, MD and Dwain L. Thiele, MD |
| MTP-05 | Drug Induced Liver Injury | Neil Kaplowitz, MD and William M. Lee, MD |
| MTP-06 | Liver Histology for the Clinician | Elizabeth M. Brunt, MD and Andrew D. Clouston, PhD |
| MTP-07 | Cholestatic Disease in Children | William F. Balistreri, MD and Frederick J. Suchy, MD |
| MTP-08 | Current and New Therapies for Hepatitis C | Mark S. Sulkowski, MD and Stefan Zeuzem, MD |
| MTP-09 | Current and New Therapies for Hepatitis B | Anna S. F. Lok, MD and John G. McHutchison, MD |
| MTP-10 | Non-Invasive Markers of Fibrosis | Nezam H. Afdhal, MD and Don C. Rockey, MD |
| MTP-11 | Difficult to Treat Hepatitis C Patients | Gary L. Davis, MD and Andrew Muir, MD |
| MTP-12 | Treatment for Alcoholic Hepatitis | Craig J. McClain, MD and Timothy R. Morgan, MD |
| MTP-13 | What to do with a Liver Mass | Gregory J. Gores, MD and Jorge A. Marrero, MD |
| MTP-14 | Treatment of Gastric Varices | Stephen H. Caldwell, MD and Bimaljit S. Sandhu, MD |
| MTP-15 | Anti-Fibrotic Therapy | Scott L. Friedman, MD and Rebecca Wells, MD |
| MTP-16 | NASH: Pathogenesis and Treatment | Paul Angulo, MD and Christopher P. Day, MD, PhD |
| MTP-17 | Complementary and Alternative Medicine | Srinivasan Dasarathy, MD and Leonard B. Seeff, MD |
| MTP-18 | HCV/HIV Co-Infection | Douglas T. Dieterich, MD and Richard K. Sterling, MD |
| MTP-19 | Autoimmune Hepatitis | Steven L. Flamm, MD and Michael P. Manns, MD |
| MTP-20 | Fulminant Hepatic Failure | Robert O’Shea, MD and Robert J. Fontana, MD |
| MTP-21 | Immunotherapy in the Transplant Patient | Paul Martin, MD and John M. Vierling, MD |
| MTP-22 | Proteomics | Emanuel F. Petricoin, PhD and Zobair M. Younossi, MD |
| MTP-23 | Hepatic Encephalopathy | Kevin D. Mullen, MD and Rajiv Jalan, MD |
| MTP-24 | Liver Transplant for Viral Hepatitis | Mitchell L. Shiffman, MD and Nizar N. Zein, MD |
| MTP-25 | Venocclusive Disease and Budd-Chiari | Laurie D. Delave, MD, PhD and George B. McDonald, MD |
| MTP-26 | Therapy for Hepatocellular Carcinoma | Adrian M. Di Bisceglie, MD and J. Michael Henderson, MD |
| MTP-27 | Current Management of PBC and PSC | Claudia O. Zein, MD and Marlyn J. Mayo, MD |
| MTP-28 | Nuclear Receptors: Potential for Clinical Medicine | Saul J. Karpen, MD, PhD and Joel E. Lavine, MD, PhD |
| MTP-29 | Hepatorenal Syndrome | Guadalupe Garcia-Tsao, MD and Pere Gines, MD |
| MTP-30 | Pulmonary Disease in Cirrhotics | Michael B. Fallon, MD and Michael J. Krowka, MD |
| MTP-31 | Immunosuppression and Transplantation in Children | George V. Mazariegos, MD and Giorgina Mieli-Vergani, MD, PhD |
Networking Breakfast for Women in Hepatology

Sunday, November 4
6:30 – 7:45 AM  Sheraton, Back Bay B/C

Women working in the field of hepatology research and medicine are invited to participate in this complimentary breakfast meeting to discuss important issues, including attracting more women to GI and liver training, and improving the understanding of liver diseases that predominately affect women.

Early Morning Workshops

Sunday, November 4
6:45 - 7:45 AM  Refer to your luncheon ticket for meeting room location.

1 CME Credit

Early Morning Workshops consist of three categories: Basic Research, Clinical Management and NIH.

Goals and Objectives:

Basic Research Workshops

• Bring together investigators in a specific area of research to discuss their ongoing work.
• Focus of discussions is on new work and not a review of previous studies.
• Allow ample time for questions from the audience.

Clinical Management Workshops

• Discuss difficult management issues utilizing acknowledged experts in the area.
• Provide an overview of the current state-of-the-art in each area.
• Intended for the clinician who is seeking the most up-to-date information on a difficult area of management.
• Allow ample time for questions from the audience.

NIH Workshop

• Evidence for mutation, selection, immune escape, and drug resistance in HCV infection.
• Evidence for reversion when selection pressures are removed.
• The characteristics and relevance of consensus sequences.
• The concept of a latent reservoir, and its potential relevance to HCV antiviral drug resistance.

Basic Research Workshops

EM-01  Experimental Fibrosis
Frank A. Anania, MD and Scott L. Friedman, MD

EM-02  Animal Models of Fatty Liver
Richard M. Green, MD and Jacquelyn J. Maher, MD

Clinical Management Workshops

EM-03  Innate Immunity in Liver Disease
I. Nicholas Crispe, PhD and Dwain L. Thiele, MD

EM-04  Methionine and the S-adenosylmethionine System in Liver Disease
Shelly C. Lu, MD and Craig J. McClain, MD

EM-05  Pathogenesis of Alcoholic Liver Disease
Gavin E. Arteel, PhD and Laura E. Nagy, PhD

EM-06  Microarray Analysis
Jorge Bezerra, MD and Michael R. Charlton, MD

EM-07  Metabolomics
Anna Mae Diehl, MD and Satish C. Kalhan, MBBS, FRCP

NIH Workshop

EM-08  Emerging Therapies for HCV
Michael W. Fried, MD and Andrew Muir, MD

EM-09  Non-traditional Therapies for HCV
Stephen A. Harrison, MD and Nizar N. Zein, MD

EM-10  How to Manage the Post-Transplant Patient
Sandy Feng, MD, PhD and Hugo E. Vargas, MD

EM-11  Expanding the Criteria for Liver Transplantation
Charles M. Miller, MD and James F. Trotter, MD

EM-12  Treatment of PBC and PSC
Cynthia Levy, MD and Keith D. Lindor, MD

EM-13  Nutrition in Chronic Liver Disease in Children
Ross Shepherd, MD, and Ronald J. Sokol, MD

EM-14  Treatment of NASH
Naga P. Chalasani, MD and Hari S. Conjeevaram, MD

NIH Workshop

EM-15  HCV Plasticity: Escape and Resistance
Rajen Koshy, PhD, Stuart C. Ray, MD and Christopher Walker, PhD
NIH/NIDDK REQUEST FOR APPLICATION

Sunday, November 4
6:45 - 7:45 am  Location: TBA
Hepatitis B Clinical Research Network
National Institutes of Health-proposed RFA

Edward Doo, MD and Jay H. Hoofnagle, MD

Staff from NIDDK will present an overview and answer questions about the recently published Request for Applications (RFA) for participation in a multi-year, multi-center North American clinical research network devoted to elucidating the pathogenesis, natural history and optimal means of management of chronic hepatitis B. The RFA will solicit applications for clinical centers, laboratory research cores and a data coordinating center. Open to all interested persons on a walk-in basis. The RFA is available at: http://www2.niddk.nih.gov/Funding/FundingOpportunities/

This meeting is open to all interested investigators, registration is not required.

AASLD Basic Research Workshop

Sunday, November 4
8:00 - 11:40 AM  Hynes, Ballroom B
Regulatory T Cells in Tolerance and Immunity to the Liver

COURSE DIRECTORS:
Kyong-Mi Chang, MD
Ethan M. Shevach, MD

4 CME Credits

Regulatory T cells (Tregs) play a key regulatory role in immune tolerance to self and non-self. With the rapidly increasing knowledge about the nature and role of Tregs in health and disease (e.g. autoimmunity, transplant tolerance, tumor tolerance and pathogen-specific immunity), this workshop will provide the current knowledge about Tregs and their potential role in liver disease pathogenesis and therapeutics.

Goals and Objectives:
• Gain knowledge in the evolving concepts about regulatory T cells and their role in immune regulation in health and disease.
• Learn about the ongoing research on how regulatory T cells may contribute to pathogenesis of liver disease.
• Learn about potential therapeutic application involving regulatory T cells in liver disease.

8:00 - 8:25 AM  Introduction to Tregs
Ethan M. Shevach, MD

Session I. Tregs in Autoimmunity and Transplant Tolerance

8:25 - 8:50 AM  Transplant Tolerance and Regulatory T cells
Wayne Hancock, MD, PhD

8:50 - 9:15 AM  Autoimmune Liver Disease and Tregs
Eric M. Gershwin, MD

9:15 - 9:40 AM  Treg Therapeutic Applications and Manipulations
Carl June, MD

9:40 - 10:00 AM  Break

Session II. Tregs in Pathogen-Specific and Tumor-Specific Immunity

10:00 - 10:25 AM  Tregs in Viral Hepatitis
Kyong-Mi Chang, MD

10:25 - 10:50 AM  IL10+ Tr1 Cells in Viral Hepatitis
Margaret J. Koziel, MD

10:50 - 11:15 AM  Tregs in Liver Cancer
Drew M. Pardoll, MD, PhD

11:15 - 11:40 AM  Panel Discussion and Wrap-up
Hepatology Associates Course

**Sunday, November 4**
8:00 AM - 2:45 PM  Sheraton, Grand Ballroom

**Hepatology Associates Course**

**COURSE DIRECTORS:** Janet Durfee, MSN, APRN  
Karen Luken, MS, APRN

6.5 CME Credits
4.5 Contact Hours

This one-day course will expand the participant’s knowledge base for the treatment of patients with liver disease.

Goals and Objectives:
- Evaluate the case management of typical clinical scenarios in the long-term, post liver transplant patient.
- Compare and contrast the benefits and limitations of the MELD score.
- Describe the association between insulin resistance and liver disease.
- Explain how a hepatology practitioner can have a positive impact on patients with alcohol use disorders.
- Discuss the management of portal hypertension.

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<th>Time</th>
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<tr>
<td>8:00 - 8:10 AM</td>
<td><strong>Opening Remarks</strong></td>
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</table>
| 8:10 - 8:35 AM | **Clinical Management of Hemochromatosis and Wilson’s Disease**  
Sarah B. Hubbard, PA-C |
| 8:35 - 9:05 AM | **New Development in Diagnosis and Treatment of Alcohol Use Disorders**  
Mark Willenbring, MD |
| 9:05 - 9:15 AM | **Abstract Presentation** Award of Distinction  
Results of a Unique Hepatitis Outreach Program in Providing Screening, Education and Referral in a MidWest State  
Suzanne Opperman, RN, MSN |

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| 9:15 - 9:25 AM | **Abstract Presentation** Award of Distinction  
Identifying Learning Needs of Adult Liver Transplant Recipients  
Jaime Myers, RN |
| 9:25 - 9:40 AM | **Discussion**                                                               |
| 9:40 - 10:00 AM | **Break**                                                                    |

**Liver Potpourri**

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<th>Time</th>
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| 10:00 - 10:25 AM | **Portal Hypertension: Pathophysiology and Evidence Based Management**  
Hugo E. Vargas, MD |
| 10:25 - 10:50 AM | **MELD Score: Can We Do Better?**  
John R. Lake, MD |
| 10:50 - 11:15 AM | **Insulin Resistance and Liver Disease: What is the Connection?**  
Stephen A. Harrison, MD |
| 11:15 - 11:30 AM | **Discussion**                                                               |
| 11:30 AM - 12:45 PM | **Recognition Luncheon (open to Hepatology Associate Course registrants)**  
Sheraton Back Bay A-D |
| 12:45 AM - 1:00 PM | **Business Meeting**                                                          |

**Special Populations: Viral Hepatitis**

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<th>Time</th>
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| 1:00 - 1:25 PM | **Future Hepatitis C Antiviral Therapy**  
Marion G. Peters, MD |
| 1:25 - 1:50 PM | **Long Term Management of the Post Transplant Patient**  
Timothy M. McCashland, MD |
| 1:50 - 2:15 PM | **Overview of Hepatitis B**  
Richard H. Davis, PA-C |
| 2:15 - 2:40 PM | **Discussion**                                                               |
| 2:40 - 2:45 PM | **Summary and Closing Remarks**                                               |

**Poster Session 2**

**Sunday, November 4, 2007**
8:00 AM – 5:30 PM  
Hynes, Exhibit Hall C

Refer to page 129A for Poster Presentations

**Exhibit Hall Opening and Reception**

**Sunday, November 4, 2007**
9:30 AM – 3:00 PM  
Hynes Convention Center, Exhibit Hall D
Plenary Sessions

Transplant Plenary I

Sunday, November 4
8:00 - 9:35 AM  Hynes, Auditorium

MODERATORS:
Douglas W. Hanto, MD, PhD
Helen S. Te, MD

8:00 AM

#1
DELAYED AND REDUCED DOSE TACROLIMUS WITH MYCOPHENOLEATE MOFETIL AND DACLIZUMAB REDUCES RENAL IMPAIRMENT AFTER LIVER TRANSPLANT: RESULTS OF A 1 YEAR PROSPECTIVE, RANDOMISED INTERNATIONAL TRIAL

James M. Neuberger, A. David Mayer
Liver Unit, Queen Elizabeth Hospital, Birmingham, United Kingdom

8:15 AM

#2
MYCOPHENOLATE MOFETIL (MMF) IN COMBINATION WITH LOW-DOSES OF CALCINEURIN INHIBITORS (CNI) FOR CHRONIC RENAL DYSFUNCTION AFTER LIVER TRANSPLANTATION (LT) : 2-YEAR RESULTS OF A PROSPECTIVE, MULTICENTER, RANDOMIZED STUDY

Georges-Philippe Pageaux1, Lionel Rostaing2, Yvon Calmus3, Christophe Duvoux4, Claire Vanlemmens5, Jean Hardgwissen6, Paul-Henri Bernard7, Francis Navarro1, Dominique Larrey1
1Digestive Department, CHU St Eloi, Montpellier, France. 2Liver Transplant Unit, CHU Rangueil, Toulouse, France. 3Liver Transplant Unit, CHU Cochin, Paris, France. 4Liver Transplant Unit, CHU H. Mondor, Créteil, France. 5Liver Transplant Unit, CHU J. Minjoz, Besançon, France. 6Liver Transplant Unit, CHU La Conception, Marseille, France. 7Liver Transplant Unit, CHU Pellegrin, Bordeaux, France

8:30 AM

#3
HOSPITALIZATION RATES BEFORE AND AFTER ADULT-TO-ADULT LIVING DONOR OR DECEASED DONOR LIVER TRANSPLANTATION

Robert M. Merion1, Tempie H. Shearon2, Carl L. Berg3, James E. Everhart4, Michael M. Abecassiss5, Abraham Shaked6, Robert A. Fisher7, James F. Trotter8, Robert S. Brown9, Norah Terrault10, Paul H. Hayashi11, R M. Ghobrial12, A2ALL Study Group*
1Surgery, University of Michigan, Ann Arbor, MI, USA. 2Biostatistics, University of Michigan, Ann Arbor, MI, USA. 3Medicine, University of Virginia, Charlottesville, VA, USA. 4NIH/NIDDK, Bethesda, MD, USA. 5Surgery, Northwestern University, Chicago, IL, USA. 6Surgery, University of Pennsylvania, Philadelphia, PA, USA. 7Surgery, Medical College of Virginia/Virginia Commonwealth University, Richmond, VA, USA. 8Medicine, University of Colorado, Denver, CO, USA. 9Medicine, Columbia University, New York, NY, USA. 10Medicine, University of California - San Francisco, San Francisco, CA, USA. 11Medicine, University of North Carolina, Chapel Hill, NC, USA. 12Surgery, University of California - Los Angeles, Los Angeles, CA, USA

8:45 AM

#4
ACHIEVEMENT IN LIVER TRANSPLANTATION AWARD PRESENTATION

8:50 AM

#5
FACTORS INFLUENCING LIVER TRANSPLANT LENGTH OF STAY AND RESOURCE UTILIZATION AT TWO LARGE VOLUME TRANSPLANT CENTERS

Sandy Feng1, John P. Roberts1, Glenn A. Hall2, Kenneth Washburn2
1Surgery, UCSF, San Francisco, CA, USA. 2Transplant Center, Univ of Texas HSC, San Antonio, TX, USA

9:05 AM

#6
DEVELOPING A LIVER TRANSPLANTATION DONOR RISK INDEX IN A NATIONAL REGISTRY

Muhammad F. Dawwas1, Collett David2, Kerri M. Barber2, Christopher J. Watson1, James M. Neuberger1, Alexander E. Gimson1
1Hepatobiliary and Liver Transplant Unit, Addenbrooke’s Hospital, Cambridge University Hospitals NHS Foundation Trust, Cambridge, United Kingdom. 2Statistics and Audit Directorate, UK Transplant, Bristol, United Kingdom. 3Liver Unit, Queen Elizabeth Hospital, Birmingham, United Kingdom

9:20 AM

#6
REGIONAL DIFFERENCES IN DECEASED DONOR LIVER UTILIZATION AND ORGAN WASTAGE

Paul H. Hayashi1, Paolo R. Salvalaggio2, David A. Axelrad3, Schnitzler Mark4
1UNC Liver Program, University of North Carolina, Chapel Hill, NC, USA. 2Surgery Department, Saint Louis University, St. Louis, MO, USA. 3Transplantation Surgery, Dartmouth-Hitchcock Medical Center, Lebanon, NH, USA. 4Center for Outcomes Research, Saint Louis University, St. Louis, MO, USA
State-of-the-Art Lecture

Sunday, November 4
9:35 - 10:05 AM  Hynes, Auditorium

Thomas E. Starzl Transplant Surgery State-of-the-Art Lecture

On the Right Track?: Expanded Criteria in Decreased Donor Liver Transplantation

SPEAKER: Robert Merion, MD
MODERATOR: Douglas W. Hanto, MD, PhD

Dr. Robert M. Merion is Professor of Surgery in the Division of Transplantation at the University of Michigan. He is the Clinical Transplant Director of the Scientific Registry of Transplant Recipients and chairs the Steering Committee of two NIH-funded research consortia, the Adult-to-Adult Living Donor Liver Transplantation Cohort Study (A2ALL) and the Clinical Outcomes of Live Organ Donors network. Dr. Merion is the Secretary of the American Society of Transplant Surgeons, has served on the Board of Directors of the Organ Procurement and Transplantation Network, and is a past president of Gift of Life Michigan. Dr. Merion has published over 200 peer-reviewed articles, books, and book chapters.

This lecture will focus on the increasing use of donated livers from deceased donors with characteristics that are associated with a higher risk of graft failure and death. The development of measures of donor risk, along with information about transplant candidate and recipient mortality risk, allows us to examine the consequences of practice patterns in the choice of donor-recipient combinations.

Goals and Objectives

• Recognize the trends in deceased donor characteristics that have occurred over time.
• Know the donor factors associated with a higher risk of graft failure and death.
• Understand the donor risk index.
• Recognize the association between donor risk factors and recipient transplant survival benefit.
• Be able to make better informed decisions about deceased donor offers for their patients.

The Thomas E. Starzl Transplant Surgery State-of-the-Art Lecture recognizes the pioneering work that Dr. Thomas E. Starzl and colleagues have done to elevate liver transplantation from an experimental procedure to one which saves thousands of lives annually. To ensure that future transplant scientists have a distinct platform to provide their valuable insights at The Liver Meeting®, a restricted fund has been established to support this lecture in perpetuity. AASLD gratefully acknowledges the following individuals and organizations that have generously contributed to this fund:

Astellas Pharma US, Inc.
Clyde F. Barker, MD
Adel Bozorgzadeh, MD
Pradip Chakrabarti, MD
Chao-Long Chen, MD
David K. C. Cooper, MD
Bijan Eghtesad, MD
Ira J. Fox, MD
John J. Fung, MD, PhD
Ashokkumar B. Jain, MD
Zakiyah Kadry, MD
Dympna Kelly, MD
Charles M. Miller, MD
Ernesto P. Molmenti, MD
Michael C. Morris, MD
Eduardo A. Santiago-Delpin, MD
Byers W. Shaw, Jr., MD
Cynthia A. Smetanka, MD
Lewis Teperman, MD
Paul Terasaki, MD

Break
10:05 – 10:30 AM
Plenary Sessions

Transplant Plenary II

Sunday, November 4
10:30 AM - 12:00 PM  Hynes, Auditorium

MODERATORS:
Thomas G. Heffron, MD
Consuelo Soldevila-Pico, MD

10:30 AM
#7
HEPATITIS C (HCV)- 3 STUDY: DOES IMMUNOSUPPRESSION (IS) AFFECT THE PROGRESSION OF FIBROSIS OF HCV RECURRENCE AFTER LIVER TRANSPLANTATION (OLT)?

Goran B. Klintmalm1, Carlos G. Fasola2, Linda Jennings1, Thomas G. Heffron2, Linda S. Sher3, David C. Mulligan4, Robert S. Brown5, John Ham6, Lewis Teperman7, Stephen Rudich8, Devin E. Eckhoff9, Kenneth Washburn10, Michael Mills11, John P. Roberts12, Michael R. Charlton13, Prabhakar Baliga14, Timothy L. Prueti15, Elizabeth Pomfret16, Baburao Koneru17, Michael M. Abecassis18

1Transplantation Services, Baylor University Medical Center, Dallas, TX, USA. 2Surgery, Emory University, Atlanta, GA, USA. 3Transplantation Services, University of Southern California, Los Angeles, CA, USA. 4Transplantation Services, Mayo Clinic Hospital Scottsdale, Scottsdale, AZ, USA. 5Transplantation Services, New York Presbyterian Hospital, New York, NY, USA. 6Transplantation Services, Oregon Health Sciences University, Portland, OR, USA. 7Transplantation Services, New York University, New York, NY, USA. 8Transplantation Services, University of Cincinnati, Cincinnati, OH, USA. 9Transplantation Services, University of Alabama, Birmingham, AL, USA. 10Transplantation Services, University of Texas Health Science Center, San Antonio, TX, USA. 11Transplantation Services, University of Chicago, Chicago, IL, USA. 12Transplantation Services, University of California, San Francisco, CA, USA. 13Transplantation Services, Mayo Clinic Hospital, Rochester, MN, USA. 14Transplantation Services, Medical University of South Carolina, Charleston, SC, USA. 15Transplantation Services, University of Virginia, Richmond, VA, USA. 16Transplantation Services, Lahey Clinic, Burlington, MA, USA. 17Transplantation Services, University of Medicine and Dentistry of New Jersey, Trenton, NJ, USA. 18Transplantation Services, Northwestern University, Chicago, IL, USA

11:00 AM
#9
ERYTHROPOIETIN IMPROVES LIVER REGENERATION OF THE DONOR AND RECIPIENT IN A RAT MODEL OF LIVING RELATED LIVER TRANSPLANTATION

Maximilian Bockhorn1, Christian Fingas1, Ursula Rauen2, Andreja Frilling1, Christoph E. Broelsch1, Joerg F. Schlaak3

1General and Transplantation Surgery, University Hospital, Essen, Germany. 2Physiological Transplantation Surgery, University Hospital, Essen, Germany. 3Gastroenterology and Hepatology, University Hospital, Essen, Germany

11:15 AM
#10
OUTCOMES AFTER HEART TRANSPLANTATION AMONG RECIPIENTS WITH CHRONIC HEPATITIS C INFECTION

Tse-Ling Fong1, James Cicciarelli2, Yong-Won Cho2

1University of Southern California, Los Angeles, CA, USA. 2National Institute of Transplantation, Los Angeles, CA, USA

11:30 AM
#11
A RANDOMIZED STUDY TO ASSESS THE SAFETY AND EFFICACY OF ADEFOVIR DIPIVOXIL SUBSTITUTION FOR HEPATITIS B IMMUNE GLOBULIN IN LIVER TRANSPLANTATION PATIENTS RECEIVING LONG-TERM LOW DOSE IMHBIG AND LAMIVUDINE PROPHYLAXIS

Peter W. Angus1, Simone I. Strasser2, Scott Patterson1, Geoff W. McCaughan3, Ed Gane3

1Liver Transplant Unit, Austin Health, Melbourne, VIC, Australia. 2Gilead Sciences, Foster City, CA, USA. 3NZ Liver Transplant Unit, Auckland Hospital, Auckland, New Zealand

11:45 AM
#12
DECLINE IN THE NEED FOR LIVER TRANSPLANTATION FOR END STAGE LIVER DISEASE SECONDARY TO HEPATITIS B IN THE US

W. Ray Kim1, Joanne T. Benson1, Andrew Hindman2, Carol Bros- gart3, Carolyn Fortner-Burton1

1Mayo Clinic College of Medicine, Rochester, MN, USA. 2Gilead Sciences, Foster City, CA, USA. 3United Network for Organ Sharing, Richmond, VA, USA
State-of-the-Art Lecture

**Sunday, November 4**
12:00 - 12:30 PM Hynes, Auditorium

**Hans Popper Basic Science State-of-the-Art Lecture**

**The Mitochondrial Pathway of Apoptosis**

**SPEAKER:** Douglas R. Green, PhD  
**MODERATOR:** Gregory J. Gores, MD

Dr. Douglas Green received his PhD in Biology and Immunology from Yale University in 1981. Following postdoctoral training in experimental surgery and marine biology, he received a faculty position at the University of Alberta in Edmonton in 1985. In 1990 he became the Head of the Division of Cellular Immunology at La Jolla Institute for Allergy and Immunology in San Diego, CA. Since 2005 he has been Chair of Immunology at St. Jude Children’s Research Hospital in Memphis, TN, where he holds the Peter Doherty Chair. Doug Green is an ISI Highly Cited Investigator and has published over 350 research papers, reviews, chapters, and books. His research focuses on the central mechanisms of apoptosis in cancer and the immune system.

The mitochondrial pathway of apoptosis is the major way in which vertebrate cells die during development, homeostasis, and aging, and upon physiological or pathological stress. We will consider the distribution of this pathway for cell death among the animals, which has implications for the evolution of apoptosis. Further, we will review the elements of this pathway and discuss the fundamental ways in which it is regulated. In this pathway, the proteins of the Bcl-2 family are responsible for controlling a critical event, mitochondrial outer membrane permeabilization (MOMP), which is responsible for triggering caspase activation and apoptosis. The interplay of the Bcl-2 family proteins is an essential aspect of MOMP.

Once MOMP has occurred, two things happen that ensure the death of the cell. One of these is caspase activation. The other is less clear, leading to cell death that occurs even if caspase activation is disrupted or blocked. The latter, caspase-independent cell death, may be less efficient than caspase-dependent apoptosis, and considering how cells may survive this form of cell death opens up intriguing new areas of investigation.

The Hans Popper Basic Science State-of-the-Art lecture recognizes Hans Popper, the founder of AASLD, and his role in the establishment of the AASLD journal, *HEPATOLOGY*, and his promotion of the intellectual spirit of the Association.

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50 Years of Interferons

**Sunday, November 4**
1:00 - 2:00 PM Hynes, Auditorium

**50 Years of Interferons**

**MODERATORS:** John G. McHutchison, MD  
Eugene R. Schiff, MD

The calendar year 2007 marks the 50th Anniversary for the discovery of Interferons. To date, no other scientific discovery has had such an impact on the care of patients with viral hepatitis. The AASLD will acknowledge and celebrate this anniversary with this 1 hour symposium.

**1:00 - 1:30 PM** Historical Overview on the Discovery of Interferons  
Adrian Reuben, MBBS, FRCP

**1:30 - 2:00 PM** Historical and Ongoing Therapeutic Application of Interferons in Viral Liver Diseases  
Jay H. Hoofnagle, MD
AASLD/NIAID NIH Corner

**Sunday, November 4**

1:00 - 4:00 PM  Hynes, Ballroom B  
Antiviral Therapy Against Hepatitis Viruses: Understanding and Managing Drug Resistance

**COURSE DIRECTORS:**  Anna S. F. Lok, MD  
Rajen Koshy, PhD

This symposium, present in collaboration with the American Association for the Study of Liver Diseases (AASLD) and the U.S. National Institute of Allergy and Infectious Diseases (NIAID), an Institute of the National Institutes of Health (NIH), will address issues important to physicians/health care providers who treat patients with chronic hepatitis, and investigators conducting research on antiviral resistance. A panel of experts (hepatologists, infectious disease specialists, and virologists) will discuss basic and clinical issues including standardization of nomenclature and assays relating to antiviral resistance, detection, and monitoring of antiviral resistance, and management of patients with antiviral-resistant HBV. The panel will also identify research gaps and provide directions for future research.

**Goals and Objectives:**

- Clarify the nomenclature of antiviral-resistant HBV, clinical criteria for diagnosis of antiviral resistance, and assays used to detect antiviral-resistant mutations.
- Discuss the monitoring of HBV infected patients receiving antiviral therapy for antiviral resistance, and the management of patients with antiviral-resistant HBV.
- Discuss these issues in the context of new drugs for the treatment of hepatitis C virus infection.
- Provide an interactive forum for researchers and NIH to discuss future research initiatives and international collaboration.

**Session I**

**CHAIRS:**  Rajen Koshy, PhD  
Jean-Michel Pawlotsky, MD, PhD

1:00 - 1:15 PM  NIAID Goals in Viral Hepatitis Research  
Rajen Koshy, PhD

1:15 - 1:30 PM  NIDDK Funding of Viral Hepatitis Research  
Edward Doo, MD

1:30 - 1:45 PM  Standardized Nomenclature and Management of Antiviral-resistant HBV  
Marc Ghany, MD

1:45 - 2:00 PM  Genotypic and Phenotypic Assays to Detect Antiviral-resistant Mutations  
Fabien Zoulim, MD

2:00 - 2:15 PM  Break

**Session II**

**CHAIRS:**  T. Jake Liang, MD  
Masashi Mizokami, MD, PhD

2:15 - 2:30 PM  Antiviral Resistance to New HCV Treatments  
John G. McHutchison, MD

2:30 - 2:50 PM  Panel Discussion: The Management of Patients with Antiviral Resistance

2:50 - 3:05 PM  Database for Antiviral Resistance Monitoring  
Stephen Locarnini, MD, PhD

3:05 - 3:25 PM  NIH HCV and HIV Database  
Carla Kuiken, PhD

3:25 - 4:00 PM  Panel Discussion: The Monitoring of Antiviral Resistance; Databases; Collaborations; Funding  
Jean-Michel Pawlotsky, Jake Liang, Jay Hoofnagle, Fabien Zoulim, Masashi Mizokami, Carla Kuiken, John McHutchison, Yun-Fan Liaw, Rajen Koshy

SHARP Lecture

**Sunday, November 4**

2:00 - 2:30 PM  Hynes, Auditorium  
Therapeutic Advances in Hepatocellular Carcinoma (HCC): Sorafenib

**MODERATOR:**  Gregory J. Gores, MD

2007 is a landmark year for treatment of HCC. The Sorafenib HCC Assessment Randomized Protocol (SHARP) demonstrated a significant survival advantage for patients with HCC. This update will provide an overview of the trial results, and its pragmatic implications for the treatment of HCC.
General Hepatology Update

Sunday, November 4
3:00 - 4:30 PM  Hynes, Auditorium
General Hepatology Update

MODERATORS: Rebecca Wells, MD
Sammy Saab, MD

This symposium will make practitioners who treat patients with liver disease aware of the impact of insulin resistance on the accumulation of hepatic fat and its potential outcome in fatty liver disease and treatment of chronic hepatitis C (HCV) infection. The symposium will also review new nucleoside analogues for chronic hepatitis B (HBV) infection and proper strategies for their use. Finally, issues unique to female patients with liver disease, including pregnancy and menopause, and how gender affects the outcomes of chronic liver disease, will be reviewed to update practitioners on the care of this population.

Goals and Objectives:
• Understand the role of insulin resistance as a systemic disease including the liver.
• Understand the new strategies in treating hepatitis B.
• Understand the unique issues in caring for the female patients with liver disease.

3:00 - 3:30 PM  Insulin Resistance And Liver Disease: More Than Just The Fatty Liver?
Frank A. Anania, MD

3:30 - 4:00 PM  A Rationale Roadmap In Using The Newer Nucleoside Analogues For Chronic Hepatitis B
Emmet B. Keeffe, MD

4:00 - 4:30 PM  Unique Aspects of Liver Disease Management in Women
Norah Terrault, MD
Parallel Sessions

HCV: Pathogenesis

Sunday, November 4
3:00 - 4:30 PM  Hynes, Room 312

MODERATORS:
Barbara Rehermann, MD
Angela Dolganiuc, MD, PhD

3:00 PM
#13
MECHANISMS OF IMMUNE EVASION BY TRANSITION VARIANTS OF HEPATITIS C VIRUS
Jane H. Wang1,2, Matthew J. Pianko1, Xiaogang Ke2, Scott Collier2, Peter F. Whittington1
1Pediatrics, Division of GI, Hepatology & Nutrition, Northwestern University, Chicago, IL, USA. 2Medicine, Section of Hepatology, University of Illinois at Chicago, Chicago, IL, USA

3:15 PM
#14
ENDOPLASMIC RETICULUM (ER) STRESS ASSOCIATED WITH DOWNREGULATION OF THE UNFOLDED PROTEIN RESPONSE (UPR) IN LIVERS OF PATIENTS WITH CHRONIC HEPATITIS C
Tarik Asselah1, Ivan Bièche2, Ingrid Laurendeau2, Dominique Cazals-Hatem3, Gérard Feldmann1, Pierre Bedossa3, Valérie Paradis3, Didier Lebrec1, Eric Ogier-Denis1, Abdel Mansouri1, Michel Vidaud2, Patrick Marcellin1, Richard Moreau1
1INSERM U773 and Liver Unit, Hôpital Beaujon, Clichy, France. 2INSERM U745, Paris, France. 3Service d’Anatomie-Pathologie, Hôpital Beaujon, Clichy, France

3:30 PM
#15
PP2A OVEREXPRESSION IN CHRONIC HEPATITIS C INHIBITS INSULIN SIGNALING IN THE LIVER
Christine Bernsmeier1, François Duong1, Verena Christen1, Luigi Terracciano2, Markus Heim1
1Gastroenterology and Hepatology, University Hospital Basel, Basel, Switzerland. 2Institute of Pathology, University Hospital, Basel, Switzerland

3:45 PM
#16
IFN-α AND TGF-BETA MODULATE THE CELLULAR UPTAKE OF HEPATITIS C VIRUS
Anna Tietjens1, Philip Hilgard1, Jean Dubuisson2, Guido Gerken1, Joerg F. Schlaak1
1Dept. for Gastroenterology and Hepatology, University-Hospital Essen, Essen, Germany. 2CNRS-UMR8161, Institut de Biologie de Lille, Institut Pasteur de Lille, Lille, France

4:00 PM
#17
DEFECTIVE T HELPER 1 RESPONSE BY HEPATOCYTE-STIMULATED CD4 T CELLS IMPAIRS ANTI-VIRAL CD8 RESPONSE AND VIRAL CLEARANCE
Johannes Herkel1, Christiane Wiegard1, Petra Wolint2, Christian Frenzel1, Edgar Schmitt2, Annette Oxenius2, Ansgar W. Lohse1
1Department of Medicine I, University Medical Centre Hamburg-Eppendorf, Hamburg, Germany. 2Institute of Microbiology, Swiss Federal Institute of Technology, Zurich, Switzerland. 3Institute of Immunology, Johannes Gutenberg-University, Mainz, Germany

4:15 PM
#18
HCV-SPECIFIC CELLULAR IMMUNE RESPONSES IN INTRAVENOUS DRUG USERS WITHOUT HCV INFECTION
Prem H. Thurairajah1, Doha M. Hegazy1, Sarah J. Richardson1, Andy G. Demaine1, Edward R. Kaminski2, Matthew E. Cramp1,3
1Institute of Biomedical and Clinical Sciences, Peninsula Medical School, Plymouth, United Kingdom. 2Department of Immunology, Derriford Hospital, Plymouth, United Kingdom. 3Department of Hepatology, Derriford Hospital, Plymouth, United Kingdom

Hepatobiliary Neoplasia: Clinical and Translational Aspects

Sunday, November 4
3:00 - 4:30 PM  Hynes, Room 302

MODERATORS:
Josep M. Llovet, MD
Jorge A. Marrero, MD

3:00 PM
#19
PREDICTORS OF DROPOUT FROM TRANSPLANT WAITING LIST AMONG PATIENTS LISTED FOR HEPATOCELLULAR CARCINOMA USING THE UNOS/OPTN DATABASE
Carlos J. Romero-Marrero1, Sherry Fu2, Veena Thyagarajan2, Robert J. Fontana2, Anna S. Lok2, Shawn Pelletier3, Jorge A. Marrero1
1Internal Medicine, Division of Gastroenterology, University of Puerto Rico, San Juan, PR, USA. 2Internal Medicine, Division of Gastroenterology, University of Michigan, Ann Arbor, MI, USA. 3Surgery, Division of Transplantation, University of Michigan, Ann Arbor, MI, USA
3:15 PM

#20
PROSPECTIVE VALIDATION OF AASLD GUIDELINES FOR THE EARLY DIAGNOSIS OF HEPATOCELLULAR CARCINOMA IN CIRRHTIC PATIENTS

Angelo Sangiovanni1, Matteo A. Manini1, Raffaella Romeo1, Massimo Iavarone1, Mirella Fraquelli2, Laura V. Forzenigo3, Guido Ronchi1, Massimo Colombo1

1A.M. & A. Migliavacca, First Division of Gastroenterology, Fondazione IRCCS Maggiore Hospital Polyclinic, Mangiagalli & Regina Elena, University of Milan, Milan, Italy. 2Second Division of Gastroenterology, Fondazione IRCCS Maggiore Hospital Polyclinic, Mangiagalli & Regina Elena, University of Milan, Milan, Italy. 3Division of Radiology, Fondazione IRCCS Maggiore Hospital Polyclinic, Mangiagalli & Regina Elena, University of Milan, Milan, Italy.

3:30 PM

#21
ARE HILAR AND INTRAHEPATIC CHOLANGIOCARCINOMAS DIFFERENT ENTITIES?

Nathalie Guedj1,2, Perigny Martine1, Françoise Degos3, Qian Zhan4, Dominique Valla5, Jacques Belghiti6, Olivier Farges7, Pierre Bedossa8, Valérie Paradis1,2

1pathology, Beaujon hospital, Clichy, France. 2liver physiopathology, CNRS, Paris, France. 3hepatology, Beaujon hospital, clichy, France. 4surgery, Beaujon hospital, clichy, France

3:45 PM

#22
ONCOGENIC PATHWAYS AND NEW THERAPEUTIC TARGETS ARE SPECIFICALLY ACTIVATED IN THE DIFFERENT HEPATOCELLULAR ADENOMA SUBTYPES OF TUMORS

Sandra Reboissoud1, Sandrine Imbeaud2, Cristel Thomas1, Charles P. Balabaud3, Paulette Bioulac-Sage4, Jessica Zucman-Rossi1

1U674, Inserm, Paris, France. 2Array s/IMAGE, Genexpress, CNRS, Villejuif, France. 3Hôpital Saint André, CHU Bordeaux, Bordeaux, France. 4hospital Pellegrin, CHU Bordeaux, Bordeaux, France.

4:00 PM

#23
CLINICAL SAFETY AND BIOACTIVITY OF OK432-STIMULATED DENDRITIC CELL TRANSFER INTO HEPATOCELLULAR CARCINOMA FOLLOWING TRANSCATHETER HEPATIC ARTERIAL EMBOLIZATION

Yasunari Nakamoto1, Eishiro Mizukoshi1, Masaaki Kitahara1, Yoshio Sakai1, Kaiehta Kakinoki1, Kunici Ari1, Tatsuya Yamashita1, Naofumi Mukaida1, Kouji Matsushima2, Osamu Matsui1, Shuichi Kaneko1

1Kanazawa University, Kanazawa, Japan. 2University of Tokyo, Tokyo, Japan

4:15 PM

#24
DIFFUSION-WEIGHTED MRI FOR DETECTION OF HEPATOCELLULAR CARCINOMA IN PATIENTS WITH HEPATIC CIRRHOSIS: A CORRELATION TO HISTOLOGY AND IMAGING FOLLOW-UP

Chris Verslype1, Frederik De Keyzer2, Louis Libbrecht2, Tania Roskams3, Ilse Roeben2, Steven Dymarkowski2, Jacques Firenne4, Frederik Nevens1, Vincent Vandecaveye2

1Hepatology, University Hospital Leuven, Leuven, Belgium. 2Radiology, University Hospital Leuven, Leuven, Belgium. 3Pathology, University Hospital Leuven, Leuven, Belgium. 4Abdominal Transplant Surgery, University Hospital Leuven, Leuven, Belgium

Liver Transplantation
Sunday, November 4
3:00 - 4:30 PM Hynes, Ballroom A

MODERATORS:
Marina Berenguer, MD
Kirti Shetty, MD

3:00 PM

#25
PROPHYLACTIC PEGINTERFERON ALFA-2A/RIBAVIRIN VS NO PROPHYLAXIS FOLLOWING ORTHOTOPIC LIVER TRANSPLANTATION (OLT) FOR HEPATITIS C: 24-WEEK VIROLOGIC AND SAFETY RESPONSES

Michael R. Charlton1, Natalie Bzowej2, Stephen Rossi3, David R. Nelson4

1Mayo Clinic College of Medicine, Rochester, MN, USA. 2California Pacific Medical Center, San Francisco, CA, USA. 3Roche Laboratories, Nutley, NJ, USA. 4University of Florida, Gainesville, FL, USA

3:30 PM

#26
HEALTH RELATED QUALITY OF LIFE (HRQOL) IN PEDIATRIC LIVER TRANSPLANT RECIPIENTS-PRELIMINARY RESULTS OF THE STUDIES OF PEDIATRIC LIVER TRANSPLANTATION (SPLIT) FUNCTIONAL OUTCOMES GROUP (FOG)

Estella M. Alonso1, Katie Neighbors1, Christine Limbers6, Karen Martz2, Thomas Webb3, John C. Bucvalas4, James W. Varni2, Group FOG Research1

1Pediatrics, Childrens Memorial Hospital, Chicago, IL, USA. 2Pediatrics & Architecture, Texas A&M University, College Station, TX. 3The EMMES Corporation, Rockville, MD, USA. 4University of Cincinnati, Cincinnati, OH, USA. 5Pediatric Liver Care Center, Cincinnati Childrens Hospital, Cincinnati, OH, USA. 6Psychology, Texas A&M University, College Station, TX, USA

4:00 PM

#27
USAGE OF DONORS WITH STEATOSIS AFTER AN INTENSIVE DIETARY TREATMENT IN LIVING DONOR LIVER TRANSPLANTATION IN ADULTS

Riccardo Volpes1,2, Lisa Randisi1,2, Salvatore Gruttadauria1,2, Marcello Castelllee1,2, Maria Rubino1,2, Marida Minervini1,2, Giovanni Vizzini1,2, Bruno Gridelli1,2

1ISMETT, Palermo, Italy. 2UPMC, Pittsburgh, PA, USA
3:45 PM
#28
SURVIVAL AND RISK OF HEPATITIS B VIRUS (HBV) RECURRENCE IN HIV-HBV COINFECTED LIVER TRANSPLANT RECIPIENTS: PRELIMINARY FINDINGS FROM THE HIV-TR STUDY

Carla S. Coffin¹, Carl L. Berg³, Lorna M. Dove⁴, Fred Poordad⁵, Michael P. Curry⁶, Fredric G. Regenstein⁷, Kenneth E. Sherman⁸, Michelle E. Roland¹, Peter G. Stock², Norah Terrault¹,²

¹Medicine, University of California San Francisco, San Francisco, CA, USA. ²Surgery, University of California San Francisco, San Francisco, CA, USA. ³Medicine, University of Virginia, Charlottesville, VA, USA. ⁴Medicine, Columbia University, New York, NY, USA. ⁵Medicine, Cedars-Sinai, Los Angeles, CA, USA. ⁶Medicine, Harvard School of Medicine, Boston, MA, USA. ⁷Medicine, Tulane University, New Orleans, LA, USA. ⁸Medicine, University of Cincinnati, Cincinnati, OH, USA

4:00 PM
#29
IMPACT OF HIV ON SURVIVAL AFTER LIVER TRANSPLANTATION. ANALYSIS OF UNITED NETWORK FOR ORGAN SHARING (UNOS) DATABASE

Ayse L. Mindikoglu¹, Arie Regev², Laurence S. Magder³

¹Department of Medicine, Division of Gastroenterology and Hepatology, University of Maryland School of Medicine, Baltimore, MD, USA. ²Global Product Safety, Eli-Lilly and Company, Indianapolis, IN, USA. ³Department of Epidemiology and Preventive Medicine, Division of Biostatistics and Bioinformatics, University of Maryland School of Medicine, Baltimore, MD, USA

4:15 PM
#30
LONG TIME FOLLOW UP FOR THE PATIENT OF AUTOLOGOUS BONE MARROW CELL INFUSION (ABMI) THERAPY FOR LIVER CIRRHOSIS

Shuji Terai, Makoto Segawa, Kaoru Omori, Takuya Iwamoto, Yoko Mizunaga, Toshihiko Matsumoto, Yohei Urita, Yoshio Marumoto, Tsuyoshi Ishikawa, Naoki Yamamoto, Koichi Uchida, Takahiro Yamasaki, Isao Sakaida

Department of Gastroenterology & Hepatology, Yamaguchi University Graduate School of Medicine, Ube, Yamaguchi, Japan

3:00 PM
#31
METRON FACTOR-1, AN HGF-MSP CHIMERA, PREVENTS LIVER INJURY WITHOUT PROMOTING TUMOR GROWTH AND METASTASIS

Terumi Takahara¹, Feng Xue¹,², Yutaka Yata¹, Kazunobu Nonome¹, Masami Kanayama¹, Kengo Kawai¹, Toshiro Sugiyama¹, Paolo Michieli³

¹Third Department of Internal Medicine, University of Toyama, Toyama City, Japan. ²Organ Transplantation Center, Renji Hospital Affiliated to Shanghai Jiao-tong University, Shanghai, China. ³Institute for Cancer Research and Treatment, University of Torino Medical School, Turin, Italy

3:15 PM
#32
DEGRADATION OF THE EGF RECEPTOR IN HEPATOCYTES IS MEDIATED BY A DYNAMIN-ASSOCIATED UBIQUITIN-BASED ENDOCYTIC MACHINERY

Barbara Schroeder¹, Jing Chen¹, Mark A. McNiven¹,²

¹Division of Gastroenterology and Hepatology, Mayo Clinic, Rochester, MN, USA. ²Biochemistry and Molecular Biology, Mayo Clinic, Rochester, MN, USA

3:30 PM
#33
PREVENTION AND TREATMENT OF ACUTE LIVER INJURY IN ANIMAL MODELS BY ENDOTHELIAL PROGENITOR CELLS

Verónica Fernández-Ruiz, Milosz P. Kawa, Maria Iñiguez, Jesús Prieto, Cheng Qian

Hepatology and Gene Therapy, Center for Applied Medical Research-University of Navarra, Pamplona, Spain

3:45 PM
#34
DYNAMIC MICROTUBULES ARE REQUIRED FOR CONSTITUTIVE CANALICULAR TRAFFICKING OF BSEP (ABCB11) IN POLARIZED WIF-B9 CELLS AND PROVIDE THE “MISSING LINK” TO THE CANALICULAR ACTIN NETWORK

Yoshiyuki Wakabayashi, Jennifer Lippincott-Schwartz, Irwin M. Arias

CBMB, NICHD, NIH, Bethesda, MD, USA
4:00 PM  
#35  
ENDOCYTIC INTERNALIZATION OF THE TRANSFERRIN RECEPTOR 1 (TFR1) IN HEPATOCYTES IS MEDIATED BY ACTIVATION OF SRC  
Hong Cao¹, Jing Chen¹, Mark A. McNiven¹,²  
¹Division of Gastroenterology and Hepatology, Mayo Clinic, Rochester, MN, USA. ²Biochemistry and Molecular Biology, Mayo Clinic, Rochester, MN, USA

4:15 PM  
#36  
EPIGENETIC GENE EXPRESSION PROFILING IDENTIFIED HGF ACTIVATOR INHIBITOR 2 (HAI-2) AS A FREQUENTLY SILENCED CANDIDATE TUMOR SUPPRESSOR GENE IN HUMAN HEPATOCELLULAR CARCINOMA  
Edmund K. Tung, Chun M. Wong, Irene O. Ng  
Pathology, The University of Hong Kong, Hong Kong, China

Portal Hypertension: Clinical  
Sunday, November 4  
3:00 - 4:30 PM  
Hynes, Room 304/306  
MODERATORS:  
Guadalupe Garcia-Tsao, MD  
Pere Gines, MD

3:00 PM  
#37  
PENTOXIFYLLINE FOR THE TREATMENT OF PATIENTS WITH ADVANCED CIRRHOSIS. A RANDOMIZED, PLACEBO-CONTROLLED, DOUBLE-BLIND TRIAL  
Didier Lebrec¹, Dominique Thabut², Frederic Oberti³, Jean-Marc Perarnau⁴, Bertrand Condat⁵, Helene Barraud⁶, Faouzi Saliba⁷, Nicolas Carbonell⁸, Philippe Renard⁹, Marie-José Ramond¹, Richard Moreau¹, Thierry Poynard²  
¹Hepatology, INSERM, Clichy, France. ²Service d’hepatogastroenterologie, Groupe hospitalier Pitié Salpêtrière, Paris, France. ³Hepatology, CHU, Angers, France. ⁴Hepatology, CHU, Tours, France. ⁵Hepatology, Hospital sainte Camille, Brie sur Marne, France. ⁶Hepatology, CHU, Nancy, France. ⁷Hepatology, Hopital Paul Brousse, Villejuif, France. ⁸Hepatology, Hopital Saint Antoine, Paris, France. ⁹Hepatology, Hopital d’Argenteuil, Argenteuil, France

3:15 PM  
#38  
GLOMERULAR FILTRATION RATE AS AN IMPROVED PROGNOSTIC INDICATOR IN PATIENTS WITH END STAGE LIVER DISEASE  
Young Suk Lim, Joanne T. Benson, Walter Kremers, Patrick S. Kamath, Terry M. Therneau, W. Ray Kim  
Mayo Clinic College of Medicine, Rochester, MN, USA

3:30 PM  
#39  
ENDOSCOPIC VARICEAL LIGATION (EVL) PLUS PROPRANOLOL (P) AND ISOSORBIDE MONONITRATE (ISMN) VERSUS EVL ALONE IN SECONDARY PROPHYLAXIS OF VARICEAL BLEEDING: A PROSPECTIVE RCT  
Sanjeev K. Jha, Ashish Kumar, Barjesh C. Sharma, Shiv Kumar Sarin  
Gastroenterology, G B Pant Hospital, New Delhi, India

3:45 PM  
#40  
PRESENCE OF BACTERIAL DNA IS A NEW SURVIVAL INDICATOR IN PATIENTS WITH CIRRHOSIS AND NON-INFECTED ASCITIC FLUID  
Pedro Zapater¹, Rubén Francés¹, José M. Gonzalez-Navajas¹, Rocio Moreu¹, Lucia Llanos¹, Sonia Pascual¹,³, David Monfort², Silvia Montoliu³, Carmen Vila³, A. Escudero³, X. Torras³, Isabel Carrera³, Carlos Guarner Jr.², José M. Palazón¹,³, Fernando Carrión¹,³, Carlos Guarner¹,³, Ramon Planas¹,³, Ricard Solà⁴, Miguel A. Serra⁴, Carlos Muñoz¹, Miguel Pérez-Mateo¹, Jose Such¹,³  
¹Liver Unit, Hospital General Universitario, Alicante, Spain. ²Sección de Hepatologia, Hospital de la Santa Creu i Sant Pau, Barcelona, Spain. ³Sección de Hepatologia, Hospital Germans Trias i Pujol, Badalona, Spain. ⁴Sección de Hepatologia, Hospital del Mar, Barcelona, Spain. ⁵Servicio de Hepatologia, Hospital Clinico Universitario, Valencia, Spain. ⁶CIBERehd, Instituto de Salud Carlos III, Madrid, Spain

4:00 PM  
#41  
A MULTI-CENTER CASE-CONTROL STUDY OF GENETIC PREDICTORS FOR HEPATOPULMONARY SYNDROME (HPS)  
Michael B. Fallon¹, Kari Roberts², Michael J. Krowka³, Vijay Shah³, James F. Trotter⁴, David Badesch⁵, Lisa Forman⁶, Robert S. Brown⁶, Dan Rabinowitz⁵, Steven Zacks⁶, Steven M. Kawut¹  
¹Medicine, University of Alabama at Birmingham, Birmingham, AL, USA. ²Medicine, Tufts-NEMC, Boston, MA, USA. ³Medicine, Mayo Clinic, Rochester, MN, USA. ⁴Medicine, University of Colorado, Denver, CO, USA. ⁵Medicine, Columbia University, New York, NY, USA. ⁶Medicine, University of North Carolina, Chapel Hill, NC, USA
4:15 PM

#42
ELTROMBOPAG RAISES PLATELET COUNTS IN TWO WEEKS IN PATIENTS WITH HCV AND SIGNIFICANT THROMBOCYTOPENIA

Nezam H. Afdhal1, John G. McHutchison2, Mitchell L. Shiffman3, Maribel Rodriguez-Torres4, Geoffrey M. Dusheiko5, Samuel Sigal6, Scott White7, Nicole Blackman7, Fiona Campbell8, Dickens Theodore9
1Beth Israel Deaconess Medical Center, Boston, MA, USA. 2Duke Clinical Research Institute, Durham, NC, USA. 3Virginia Commonwealth University Health System, Richmond, VA, USA. 4Fundacion de Investigacion de Diego, San Juan, PR, USA. 5Royal Free Hospital, London, United Kingdom. 6Weill Medical College of Cornell University, New York, NY, USA. 7GlaxoSmithKline, Collegeville, PA, USA. 8GlaxoSmithKline, Greenford, United Kingdom. 9GlaxoSmithKline, Research Triangle Park, NC, USA

Experimental Hepatotoxicity
Sunday, November 4

4:45 - 6:15 PM  
Hynes, Room 309
Moderators:  
Gavin E. Arteel, PhD  
Natalia Nieto, PhD

4:45 PM

#43
ANALYSIS OF HEPATOCYTE-SPECIFIC CASPASE-8 KNOCKOUT MICE IN EXPERIMENTAL MODELS OF LIVER INJURY

Christian Liedtke1, Julia Freimuth1, Konrad L. Steeetz1, Naiara Beraza1, Daniela Lambertz1, Dieter Riemthacher2, Christian Trautwein1
1Department of Medicine III, University Hospital Aachen, Aachen, Germany. 2Centre for Molecular Neurobiology, Hamburg, Germany

5:00 PM

#44
APOPTOSIS-INDUCING FACTOR CAUSES MITOCHONDRIAL OXIDANT STRESS AND NUCLEAR DNA FRAGMENTATION IN ACETAMINOPHEN-INDUCED LIVER INJURY

Hartmut W. Jaeschke1, Margitta Lebofsky1, John J. Lemasters2, Mary Lynn Baij1
1Pharmacology, Toxicology & Therapeutics, University of Kansas Medical Center, Kansas City, KS, USA. 2Pharmaceutical Sciences, Medical University of South Carolina, Charleston, SC, USA

5:15 PM

#45
ETHANOL SUPPRESSES MHC CLASS I-RESTRICTED ANTIGEN PRESENTATION IN LIVER CELLS: ROLE OF PROTEASOME

Natalia A. Osna1,2, Kusum K. Kharbanda1,2, Ronda L. White1,2, Geoffrey M. Thiele1,2, Terrence M. Donohue1,2
1Internal Medicine, University of Nebraska Medical Center, Omaha, NE, USA. 2VA Medical Center, Omaha, NE, USA

5:30 PM

#46
ACUTE ETHANOL CAUSES LIVER MITOCHONDRIAL DEPOLARIZATION IN VIVO INDEPENDENT OF THE MITOCHONDRIAL PERMEABILITY TRANSITION (MPT) AND POTASSIUM CHANNEL OPENING

Zhi Zhong1, Venkat K. Ramshesh1, Hasibur Rehman1, John J. Lemasters1,2
1Pharmaceutical Sciences, Medical University of South Carolina, Charleston, SC, USA. 2Biochemistry & Molecular Biology, Medical University of South Carolina, Charleston, SC, USA

5:45 PM

#47
BILE ACIDS REGULATE HEPATOCYTE CELL DEATH BY DIFFERENTIAL ACTIVATION OF CLASS 1A PHOSPHOINOSITIDE-3-KINASE P110 ISOFORMS

Simon Hohenester1, Ulrich Beuers2, Sawkat Anwer3, Cynthia R. Webster4
1University of Munich, Munich, Germany. 2Gastroenterology and Hepatology, University of Amsterdam, Amsterdam, Netherlands. 3Biomedical Sciences, Tufts Cummings School of Veterinary Medicine, N Grafton, MA, USA. 4Clinical Science, Tufts Cummings School of Veterinary Medicine, N Grafton, MA, USA

6:00 PM

#48
INDUCED OVEREXPRESSION OF FIBROBLAST GROWTH FACTOR 10 PARTIALLY RESCUES ACUTE ETHANOL TOXICITY IMPAIRMENT OF LIVER REGENERATION

Joaquin J. Estrada1, Tove A. Berg1, Lily Lee1, Travis Chong1, Shigang Xiong2, Pierre Del Morall, Saverio Belluscil, Hidekazu Tsukamoto2, Kasper Wang1
1Pediatric Surgery, Childrens Hospital Los Angeles, Los Angeles, CA, USA. 2Department of Pathology, Keck School of Medicine, University of Southern California, Los Angeles, CA, USA
HCV: Clinical Development Strategies I
Sunday, November 4
4:45 - 6:15 PM Hynes, Auditorium
MODERATORS: Jean-Michel Pawlotsky, MD, PhD
Charles D. Howell, MD

4:45 PM
#49
ANTIVIRAL, PHARMACOKINETIC AND SAFETY DATA FOR GS-9190, A NON-NUCLEOSIDE HCV NS5B POLYMERASE INHIBITOR, IN A PHASE-1 TRIAL IN HCV GENOTYPE 1 INFECTED SUBJECTS
Linda Bavisotto1, Chia C. Wang2, Ira M. Jacobson3, Patrick Marcellin4, Stefan Zeuzem5, Eric J. Lawitz6, Martin Lunde7, Patrick Sereni8, Christopher O'Brien9, David W. Oldach10, Gerry Rhodes10
1Charles River Laboratories, Northwest Kinetics, Inc., Tacoma, WA, USA. 2U of Washington, Seattle, WA, USA. 3Weill Cornell Medical College, New York, NY, USA. 4Hospital Beaumont, Clichy, France. 5Saarland University Hospital, Homburg, Germany. 6Alamo Medical Research, San Antonio, TX, USA. 7Prism Research, St. Paul, MN, USA. 8Hospital St. Louis, Paris, France. 9U of Miami, Miami, FL, USA. 10Gilead Sciences, Inc., Foster City, CA, USA

5:00 PM
#50
TELAPREVIR RESISTANCE MUTATIONS IN PATIENTS WITH HEPATITIS C WHO RELAPSED AFTER SEQUENTIAL THERAPY WITH TELAPREVIR, PEG-INF xERON ALFA 2A AND RIBAVIRIN
Nicole Forestier1,2, Simone Susser2, Martin W. Welker2, Christine J. Weegink3, Hendrik W. Reesink3, Stefan Zeuzem1,2, Christoph Sarrazin1,2
1Internal Medicine I, J. W. Goethe University Hospital, Frankfurt, Germany. 2Internal Medicine II, Saarland University Hospital, Homburg, Germany. 3Department of Gastroenterology and Hepatology, Academic Medical Center, Amsterdam, Netherlands

5:15 PM
#51
SUSTAINED VIROLOGIC RESPONSE WITH ALBINTERFERON ALFA-2B/RIBAVIRIN TREATMENT IN PRIOR INTERFERON THERAPY NON-RESPONDERS
David R. Nelson1, Vinod K. Rustgi2, Vijayan Balan3, Mark Sulkowski4, Gary L. Davis5, Andrew Muir6, Louis Lambiase7, Roland C. Dickson8, Russell H. Wiesner9, John G. McHutchison10, Erik Pulkstenis11, Patrick Cronin11, G M. Subramanian11
1University of Florida, Gainesville, FL, USA. 2Metropolitan Research, Arlington, VA, USA. 3Mayo Clinic, Scottsdale, AZ, USA. 4Johns Hopkins University, Baltimore, MD, USA. 5Baylor University Medical Center, Dallas, TX, USA. 6Duke University, Durham, NC, USA. 7University of Florida, Jacksonville, FL, USA. 8University of Florida, Jacksonville, FL, USA. 9Mayo Clinic, Rochester, MN, USA. 10Duke Clinical Research Institute, Durham, VA, USA. 11Human Genome Sciences, Rockville, MD, USA
Mechanisms and Animal Models of Immune-mediated Liver Injury

Sunday, November 4
4:45 - 6:15 PM  Hynes, Room 311
MODERATORS:
Bin Gao, MD, PhD
Cara Mack, MD

4:45 PM
#55
DIET-INDUCED STEATOHEPATITIS IS PREVENTED BY MYD88 KNOCKOUT PHENOTYPE BUT NOT BY SELECTIVE MYD88 DEFICIENCY IN THE BONE MARROW-DERIVED CELLS IN MICE

Arunugam Velayudham, Istvan Hritz, Angela Dolganiuc, Evelyn Kurt-Jones, Donna Catalano, Pranoti Mandrekar, Gyongyi Szabo
Department of Medicine, Division of Gastroenterology, University of Massachusetts Medical School, Worcester, MA, USA

5:00 PM
#56
ENHANCED INNATE IMMUNE RESPONSE AND ACCELERATED LIVER REGENERATION IN HEPATOCYTE-SPECIFIC IKK2 DELETED MICE

Yann Malato¹, Naiara Beraza¹, Leif Sander¹, Malika Almasaoudi¹, Manolis Pasparakis², Christian Trautwein¹
¹Internal Medicine III, Universitätsklinikum Aachen, Aachen, Germany. ²Department Of Genetics, University Of Cologne, Cologne, Germany

5:15 PM
#57
DEPENDENCE OF THE ANTIVIRAL RIG-I-MAVS-IKK: SIGNALING PATHWAY ON MITOCHONDRIAL RESPIRATION

Ting Wang, Kui Li, Stanley M. Lemon, Steven A. Weinman
University of Texas Medical Branch, Galveston, TX, USA

5:30 PM
#58
HEPATOCYTE CYCLOOXYGENASE-2 MEDIATES ENDOTOXIN-INDUCED ACUTE LIVER FAILURE

Chang Han, Kyu Lim, Quiying Li, Lihong Xu, Tong Wu
Department of Pathology, University of Pittsburgh, School of Medicine, Pittsburgh, PA, USA

5:45 PM
#59
LIVER DAMAGE IN A MOUSE MODEL OF FULMINANT AUTOIMMUNE HEPATITIS IS DEPENDENT UPON CD4+ T CELL PRODUCTION OF IFN-γ, BUT INDEPENDENT OF BOTH CD8+ T CELLS AND FAS

Richard T. Robinson², M. Wesley Milks³, James D. Gorham¹,²
¹Pathology, Dartmouth Medical School, Lebanon, NH, USA. ²Microbiology and Immunology, Dartmouth Medical School, Lebanon, NH, USA. ³Dartmouth College, Hanover, NH, USA

Mechanisms of Hepatobiliary Neoplasia

Sunday, November 4
4:45 - 6:15 PM  Hynes, Room 304/306
MODERATORS:
Tong Wu, MD, PhD
Shelly C. Lu, MD

4:45 PM
#61
ORPHAN NUCLEAR RECEPTOR SHP IS A NOVEL TUMOR SUPPRESSOR OF HEPATOCELLULAR CARCINOMA

Nan He, Kyungtae Park, Yuxia Zhang, Jiansheng Huang, Li Wang
Medicine & Pharmacology, University of Kansas Medical Center, Kansas, KS, USA

5:00 PM
#62
TUMORIGENIC LIVER STEM CELLS EXPAND DURING AGING IN METHIONINE ADENOSYLTRANSFERASE 1A DEFICIENT MICE

Carl B. Rountree²,³, Shantha Senadheera², Gay M. Crooks³, Shelly C. Lu¹
¹Medicine, Keck School of Medicine USC, Los Angeles, CA, USA. ²Division of Gastroenterology, Hepatology, and Nutrition, Childrens Hospital Los Angeles, Los Angeles, CA, USA. ³Gene, Immunology, and Stem Cell Program, Childrens Hospital Los Angeles, Los Angeles, CA, USA

5:15 PM
#63
COX-2-DERIVED PGE2 ACTIVATES β-CATENIN IN HUMAN HEPATOCELLULAR CARCINOMA CELLS: IMPLICATION FOR TARGETED CHEMOPREVENTION AND TREATMENT

Kyu Lim¹, Chang Han¹, Yifan Dai², Lihong Xu¹, Tong Wu¹
¹Department of Pathology, University of Pittsburgh School of Medicine, Pittsburgh, PA, USA. ²Department of Surgery, University of Pittsburgh, School of Medicine, Pittsburgh, PA, USA
5:30 PM

#64
LEPTIN IS A GROWTH FACTOR FOR CHOLANGIOCARCINOMA: AN IN VIVO EXPERIMENTAL STUDY
Giammarco Fava1, Gianfranco Alpini2, Gianluca Svegliati-Baroni1, Luciano Trozzi1, Stefania Saccomanno1, Chiara Rychlicki1, Cinzia Candelaresi1, Antonio Di Sario1, Marco Marzioni1, Antonio Benedetti1
1Gastroenterology, Università Politecnica delle Marche, Ancona, Italy. 2Department of Medicine and Systems Biology and Translational Medicine, Central Texas Veterans HCS, Scott & White and The Texas A&M HSC, Temple, TX, USA

5:45 PM

#65
OVER-EXPRESSION OF IL-6 CONTRIBUTES TO CHOLANGIOCARCINOMA INVASION BY MIR-21 DEPENDENT PATHWAYS
Fanyin Meng1,2, Roger Henson2, Hania Wehbe-Janek2, Heather Smith2, Tushar Patel1,2
1Internal Medicine, Ohio State University Medical Center, Columbus, OH, USA. 2Scott & White Clinic, Temple, TX, USA

6:00 PM

#66
ESTABLISHMENT OF RAT LIVER CANCER STEM CELL LINES EXPRESSING GRANULOCYTE-COLONY STIMULATING FACTOR RECEPTOR (G-CSFR) AND ROLE OF G-CSF/G-CSFR AXIS IN MODULATING THEIR PROLIFERATION AND MIGRATION POTENTIAL
Anna C. Piscaglia1,2, Thomas D. Shupe2, Seh-Hoon Oh2, Nicole Steiger2, Antonio Gasbarrini1, Bryon E. Petersen2
1Internal Medicine and Gastroenterology, Catholic University of Rome, Rome, Italy. 2Pathology, University of Florida, Gainesville, FL, USA

5:00 PM

#68
HLA ASSOCIATION IN PRIMARY SCLEROSING CHOLANGITIS: DETECTION AND FINE MAPPING OF AN HLA INDEPENDENT SIGNAL IN THE COMPLEMENT GENE CLUSTER
Tom H. Karlsen1,2, Peter Croucher3,4, Jochen Hampe4,5, Andre Franke4, Erik Schrumpf4, Annika Bergquist6, Erik Thorsby2, Benedicte A. Lie2, Kirsten M. Boberg1, Stefan Schreiber4
1Medical Department, Rikshospitalet-Radiumhospitalen Medical Center, Oslo, Norway. 2Institute of Immunology, Rikshospitalet-Radiumhospitalen Medical Center, Oslo, Norway. 3Institute of Medical Informatics and Statistics, Christian-Albrechts-University, Kiel, Germany. 4Institute for Clinical Molecular Biology, Christian-Albrechts-University, Kiel, Germany. 5Department of General Internal Medicine, University Hospital Schleswig-Holstein, Kiel, Germany. 6Department of Gastroenterology and Hepatology, Karolinska University Hospital, Stockholm, Sweden

5:15 PM

#69
THE NATURAL HISTORY OF SMALL-DUCT PRIMARY SCLEROSING CHOLANGITIS
Einar Bjornsson1, Rolf Olsson1, Annika Bergquist2, Stefan Lindgren3, Barbara Braden4, Roger Chapman4, Kirsten Boberg5, Paul Angulo6
1Section of Gastroenterology and Hepatology, Department of Internal Medicine, Gothenburg, Sweden. 2Section of gastroenterology and Hepatology, Karolinska University Hospital, Stockholm, Sweden. 3Department of Medicine, Malmo General hospital, Malmo, Sweden. 4Department of Medicine, John Radcliffe hospital, Oxford, United Kingdom. 5Department of Medicine, Rikshospitalet, Oslo, Norway. 6Department of Medicine, Mayo Clinic College of Medicine, Rochester, MN, USA

5:30 PM

#70
MULTI-CENTER, DOUBLE BLIND, RANDOMIZED CONTROLLED TRIAL OF ZIDOVUDINE AND LAMIVUDINE (COMBIVIR) THERAPY FOR PATIENTS WITH PRIMARY BILIARY CIRRHOSIS
Andrew L. Mason1, Keith D. Lindor2, Bruce R. Bacon3, Catherine Vincent4, James M. Neuberger5, Shawn T. Wassenenko1
1Medicine, University of Alberta, Edmonton, AB, Canada. 2Medicine, Mayo Clinic, Rochester, MN, USA. 3Medicine, St. Louis University, St. Louis, MO, USA. 4Medicine, University of Montreal, Montreal, QC, Canada. 5Medicine, University of Birmingham, Birmingham, United Kingdom

Molecular Advances in Human Cholestatic Liver Disease
Sunday, November 4
4:45 - 6:15 PM Hynes, Room 312
MODERATORS:
Marco A. Arrese, MD
Laura Bull, PhD

4:45 PM

#67
VARIABILITY OF THE ABCB4 GENE IN YOUNG ADULT CHOLECYSTECTOMIZED PATIENTS
Karl Esten Nakken1,2, Knut J. Labori2, Olaug Rødningen3, Sigve Nakken4, Kristin Eiklid3, Morten G. Roaeder1,2
1Institute for Experimental Medical Research, Ulleval University Hospital, Oslo, Norway. 2Department of Gastrointestinal Surgery, Ullevaal University Hospital, Oslo, Norway. 3Department of Medical Genetics, Ulleval University Hospital, Oslo, Norway. 4Centre for Molecular Biology and Neuroscience & Institute of Medical Microbiology, Rikshospitalet-Radiumhospitalen Medical Centre, Oslo, Norway
5:45 PM

#71
CONTRIBUTION OF THE VARIANT P.V444A OF ABCB11 (BSEP) TO INTRAHEPATIC CHOLESTASIS OF PREGNANCY

Peter Dixon1, Jennifer Chambers1, Sandra Strautnieks2, Richard J. Thompson2, Frank Lammert3, Ralf Kubitz3, Anna G. Glantz5, Lars-Ake Mattsson6, Gudrun E. Moore6, Catherine Williamson1
1Maternal and Fetal Medicine, Imperial College, London, United Kingdom. 2Liver Studies and Transplantation, Kings College, London, United Kingdom. 3Internal Medicine, University Hospital, Bonn, Germany. 4Gastroenterology, Hepatology and Infectious Disease, University of Dusseldorf, Dusseldorf, Germany. 5Obstetrics and Gynecology, Sahlgrenska University Hospital, Goeteborg, Sweden. 6Clinical and Molecular Genetics, Institute of Child Health, London, United Kingdom

6:00 PM

#72
VARIATION IN THE MDR3 GENE INFLUENCES DISEASE PROGRESSION IN PSC PATIENTS AND DISEASE SUSCEPTIBILITY IN EPISISTIC INTERACTION WITH A POLYMORPHISM IN THE OST-α GENE

Espen Melum1,2, Kirsten M. Boberg1, Andre Franke3, Annika Bergquist4, Jochen Hampe5, Stefan Schreiber3, Benedicte A. Lie2, Erik Schrumpf1, Tom H. Karlsen1,2
1Medical Department, Rikshospitalet-Radiumhospitalet Medical Center, Oslo, Norway. 2Institute of Immunology, Rikshospitalet-Radiumhospitalet Medical Center, Oslo, Norway. 3Institute for Clinical Molecular Biology, Christian-Albrechts University, Kiel, Germany. 4Department of Gastroenterology and Hepatology, Karolinska University Hospital, Huddinge, Stockholm, Sweden. 51st Department of Medicine, Christian-Albrechts University, Kiel, Germany

Portal Hypertension: Basic
Sunday, November 4
4:45 - 6:15 PM
Hynes, Room 302

MODERATORS:
Srinivasan Dasarathy, MD
Don C. Rockey, MD

4:45 PM

#73
THE PROTEIN KINASE G-VASODILATOR STIMULATED PHOSPHOPROTEIN PATHWAY DISRUPTS HEPATIC STELLATE CELL DRIVEN ANGIOGENESIS BY DISRUPTING FOCAL ADHESION FORMATION

Ningling K. Decker1, Kenneth D. Bloch2, Vijay Shah1
1Gastroenterology Research Unit, Mayo Clinic, Rochester, MN, USA. 2Massachusetts General, Charlestown, MA, USA

5:00 PM

#74
EXPRESSION, LOCALIZATION, AND FUNCTION OF NEUROPLIN-1 IN HEPATIC STELLATE CELLS

Robert C. Huebert1, Sheng Cao1, Usman Yaqoob3, Debabrata [Dev] Mukhopadhyay2, Vijay Shah1
1Gastroenterology Research Unit, Mayo Clinic, Rochester, MN, USA. 2Biochemistry & Molecular Biology, Mayo Clinic, Rochester, MN, USA

5:15 PM

#75
ANGIOGENESIS IN EXPERIMENTAL HEPATOPULMONARY SYNDROME (HPS)

Junlan Zhang1, Bao Luo1, Yongming Wang1, Joseph Barney2, Selvarangan Ponnapazhagan3, Michael B. Fallon1
1Medicine, University of Alabama at Birmingham, Birmingham, AL, USA. 2Pulmonary/Allergy/Critical Care, University of Alabama at Birmingham, Birmingham, AL, USA. 3Molecular & Cellular Pathology, University of Alabama at Birmingham, Birmingham, AL, USA

5:30 PM

#76
FXR BILE ACID RECEPTOR ACTIVATES FOCAL ADHESION KINASE AND STRESS FIBER-MEDIATED MOTILITY IN ENDOTHELIAL CELLS

Amitava Das, Usman Yaqoob, Ningling K. Decker, Vijay Shah

5:45 PM

#77
ISCHEMIA MODIFIED ALBUMIN PREDICTS MORTALITY IN LIVER DISEASE PATIENTS

Nathan Davies1, Stephen Hodges1, Lisa Cheshire1, Rajeshwar P. Mookerjee1, Gert Matthies2, Rajiv Jalan1
1The UCL Institute of Hepatology, University College London, London, United Kingdom. 2University of Leipzig, Leipzig, Germany

6:00 PM

#78
VASCULAR HYPORESPONSIVENESS TO ANGIOTENSIN-II IN RATS WITH CCL4-INDUCED CIRRHOSIS IS ASSOCIATED WITH COMPLEXATION OF THE ANGIOTENSIN-II TYPE1 RECEPTOR WITH THE RECEPTOR-DESENSITIZING PROTEIN, BETA-ARRESTIN 2

Martin Hennenberg, Jonel Trebicka, Andreas Eckhardt, Tilman Sauerbruch, Jörg Heller
Department of Internal Medicine I, University of Bonn, Bonn, Germany
Early Morning Workshops

Monday, November 5
6:45 - 7:45 AM Refer to your luncheon ticket for meeting room location.

Basic Research Workshops

EM-16 Toll-like Receptors
David A. Brenner, MD and Gyongyi Szabo, MD, PhD

EM-17 Experimental Hepatocarcinogenesis
Josep M. Llovet, MD and Lewis R. Roberts, MD, PhD

EM-18 Pathogenesis of NASH
Arun J. Sanay, MD and Frank A. Anania, MD

EM-19 Oxidative Liver Injury
Mario Chojkier, MD and John J. Lemasters, MD, PhD

EM-20 Stem Cells
Stewart Sell, MD and Neil D. Theise, MD

EM-21 Nuclear Receptors
John Chiang, PhD and Michael Trauner, MD

EM-22 Signaling Mechanisms in the Liver
Michael H. Nathanson, MD, PhD and Rebecca Wells, MD

Clinical Management Workshops

EM-23 Obesity Surgery and the Liver
Vlad Ratziu, MD and Pierre M. Gholam, MD

EM-24 Treatment of Autoimmune Hepatitis
Steven L. Flamm, MD and Jorge L. Rakela, MD

EM-25 Epidemiology of NASH
Stephen H. Caldwell, MD and Zobair M. Younossi, MD

EM-26 Therapeutic Use of Immunosuppressive Drugs
Paul Martin, MD and Russell H. Wiesner, MD

EM-27 Therapy for Alcoholic Hepatitis
Michael R. Lucey, MD and Timothy R. Morgan, MD

EM-28 Acute Liver Failure Due to Drugs
Robert J. Fontana, MD and William M. Lee, MD

EM-29 How to Treat Portal Hypertension
Guadalupe Garcia-Tsao, MD and Vijay Shah, MD

NIH/NIDDK REQUEST FOR APPLICATION

Monday, November 5
6:45 - 7:45 am Location: TBA

Drug-Induced Liver Injury Network (DILIN).
Re-Competition and Expansion, a National Institutes of Health RFA.

Jose Serrano, MD and Leonard B. Seeff, MD

Staff from the NIDDK will present an overview and answer questions about the recently published Request for Applications (RFA) for participation in a multi-year, multi-center clinical research network devoted to establishing a large database with serum, tissue and DNA samples from well characterized cases of drug-induced liver injury. The RFA will solicit applications for clinical centers and a data coordinating center to continue and expand the current “Drug-Induced Liver Injury Network” (DILIN) for another 5 years. Open to all interested persons on a walk-in basis. The text of the RFA is available at: http://www2.niddk.nih.gov/Funding/FundingOpportunities/

This meeting is open to all interested investigators, registration is not required.

Poster Session 3

Monday, November 5, 2007
8:00 AM – 5:30 PM
Hynes, Exhibit Hall C

Refer to page 165A for Poster Presentations

Exhibit Hall Opening and Reception

Monday, November 5, 2007
9:30 AM – 3:00 PM
Hynes, Exhibit Hall D
Plenary Sessions

Presidential Plenary I
Monday, November 5
8:00 - 9:30 AM  Hynes, Auditorium
MODERATORS:  Gregory J. Gores, MD
Arthur J. McCullough, MD

8:00 AM
#79
INTRAVENOUS N-ACETYLCYSTEINE IMPROVES SPONTANEOUS SURVIVAL IN EARLY STAGE NON-ACETAMINOPHEN ACUTE LIVER FAILURE
William M. Lee1, Lorenzo Rossaro2, Robert J. Fontana3, Anne M. Larson4, Richard T. Stravitz5, Timothy J. Davern6, Linda S. Hynan1, Joan S. Reisch1, Patricia R. Robuck7
1University of Texas Southwestern Medical Center, Dallas, TX, USA. 2University of California, Davis, Davis, CA, USA. 3University of Michigan Medical Center, Ann Arbor, MI, USA. 4University of Washington, Seattle, WA, USA. 5Virginia Commonwealth University, Richmond, VA, USA. 6University of California, San Francisco, San Francisco, CA, USA. 7National Institutes of Health, Bethesda, MD, USA

8:15 AM
#80
PROVE2: PHASE II STUDY OF VX950 (TELAPREVIR) IN COMBINATION WITH PEGINTERFERON ALFA2A WITH OR WITHOUT RIBAVIRIN IN SUBJECTS WITH CHRONIC HEPATITIS C, FIRST INTERIM ANALYSIS
Christophe Hezode1, Peter Ferenci2, Geoffrey M. Dusheiko3, Stanislav Pol4, Tobias Goeser5, Jean-Pierre Bronowicki6, Shahin Gharakhanian7, Desiree Devonish7, Robert Kauffman7, John Alam7, Jean-Michel Pawlotsky8, Stefan Zeuzem9
1AP-HP Hepatogastroenterology Services, Henri Mondor Hospital, Creteil, France. 2Internal Medicine III, Medical University, Vienna, Austria. 3Center for Hepatology, Royal Free Hospital, London, United Kingdom. 4AP-HP Liver Unit, Necker-Cochin Hospitals, Paris, France. 5Gastroenterology & Hepatology, University of Cologne, Cologne, Germany. 6CHU, Nancy University Hospital Center, Nancy, France. 7Medicinal Development Group, Vertex Pharmaceuticals, Inc, Cambridge, MA, USA. 8AP-HP Dept of Bacterology & Virology, Henri Mondor Hospital, Creteil, France. 9Internal Medicine II, Saarland University Hospital, Homburg/Saar, Germany

8:30 AM
#81
MULTICENTER RANDOMIZED CONTROLLED TRIAL OF CARVEDIILOL VERSUS VARICEAL BAND LIGATION FOR THE PREVENTION OF THE FIRST VARICEAL BLEED
Dhiraj Tripathi1, James W. Ferguson1, Narendra Kocher1, Joanna A. Leithed1, George Therapondos1, Norma C. McAvoy1, Adrian J. Stanley2, Ewan H. Forrest2,3, S. W. Hislop5, Peter R. Mills4, Peter C. Hayes1
1Dept of Hepatology, Royal Infirmary, Edinburgh, United Kingdom. 2Dept of Gastroenterology, Victoria Infirmary, Glasgow, United Kingdom. 3Dept of Gastroenterology, Royal Infirmary, Glasgow, United Kingdom. 4Dept of Gastroenterology, Gartnavel Hospital, Glasgow, United Kingdom. 5Dept of Gastroenterology, Royal Alexandria Hospital, Paisley, United Kingdom

8:45 AM
#82
PRESERVING RENAL FUNCTION IN LIVER TRANSPLANTATION. EFFICACY AND SAFETY OF A MYCOPHENOLATE MOFETIL (MMF)/SIROLIMUS MAINTENANCE REGIMEN FOLLOWING CALCINEURIN INHIBITOR (CNI) WITHDRAWAL: INTERIM DATA FROM THE SPARE-THE-NEPHRON (STN) TRIAL
Lewis Teperman1, Anthony Sebastian2, Juan Arenas2, Linda Sher4, Baburao Koneru5, Paul Marotta6, John P. Roberts7, Dharmesh Patel8
1NYU School of Medicine, New York, NY, USA. 2Integris Baptist Medical Center, Oklahoma City, OK, USA. 3Henry Ford Hospital, Detroit, MI, USA. 4University of Southern California, Los Angeles, CA, USA. 5University of Medicine and Dentistry of New Jersey, Newark, NJ, USA. 6London Health Sciences Hospital, London, ON, Canada. 7University of California, San Francisco, CA, USA. 8Roche Laboratories Inc., Nutley, NJ, USA
9:00 AM
#83
FIRST MULTICENTER EVALUATION OF THE EFFICACY OF TENOFEVIR IN NUCLEOS(T)IDE ANALOG EXPERIENCED PATIENTS WITH HBV MONOINFECTION

Florian van Bömmel¹, Robert A. De Man², Andreas Erhardt³, Dietrich Hüppe⁴, Kerstin Stein⁵, Peter Buggisch⁶, Wulf Böcher⁷, Christoph Sarrazin⁸,⁹, Jörg Trojan⁸, Ulrich Spengler¹⁰, Jurrien G. Reijniers¹¹, Bernd Möller¹¹, Hermann E. Wasmuth¹², Peter Rohde¹³, Heinz-Hubert Feucht¹⁴, Bertram Wiedenmann⁵, Thomas Berg¹

¹Medizinische Klinik m. S. Hepatologie und Gastroenterologie, Charité-Universitätsmedizin Berlin, Berlin, Germany. ²Department of Gastroenterology and Hepatology, Erasmus MC, University Medical Center Rotterdam, Rotterdam, Netherlands. ³Klinik für Gastroenterologie, Hepatologie und Infektiologie, Universitätsklinikum der Heinrich-Heine-Universität Düsseldorf, Düsseldorf, Germany. ⁴Gastroenterologische Gemeinschaftspraxis, Herne, Germany. ⁵Innere Medizin IV, Universitätsklinikum Heidelberg, Heidelberg, Germany. ⁶Medizinische Klinik, Universitätsklinikum Hamburg-Eppendorf, Hamburg, Germany. ⁷Kliniken für Innere Medizin II, Universitätsklinikum des Saarlandes, Homburg an der Saar, Germany. ⁸Medizinische Klinik III, Johann Wolfgang Goethe Universität, Frankfurt am Main, Germany. ⁹Zentrum für Innere Medizin, Universitätsklinikum Bonn, Oklahoma, OK, USA. ¹⁰Hepatologische Schwerpunktpraxis, Berlin, Germany. ¹¹Medizinische Klinik und Poliklinik, Johannes Gutenberg Universität Mainz, Mainz, Germany. ¹²Klinik für Innere Medizin II, Universitätsklinikum des Saarlandes, Homburg an der Saar, Germany. ¹³Medizinische Klinik I, Johann Wolfgang Goethe Universität, Frankfurt am Main, Germany. ¹⁴Zentrum für Innere Medizin, Universitätsklinikum Bonn, Oklahoma, OK, USA. ¹⁵Medizinische Klinik III, Universitätsklinikum Aachen, Aachen, Germany. ¹⁶Abteilung für Gastroenterologie, St. Marien Hospital, Hamm, Germany. ¹⁷Institut für Medizinische Mikrobiologie und Immunologie, Universitäts-Krankenhaus Eppendorf, Hamburg, Germany.

9:15 AM
#84
A PILOT RANDOMIZED CONTROLLED STUDY EVALUATING EFFICACY OF AUTOLOGOUS BONE MARROW MONONUCLEAR CELLS TRANSPLANTATION IN PATIENTS WITH ADVANCED CHRONIC LIVER DISEASE

Andre C. Lyra¹, Milena B. Soares², Luiz F. da Silva², Eduardo L. Braga¹, Sheila A. Oliveira³, Marcos F. Fortes³, André G. Silva¹, Patrícia S. Brandão¹, Bernd Gensen³, Ricardo R. dos Santos², Luiz G. Lyra¹

¹Internal Medicine - GastroHepatology Service, Hospital Sao Rafael and Federal University of Bahia, Salvador-Bahia, Brazil. ²Fundacao Oswaldo Cruz - FioCruz, Salvador, Brazil. ³BGStats Consulting, Graz, Belgium.
State-of-the-Art Lecture

Monday, November 5
10:00 - 10:30 AM Hynes, Auditorium

Hyman J. Zimmerman Hepatotoxicity State-of-the-Art Lecture

Endocannabinoids and the Liver: Unlikely Partners in Multiple Functions

SPEAKER: George Kunos, MD, PhD
MODERATOR: Jacquelyn J. Maher, MD

Dr. George Kunos is Scientific Director at the National Institute on Alcohol Abuse and Alcoholism (NIAAA), National Institutes of Health (NIH) in Bethesda, Maryland. His research interests include autonomic pharmacology, receptor mechanisms, cardiovascular regulation, biology of the endocannabinoid system, regulation of energy homeostasis, and the biology of alcoholism. Dr. Kunos has published 160 peer-reviewed papers (including papers in Nature, Nature Medicine Science, Journal of Clinical Investigation, Proceedings of the National Academy of Sciences, Circulation) and 25 book chapters, and edited 4 monographs, and received numerous awards and honors. He is the owner of 3 U.S. patents.

This lecture will begin with a brief historical overview of the discovery of the key components of the endocannabinoid system, its physiological functions and its role in various pathological states. The emerging role of hepatic endocannabinoids and their CB1 receptors in liver biology will then be discussed, including their involvement in non-alcoholic and alcoholic fatty liver, in hepatic fibrogenesis and in the hemodynamic consequences of advanced liver cirrhosis.

Goals and Objectives:
- Understand how endocannabinoids are generated, released, activate receptors and metabolized.
- Understand the key physiological functions of endocannabinoids in the central nervous system and in peripheral tissues.
- Understand the mechanism, underlying both the therapeutic and the undesired side effects of novel medications that interact with components of the endocannabinoid system.

The Hyman J. Zimmerman Hepatotoxicity State-of-the-Art Lecture recognizes Dr. Zimmerman’s work in toxic liver injury. A restricted fund supporting this lecture ensures that future investigators will have a distinct platform to present their valuable work on liver toxicity at The Liver Meeting®. AASLD gratefully acknowledges Eli Lilly and Company for its generous support of this fund.

Break
10:30 – 11:00 am

Advances for Practitioners

Monday, November 5
11:00 AM - 12:30 PM Hynes, Ballroom B

Advances for Practitioners

MODERATOR: Thomas D. Boyer, MD

Advances for Practitioners will focus on recent publications which impact the practice of Hepatology. An emphasis will be placed on therapeutic studies. The lessons learned from the study, the clinical context and its limitations will be discussed. An expert panel will answer the question for each study: Should I incorporate this information into my practice and if so, when?

Goals and Objectives:
- Review recent published manuscripts in clinical hepatology which impact our practice.
- Place the lessons learned from these studies into context.
- Define how this information may immediately impact our clinical practice.

11:00- 11:10 AM Introduction


12:10- 12:30 PM Panel Discussion

Arun J. Sanyal, MD, Thomas D. Boyer, MD and Eugene R. Schiff, MD
Plenary Sessions

Presidential Plenary II
Monday, November 5
11:00 AM - 12:35 PM Hynes, Auditorium
MODERATORS: Scott L. Friedman, MD
Laurie D. DeLeve, MD, PhD

11:00 AM
#85
THE BASOLATERAL TRANSPORTER OSTα-OSTβ IS ESSENTIAL FOR INTESTINAL BILE ACID ABSORPTION AND HOMEOSTASIS
Anuradha Rao¹, Jamie Haywood¹, Ann L. Craddock¹, Martin G. Belinsky², Gary D. Kruh², Paul A. Dawson¹
¹Department of Internal Medicine - GI, Wake Forest University School of Medicine, Winston-Salem, NC, USA. ²Medical Science Division, Fox Chase Cancer Center, Philadelphia, PA, USA

11:15 AM
#86
CHOLANGIOCYTE CILIA EXPRESS THE OSMOSENSORIY PROTEIN TRPV4 AND DETECT CHANGES IN LUMINAL TONICITY THAT INDUCE DUCTAL BICARBONATE SECRETION
Sergio A. Gradilone, Anatoliy I. Masyuk, Patrick L. Splinter, Bing Q. Huang, Pamela S. Tietz, Jesus M. Banales, Tatyana V. Masyuk, Nicholas F. LaRusso
Internal Medicine, Mayo Clinic College of Medicine, Rochester, MN, USA

11:30 AM
#87
DUAL OVER-EXPRESSION OF INSULIN RECEPTOR SUBSTRATE-1 AND HEPATITIS BX GENE CAUSES PRE-MALIGNANT CHANGES IN LIVER
Lisa Longato¹, Suzanne de la Monte¹, Noriyoshi Kuzushita¹, Masayoshi Horimoto¹, Betty Slagle², Arlin Rogers³, Jack R. Wands¹
¹Liver Research Center, Rhode Island Hospital and The Warren Alpert Medical School of Brown University, Providence, RI, USA. ²The Baylor College of Medicine, Houston, TX, USA. ³Massachusetts Institute of Technology, Cambridge, MA, USA

11:45 AM
PRESENTATION OF THE JAN ALBRECHT COMMITMENT TO CLINICAL RESEARCH IN LIVER DISEASES AWARD
Presented to: Patrick G. Northup, MD

PRESENTATION OF THE SHEILA SHERLOCK CLINICAL AND TRANSLATIONAL AWARDS IN LIVER DISEASE
Presented to: Matthew Cave, MD and Tamar H. Takkei, MD, PhD

11:50 AM
#88
DISTINCT EFFECTS OF RAGE & MYD88 SIGNALING IN MASSIVE HEPATECTOMY
Shan Zeng¹, Hao Dun¹, Nikalesh Ippagunta¹, Rosa H Rosaria², Ann Marie Schmidt², Jean C Emond¹
¹Division of Liver Diseases and Transplantation, Department of Surgery, College of Physicians & Surgeons, Columbia University, New York, NY, USA. ²Division of Surgical of Science, Department of Surgery, College of Physicians & Surgeons, Columbia University, New York, NY, USA

12:05 PM
#89
ACUTE LIVER INJURY UPREGULATES MICRONA-491 IN MICE, AND ITS OVEREXPRESSION IN HEP G2 CELLS CAUSES APOPTOSIS
Sangjeong Yoon, Ping Guo Liu, Jian Wu, Mark A. Zern, Senthil K. Venugopal
Internal Medicine, UCDavis Medical Center, Sacramento, CA, USA

12:20 PM
#90
ROLE OF HEPATIC STELLATE CELL AND ANGIOPOIETIN ON ANGIOGENESIS DURING LIVER FIBROSIS
KOJIRO TAURA, David A. Brenner
Medicine, University of California, San Diego, La Jolla, CA, USA
Parallel Sessions

Acute Liver Failure

Monday, November 5
3:00 - 4:30 PM  Hynes, Room 302
MODERATORS:  J. Eileen Hay, MD
              Andres T. Blei, MD

3:00 PM
#91
INTERIM RESULTS OF RANDOMIZED CONTROLLED
TRIAL OF ELAD™ IN ACUTE ON CHRONIC LIVER
DISEASE
Zhong-ping Duan1, Jing Zhang2, Shaojie Xin3, Ju Mei Chen4, Dar
He2, John D. Brotherton2, Kameron Maxwell2, Michael Millis1
1Transplantation, University of Chicago, Chicago, IL, USA. 2Vital
Therapies, Inc, San Diego, IL, USA. 3Beijing Youan Hospital, Bei-
ing, China. 4302 Military Hospital, Beijing, China

3:15 PM
#92
OLDER AGE IS NOT ASSOCIATED WITH WORSE
OUTCOMES IN ACUTE LIVER FAILURE
Frank V. Schiodt1,6, Raymond T. Chung2, Schilsky L. Michael13, J.
Eileen Hay4, Paul Martin5, William M. Lee6
1Dept of Hepatology A, Rigshospitalet, Copenhagen, Denmark. 2Gastrointestinal Unit, Massachusetts General Hospital, Boston,
MA, USA. 3Liver Transplantation, Weill Cornell Medical Center,
New York, NY, USA. 4Mayo Clinic, Rochester, MN, USA. 5Mount
Sinai School of Medicine, New York, NY, USA. 6Division of Digestive
and Liver Diseases, UT Southwestern Medical Center, Dallas,
TX, USA

3:30 PM
#93
L-ORNITHINE PHENYLACETATE ATTENUATES
INCREASE IN ARTERIAL AMMONIA AND
INTRACRANIAL PRESSURE IN A DEVASCULARISED
PIG MODEL OF ACUTE LIVER FAILURE: A NOVEL
AMMONIA-LOWERING STRATEGY FOR HEPATIC
ENCEPHALOPATHY
Lars M. Ytrebø1, Rune Gangsøy Kristiansen1, Ole Martin
Fuskevåg2, Hanne Mæhre3, Arthur Revhaug4, Rajiv Jalan5, Christopher
Rose6
1Department of Anesthesiology, University Hospital of North Nor-
way and University of Tromsø, Tromsø, Norway. 2Department of
Clinical Pharmacology, University Hospital of North Norway and
University of Tromsø, Tromsø, Norway. 3Department of Marine
Biotechnology, University Hospital of North Norway and University
of Tromsø, Tromsø, Norway. 4Department of Digestive Surgery,
University Hospital of North Norway and University of Tromsø,
Tromsø, Norway. 5Liver Failure Group, Institute of Hepatology, Di-
vision of Medicine, University College London, London, United King-
dom. 6Neuroscience Research Unit, CHUM (Hôpital Saint-Luc),
Montreal, QC, Canada

3:45 PM
#94
STARVATION-INDUCED HEPATOCYTE
AUTOPHAGY: AN ORIGINAL MECHANISM OF
ACUTE LIVER CELL DAMAGE IN PATIENTS WITH
ANOREXIA NERVOSA
Pierre-Emmanuel Rautou1, Dominique Cazals-Hatem2, Richard
Moreau1,3, Claire Francoz1, Gérard Feldmann3, Didier Lebrec1,3,
Eric Ogier-Denis3, Dominique Valla1, Francois Durand1
1Hepatology unit, Beaujon Hospital, Clichy, France. 2Pathology,
Beaujon Hospital, Paris, France. 3INSERM U773, Bichat Hospital,
Paris, France

4:00 PM
#95
USE OF NUCLEOSIDE ANALOGUES IN HBV
RELATED ACUTE LIVER FAILURE
Emmanuel Seremba1, Corron M. Sanders1, Mamta K. Jain1, Timo-
thy J. Davern2, Lorenzo Rossaro3, Robert J. Fontana4, Steven Han5,
William M. Lee1
1University of Texas Southwestern Medical Center, Dallas, TX, USA.
2University of California, San Francisco, San Francisco, CA, USA.
3University of California, Davis, Davis, CA, USA. 4University of
California, Los Angeles, Los Angeles, CA, USA

4:15 PM
#96
EVALUATION OF A SCORING SYSTEM FOR
ASSESSING PROGNOSIS IN PEDIATRIC ACUTE
LIVER FAILURE
Brandy Lu, Jane Gralla, Edwin Liu, Emily Dobyns, Michael
Narkewicz, Ronald Sokol
Pediatrics, The University of Colorado School of Medicine and the
Children’s Hospital, Denver, CO, USA
Advances in Pediatric Hepatology

Monday, November 5

3:00 - 4:30 PM  Hynes, Room 311

MODERATORS: Ronald J. Sokol, MD
Kathleen M. Loomes, MD

3:00 PM

#97
A DEDICATED FIBROSCAN® PROBE TO EVALUATED LIVER FIBROSIS IN CHILDREN: FEASIBILITY AND PERFORMANCE FOR THE DIAGNOSIS OF CIRRHOSIS

Thierry Lamireau1, Stéphanie Franchi2, Monique Fabre3, Brigitte Le Bail4, Jean-François Chatel5, Daniele Pariente2, Juliette Foucher1, Olivier Bernard7, Victor de Ledinghen5
1Pediatry, Haut Leveque Hospital, Pessac, France. 2Pediatric Radiology, Bicetre Hospital, Le Kremlin Bicetre, France. 3Pathology, Bicetre Hospital, Le Kremlin Bicetre, France. 4Pathology, Haute Leveque Hospital, Pessac, France. 5Pediatric Radiology, Haute Leveque Hospital, Pessac, France. 6Hepato Gastroenterology, Haute Leveque Hospital, Pessac, France. 7Pediatric Hepato Gastroenterology, Bicetre Hospital, Le Kremlin Bicetre, France

3:15 PM

#98
OVERWEIGHT IS ASSOCIATED WITH DIMINISHED ANTIVIRAL RESPONSE IN HCV-INFECTED CHILDREN

Aymin Delgado-Borrego 1,2, Marielle Christofe 2, David Healey 2, David A. Ludwig1, Maureen M. Jonas2,4, Raymond T. Chung3,4
1Pediatrics, University of Miami, Miami, FL, USA. 2Medicine/GI and Nutrition, Children’s Hospital Boston, Boston, MA, USA. 3Medicine/GI, Massachusetts General Hospital, Boston, MA, USA. 4Harvard University, Boston, MA, USA

3:30 PM

#99
HIGH THROUGHPUT SEQUENCE ANALYSIS IDENTIFIES INDIVIDUAL AND COMBINED GENETIC DEFECTS IN CHILDREN WITH SYNDROMES OF INTRAHEPATIC CHOLESTASIS

Ursula Matte1, Cong Liu1, Alexander Miethke1, Reena Mourya1, Gregory Kauffmann1, Katie D. Mayer1, Laura Bull2, Nancy Spinner3, Richard J. Thompson4, Jorge Bezerra1
1Pediatrics, Cincinnati Children’s Hospital Medical Center, Cincinnati, OH, USA. 2UCSF Liver Center Laboratory, San Francisco General Hospital, San Francisco, CA, USA. 3Human Genetics, University of Pennsylvania School of Medicine, Philadelphia, PA, USA. 4Liver Studies and Transplantation, King’s College London School of Medicine, London, United Kingdom

3:45 PM

#100
COMPARATIVE PROTEOMIC ANALYSIS OF PARENTERAL NUTRITION-ASSOCIATED LIVER DISEASE (PNALD) IN INFANTS WITH INTESTINAL FAILURE

Ronald J. Sokol1,2, Kerri Murray2, Jenna Boyd2, Heather Thompson2, Ross Shepherd3, Frederick M. Karrer1, Cara Mack1, Mark Duncan2
1Pediatrics, University of Colorado School of Medicine and The Children’s Hospital, Denver, CO, USA. 2Pediatrics, University of Colorado at Denver and Health Sciences Center, Aurora, CO, USA. 3Pediatrics, Washington University School of Medicine, St. Louis, MO, USA

4:00 PM

#101
HCV-SPECIFIC PRODUCTION OF IL-10 AND IFN-GAMMA-INDUCIBLE PROTEIN-10 (IP-10) LEVELS PREDICT TREATMENT RESPONSE TO PEGYLATED INTERFERON AND RIBAVIRIN IN CHILDREN WITH CHRONIC HEPATITIS C INFECTION

Ivana Carey, Maria Mytilinaiou, Munther Hussain, Sanjay Bansal, Pushpa Subramaniam, Mary Horner, Maria Serena Longhi, Francesca Meda, Giorgina Mieli-Vergani, Diego Vergani
Institute of Liver Studies, King’s College London School of Medicine at King’s College Hospital, London, United Kingdom

4:15 PM

#102
DISCRIMINATING FEATURES OF BILIARY ATRESIA - A PROSPECTIVE MULTI-CENTERED ANALYSIS

Benjamin Shneider1, Ross Shepherd2, John Magee3, John C. Bucuvalas4, Barbara Haber5, Saul J. Karpen6, Phil Rosenthal7, Kathleen B. Schwarz8, Frederick J. Suchy9, Peter F. Whittington9, Ronald J. Sokol11, C. For Bar3
1Children’s Hospital Pittsburgh, Pittsburgh, PA, USA. 2Washington University, St. Louis, MO, USA. 3University of Michigan, Ann Arbor, MI, USA. 4Cincinnati Children’s Medical Center, Cincinnati, OH, USA. 5Children’s Hospital of Philadelphia, Philadelphia, PA, USA. 6Texas Children’s Hospital, Houston, TX, USA. 7University of California San Francisco, San Francisco, CA, USA. 8Johns Hopkins University, Baltimore, MD, USA. 9Mount Sinai School of Medicine, New York, NY, USA. 10Children’s Memorial Hospital, Chicago, IL, USA. 11Children’s Hospital Denver, Denver, CO, USA
Basic Mechanisms of Cell Death
Monday, November 5
3:00 - 4:30 PM Hynes, Room 309
MODERATORS: Christian Trautwein, MD
Xiao-Ming Yin, MD

3:00 PM
#103 CASPASE INHIBITION SWITCHED TNF-ALPHA-INDED APOPTOSIS TO AUTOPHAGIC CELL DEATH IN HEPATOCYTES
Wen-Xing Ding1, Hong-Min Ni1, Xiaoyun Chen1, Donna B. Stolz2, Xiao-Ming Yin1
1Pathology, University of Pittsburgh, Pittsburgh, PA, USA. 2Cell Biology and Physiology, University of Pittsburgh, Pittsburgh, PA, USA

3:15 PM
#104 IMPAIRED AUTOPHAGY IS THE MECHANISM OF MITOCHONDRIAL DYSFUNCTION IN ISCHEMIC RAT HEPATOCYTES
Jae-Sung Kim1, Takashi Nitta1, Insil Kim3, John J. Lemasters3, William A. Dunn2, Kevin E. Behrns1
1Surgery, University of Florida, Gainesville, FL, USA. 2Anatomy and Cell Biology, University of Florida, Gainesville, FL, USA. 3Pharmaceutical Sciences, Medical University of South Carolina, Charleston, SC, USA

3:30 PM
#105 CASPASE 8-INDUCED ENDOYSMAL ACIDIFICATION IS AN UPSTREAM EVENT IN CD95-DEPENDENT HEPATOCYTE APOPTOSIS
Roland Reinehr1, Stephan Becker1, Annika Sommerfeld1, Susanne Grether-Beck2, Dieter Haussinger1
1Gastroenterology, Hepatology and Infectiology, University Hospital Dusseldorf, Dusseldorf, Germany. 2Institut fuer Umweltmedizinische Forschung (IUF), Heinrich-Heine-University Dusseldorf, Dusseldorf, Germany

3:45 PM
#106 INTERNALIZATION OF DEATH RECEPTOR 5 IS REQUISITE FOR LYSOSOMAL PERMEABILIZATION BY TRAIL
Yuko Akazawa, Steven F. Bronk, Nathan W. Werneburg, Justin L. Mott, M. Eugenia Guicciardi, Xue W. Meng, Mark A. McNiven, Vijay Shah, Gregory Gores
Mayo Clinic College of Medicine, Rochester, MN, USA

4:00 PM
#107 GLUTATHIONE DEPLETION DOWN-REGULATES TUMOR NECROSIS FACTOR (TNF) α-INDUCED NF-κB ACTIVITY VIA ikB KINASE (IKK)-INDEPENDENT AND –DEPENDENT MECHANISMS
Huan Lou, Neil Kaplowitz
Medicine, University of Southern California, Los Angeles, CA, USA

4:15 PM
#108 ACTIVATION OF FOCAL ADHESION KINASE AND JNK CONTRIBUTES TO THE EXTRACELLULAR MATRIX MEDIATED DIFFERENTIAL SENSITIVITY TO BILE ACID INDUCED APOPTOSIS IN RAT HEPATOCYTES
Paul Usechak, Anna Gates, Cynthia R. Webster
Clinical Science, Tufts Cummings School of Veterinary Medicine, North Grafton, MA, MA, USA

HBV: Pre-clinical
Monday, November 5
3:00 - 4:30 PM Hynes, Ballroom A
MODERATORS: Daryl Lau, MD
Stephen Locarnini, MD, PhD

3:00 PM
#109 IN VITRO AND IN VIVO INHIBITION OF HBV REPLICATION BY PEPTIDE APTAMERS VIA DISRUPTION OF THE HBX-PROTEASOME INTERACTION
Uwais Zaid, Zhensheng Zhang, T. Jake Liang
Liver Diseases Branch, National Institutes of Health, Bethesda, MD, USA

3:15 PM
#110 INDUCTION OF ANTIGEN-SPECIFIC HUMORAL AND CELLULAR IMMUNE RESPONSES BY ANTIGEN-PULSED HUMAN BLOOD DENDRITIC CELLS IN HEPATITIS B VACCINE NONRESPONDERS AND PATIENTS WITH CHRONIC HEPATITIS B
Sk. Md. Fazle Akbar, Osamu Yoshida, Shinya Furukawa, Yoichi Hisa, Norio Horiike, Morikazu Onji
Department of Gastroenterology and Metabolism, Ehime University Graduate School of Medicine, Toon, Japan

3:30 PM
#111 POWERFUL INHIBITION OF HEPATITIS B VIRUS REPLICATION BY CPG-INDUCED CYTOKINES IN COMBINATION WITH LAMIVUDINE IN VITRO
Isabelle E. Vincent, Julie Lucifora, Isabelle Chemin, David Duranteau, Fabien Zoulim, Christian Trepo
INSERM U871, Lyon, France

3:45 PM
#112 LONG TERM INHIBITION OF HEPATITIS B VIRUS REPLICATION USING THE NON VIRAL EPISOMAL VECTOR PEPI-1
Andreas C. Jenke1, Valerie Orth1, Hans Joachim Lipps2, Stefan Wirth1
1Children’s Hospital, HELIOS Klinikum Witten/Herdecke University, Wuppertal, Germany. 2Institute of Cell Biology, Witten/Herdecke University, Witten, Germany
4:00 PM  
**#113**  
**ADENO-ASSOCIATED VIRUS REP78 PROTEIN INHIBITS HUMAN HEPATITIS B VIRUS REPLICATION IN HUMAN CELLS BY BINDING TO X PROMOTER AND C PROMOTER OF HBV**  
Tianhui Liu, Hong You, Min Cong  
Liver research center, Beijing Friendship Hospital, Capital Medical University, Beijing, China

3:00 PM  
**HCV: Virology**

**Monday, November 5**  
3:00 - 4:30 PM  
Hynes, Room 312  
**MODERATOR:** Takaji Wakita, MD, PhD

3:00 PM  
**#115**  
**CHARACTERIZATION OF HEPATITIS C RNA CONTAINING PARTICLES FROM HUMAN LIVER BY DENSITY AND MORPHOLOGY**  
Soren U. Nielsen¹, Margaret Bassendine¹, Barnabas J. King², Dermot Neely³, Geoffrey L. Toms¹  
¹Liver Research Group, Newcastle University, Newcastle upon Tyne, United Kingdom. ²Clinical Medical Sciences, Newcastle University, Newcastle upon Tyne, United Kingdom. ³Clinical Biochemistry, Royal Victoria Infirmary, Newcastle upon Tyne, United Kingdom

3:15 PM  
**#116**  
**DETERMINANTS FOR MEMBRANE ASSOCIATION OF THE HEPATITIS C VIRUS NS3-4A COMPLEX**  
Jan Martin Berke¹, Volker Brass², Roland Montserret³, Hubert E. Blum⁴, François Perin⁵, Darius Moradpour¹  
¹Division of Gastroenterology and Hepatology, CHUV, University of Lausanne, Lausanne, Switzerland. ²Department of Medicine II, University of Freiburg, Freiburg, Germany. ³Institut de Biologie et Chimie des Protéines, CNRS-UMR 5086, University of Lyon, Lyon, France

3:45 PM  
**#118**  
**ANALYSIS OF NS5A REGION IMPORTANT FOR HEPATITIS C VIRUS PARTICLE PRODUCTION**  
Takahiro Masaki, Takaji Wakita  
Virology II, National Institute of Infectious Diseases, Tokyo, Japan

4:00 PM  
**#119**  
**INHIBITION OF HCV 5’NCR AND CORE EXPRESSION USING SMALL HAIRPIN RNA MEDIATED THROUGH AN NEW GENE CARRIER HPHA**  
Yanhua Ding¹, Junqi Niu¹, Runping Gao¹, Di Wu²  
¹Department of Infectious Disease, First Hospital, Jilin University, Changchun, China. ²Cancer Hospital of Jilin Province, Changchun, China

4:15 PM  
**#120**  
**INTERACTION OF APOLIPOPROTEIN E WITH STEM LOOP E1 OF THE 3’ END OF HEPATITIS C VIRUS MINUS STRAND RNA**  
Waganë J. Benga¹,², Maria Dimitrova¹,², Sophie Krieger¹,², Thomas F. Baumert¹,³, Catherine Schuster¹,²  
¹Inserm, U748, Strasbourg, France. ²Université Louis Pasteur, Strasbourg, France. ³Service d’Hépatogastroentérologie, Hôpitaux Universitaires de Strasbourg, Strasbourg, France
Hepatic Stellate Cell Biology
Monday, November 5
3:00 - 4:30 PM  Hynes, Room 304/306
MODERATORS:  Natalie Torok, MD
              Rebecca Wells, MD

3:00 PM  
#121  THE CC CHEMOKINE RANTES (CCL5) IS AN
      IMPORTANT MEDIATOR OF LIVER FIBROSIS IN
      MICE AND HUMANS
Anna Rueland¹, Mirko Moreno Zaldivar¹, David Scholten¹, Niko-
laus Gassler², Christian Weber³, Christian Trautwein¹, Hermann E.
Wasmuth¹
¹Medical Department III, University Hospital Aachen, Aachen, Ger-
many.  ²Department of Pathology, University Hospital Aachen,
Aachen, Germany.  ³Department of Molecular Cardiovascular Med-
icine, University Hospital Aachen, Aachen, Germany

3:15 PM  
#122  FUNCTIONAL CONSEQUENCES OF SINGLE
      NUCLEOTIDE POLYMORPHISMS OF TOLL-LIKE
      RECEPTOR 4 ON HEPATIC STELLATE CELLS
Jinsheng Guo¹,², Johnny C. Loke¹, Feng Zheng³, Feng Hong¹,Steven Yea¹, Hongjin Huang², Scott L. Friedman¹
¹Division of Liver Diseases, Mount Sinai School of Medicine, New
York, NY, USA.  ²Division of Digestive Diseases, Zhongshan Hospi-
tal, Department of Internal Medicine, Shanghai Medical College,
Fudan University, Shanghai, China.  ³Department of Geriatrics,
Mount Sinai School of Medicine, New York, NY, USA.  ⁴Celera
Diagnostics, Alameda, CA, USA

3:30 PM  
#123  HEPATOCYTE GROWTH FACTOR SUPPRESSES
      COLLAGEN GENE TRANSCRIPTION VIA NUCLEAR
      EXPORT OF SMAD3 WITH GALECTIN-7
Yutaka Inagaki¹, Miwa Kushida¹, Goshi Shiotani², Ichiro
Kuwabara², Jouhbu Itoh¹, Yun Yu Hong¹, Sachie Nakao¹, Reichi
Higashiyma¹, Tadashi Moro¹,², Isao Okazaki¹,⁴, Toshiyuki
Mikami⁴, Toru Kimura⁴, Kiyoshi Higashi⁷
¹Liver Fibrosis Research Unit, Tokai University School of Medicine,
Isehara, Japan.  ²Department of Genetic Medicine and Regenera-
tive Therapeutics, Graduate School of Medicine, Tottori University,
Yonago, Japan.  ³Department of Dermatology, University of Cali-
fornia, Davis, Sacramento, CA, USA.  ⁴Research Laboratory,
Minophagen Pharmaceutical Co. Ltd., Zama, Japan.  ⁵Sanno Hos-
pital, International University of Health and Welfare, Tokyo, Japan.
⁶Genomic Science Laboratories, Dainippon Sumitomo Pharma Co.,
Ltd., Osaka, Japan.  ⁷Environmental Health Science Laboratory,
Sumitomo Chemical Co., Osaka, Japan

3:45 PM  
#124  ACIDIC SPHINGOMYELINASE-MEDIATED
      CATHEPSINS REGULATION MODULATES THE
      FIBROGENIC POTENTIAL OF HEPATIC STELLATE
      CELLS
Anna Males, Jose C. Fernandez-Checa, Montserrat Mari
Liver Unit, Hospital Clinic, Barcelona, Spain

4:00 PM  
#125  GP120 INDUCES DIRECTIONAL MIGRATION OF
      HUMAN HEPATIC STELLATE CELLS: A LINK
      BETWEEN HIV INFECTION AND LIVER
      FIBROGENESIS
Raffaele Bruno¹, Sara Galastri², Fabio Marrà²
¹University of Pavia, Pavia, Italy.  ²University of Florence, Florence,
Italy

4:15 PM  
#126  APOLIPOPROTEIN AI REGULATORY PROTEIN 1
      (ARP-1) COORDINATES PROFIBROGENIC
      RESPONSES DURING LIVER FIBROSIS
Elisabetta Ceni¹, Simone Polvani², Tommaso Mello¹, Laura Cioni¹,
Francesca Buccoliero¹, Barbara Ottanelli¹, Francesca Lisi¹, Stefano
Milani¹, Andrea Galli¹
¹Department of Clinical Pathophysiology, University of Florence,
Florence, Italy.  ²FiorGen, Farmacogenomic foundation, Florence,
Italy
Late-breaking Abstracts

Monday, November 5
3:00 - 4:30 PM Hynes, Auditorium
MODERATORS: Jacqueline J. Maher, MD
T. Jake Liang, MD

Late-breaking Abstracts

3:00 PM
LB1
PROLONGED ANTIVIRAL THERAPY WITH PEGINTERFERON TO PREVENT COMPLICATIONS OF ADVANCED LIVER DISEASE ASSOCIATED WITH HEPATITIS C: RESULTS OF THE HEPATITIS C ANTIVIRAL LONG-TERM TREATMENT AGAINST CIRRHOSIS (HALT-C) TRIAL
Adrian M. Di Bisceglie1, Mitchell L. Shiffman2, Gregory T. Ever-son3, Karen L. Lindsay4, James E. Everhart5, Elizabeth C. Wright6, William M. Lee7, Anna S. Lok8, Herbert Bonkovsky9, Timothy R. Morgan10, Jules L. Dienstag11, Marc Ghany12, Chihiro Mor-ishima13, Kristin K. Snow14
1Division of Gastroenterology and Hepatology, Saint Louis University School of Medicine, St. Louis, MO, USA. 2Hepatology Section, Virginia Commonwealth University Medical Center, Richmond, VA, USA. 3Section of Hepatology, Division of Gastroenterology and Hepatology, University of Colorado School of Medicine, Denver, CO, USA. 4Division of Gastrointestinal and Liver Diseases, Keck School of Medicine, University of Southern California, Los Angeles, CA, USA. 5Division of Digestive Diseases and Nutrition, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD, USA. 6Office of the Director, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Department of Health and Human Services, Bethesda, MD, USA. 7Department of Medicine and Molecular & Structural Biology and The Liver-Biliary-Pancreatic Center, University of Connecticut Health Center, Farmington, CT, USA. 8Division of Gastroenterology and Gastroenterology Service, University of California - Irvine and VA Long Beach Healthcare System, Irvine, CA, USA. 9Gastrointestinal Unit (Medical Services) and the Department of Medicine, Massachusetts General Hospital and Harvard Medical School, Boston, MA, USA. 10Liver Diseases Branch, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Department of Health and Human Services, Bethesda, MD, USA. 11Virology Division, Department of Laboratory Medicine, University of Washington, Seattle, WA, USA. 12Liver Function and the Central Nervous System, New England Research Institutes, Watertown, MA, USA.
TOTAL TUMOR VOLUME PREDICTS RISK OF RECURRENCE FOLLOWING LIVER TRANSPLANTATION IN PATIENTS WITH HEPATOCELLULAR CARCINOMA

Christian Toso¹, James F. Trotter², Alice Wei³, David Bigam¹, Shimul Shah³, Joshua Lancaster³, David Grant³, Paul Greig³, James Shapiro¹, Norman Kneteman¹

¹Department of Surgery, University of Alberta, Edmonton, AB, Canada. ²Division of Gastroenterology/Hepatology, University of Colorado Health Sciences Center, Denver, CO, USA. ³Department of Surgery, Toronto General Hospital, University Health Network, Toronto, ON, Canada
Parallel Sessions

Steatohepatitis: Clinical
Monday, November 5
3:00 - 4:30 PM  Hynes, Ballroom B
MODERATORS:  Paul Angulo, MD
               Manal F. Abdelmalek, MD, MPH

3:00 PM  
#127  HEREDITABILITY OF NONALCOHOLIC FATTY LIVER DISEASE
Jeffrey B. Schwimmer1, Manuel Celedon1, Rany Salem2, Nicholas J. Schork2, Takeshi Yokoo3, Alyssa Chavez3, Michael S. Middleton3, Claude B. Sirlin3
1Pediatrics, University of California, San Diego, San Diego, CA, USA. 2Psychiatry, University of California, San Diego, San Diego, CA, USA. 3Radiology, University of California, San Diego, San Diego, CA, USA

3:15 PM  
#128  FRUCTOSE INDUCED HYPERURICEMIA AS A CAUSAL MECHANISM FOR NONALCOHOLIC FATTY LIVER DISEASE
Manal F. Abdelmalek1, Ayako Suzuki1, Cynthia Guy1, Richard J. Johnson2, Anna Mae Diehl1, for the NASH Clinical Research Group3
1Division of Gastroenterology, Duke University, Durham, NC, USA. 2Division of Nephrology, University of Florida, Gainesville, FL, USA. 3NIDDK, National Institutes of Health, Baltimore, MD, USA

3:30 PM  
#129  DECREASED GM1 EXPRESSION IN MEMBRANE LIPID RAFT IN CD4 AND CD8 CELLS OF PATIENTS WITH NASH: A POTENTIAL BIOMARKER AND THERAPEUTIC TARGET
Gadi Lalazar1, Ehud Zigmond1, Ami Ben Ya’acov1, Dan M. Livovsky1, Esther Admon2, Leah Gheber3, Alexander Fich2, Orit Pappo4, Yaron Ilan1
1Liver Unit, Hadassah Hebrew University Medical Center, Jerusalem, Israel. 2Department of Gastroenterology, Ben-Gurion University of the Negev and Soroka University Medical Center, Beer Sheva, Israel. 3Departments of Clinical Biochemistry and Chemistry, Ben-Gurion University of the Negev, Beer Sheva, Israel. 4Department of Pathology, Hadassah Hebrew University Medical Center, Jerusalem, Israel

3:45 PM  
#130  EFFECT OF BARIATRIC SURGERY ON NONALCOHOLIC FATTY LIVER DISEASE (NAFLD): A META-ANALYSIS
Rajasekhara r. Mummadi1, Krishna S. Kasturi2, Gagan Sood1
1Gastroenterology & Hepatology, UTMB, Galveston, TX, USA. 2Internal Medicine, UTMB, GALVESTON, TX, USA

4:00 PM  
#131  DIFFERENTIAL HEPATIC EXPRESSION OF LUMICAN AND FATTY ACID BINDING PROTEIN-1 IN HISTOLOGICALLY PROGRESSIVE NAFLD – NOVEL POTENTIAL INSIGHTS INTO HISTOLOGICAL SPECTRUM OF DISEASE
Michael R. Charlton, Allard Jan Kalsbeek, Kimberly Viker, Anuradha Krishnan, Bart J. Veldt, Michael Kendrick, Geoffrey Thompson, Florencia G. Que, Michael Sarr
Liver Transplant, Mayo Clinic, Rochester, MN, USA

4:15 PM  
#132  A RANDOMIZED, DOUBLE BLIND, PLACEBO CONTROLLED TRIAL OF ONE YEAR OF PIOGLITAZONE IN NON-DIABETIC SUBJECTS WITH NONALCOHOLIC STEATOHEPATITIS
Guruprasad P. Athal1, James A. Thomas1, Philip Kaye2,1, Adam Lawson1, Stephen D. Ryder1, Andrew S. Austin1, John G. Freeman1,1, Linda Morgan3, Webber Jonathan5
1Wolfson Digestive Diseases Centre, University Hospital, Nottingham, United Kingdom. 2Histopathology, University Hospital, Nottingham, United Kingdom. 3Clinical Chemistry, Nottingham University, Nottingham, United Kingdom. 4Gastroenterology, Derby Hospital, Derby, United Kingdom. 5Diabetes Centre, Selly Oak Hospital, Birmingham, United Kingdom

HCV: Epidemiology
Monday, November 5
4:45 - 6:15 PM  Hynes, Auditorium
MODERATORS:  Hashem B. El-Serag, MD, MPH
               Miriam J. Alter, PhD, MPH

4:45 PM  
#133  CHRONIC HEPATITIS C VIRUS INFECTION AND RISK OF HEPATOCELLULAR CARCINOMA: A COMMUNITY-BASED PROSPECTIVE STUDY ON 19,565 RESIDENTS IN TAIWAN
Mei-Hsuan Lee1, Hwa-I Yang2, Chun-Jen Liu3, San-Lin You2, Pei-Jer Chen3, Chien-Jen Chen1,2
1Institute of Epidemiology, National Taiwan University, Taipei city, Taiwan. 2Genomics Research Center, Academia Sinica, Taipei city, Taiwan. 3Department of Internal Medicine, National Taiwan University Hospital, Taipei city, Taiwan
#134
LOW UPTAKE OF TREATMENT FOR HEPATITIS C VIRUS (HCV) INFECTION IN A LARGE COMMUNITY-BASED COHORT OF ILLICIT DRUG USERS IN VANCOUVER

Jason Grebely1,8, Jesse D. Raffa2, Calvin Lai3, Mel Krajden4, Benedikt Fischer5, Thomas Kerr6, Mark W. Tyndall7,8

1Anesthesiology, Pharmacology and Therapeutics, University of British Columbia, Vancouver, BC, Canada. 2Statistics and Actuarial Science, University of Waterloo, Waterloo, ON, Canada. 3BC Centre for Excellence in HIV/AIDS, Vancouver, BC, Canada. 4BC Centre for Disease Control, Vancouver, BC, Canada. 5Centre for Addictions Research BC, University of Toronto, Toronto, ON, Canada. 6Department of Medicine, University of British Columbia, Vancouver, BC, Canada. 7Vancouver Coastal Health, Vancouver, BC, Canada

#135

Eric Rosenthal1,10, Dominique Salmon2, Charlotte Lewden3, Fabrice Bonnet4,8, Thierry May5, Philippe Morlat6,10, Muriel Francois5, Christine Burty4, Eric Jouglar4, Dominique Costagliola7,8, Geneviève Chêne3, Patrice Cacoub9,10

1Médecine Interne, CHU de Nice, Université de Nice Sophia Antipolis, Nice, France. 2Médecine Interne, Groupe Hospitalier Cochin-Tarnier, Paris, France. 3Inserm U593 et ISPED, Bordeaux, France. 4Médecine Interne, Hôpital Saint-André, Bordeaux, France. 5Maladies Infectieuses, CHU Vandoeuvre-Les-Nancy, Nancy, France. 6Inserm CépiDc, Le Vésinet, France. 7Université Pierre et Marie Curie, Paris, France. 8Inserm U720, Paris, France. 9Médecine Interne, Groupe Hospitalier Pitié Salpêtrière, Paris, France. 10Germivic, Paris, France

#136
STRONG ASSOCIATION BETWEEN TATTOOS AND HEPATITIS C VIRUS INFECTION: A MULTICENTER STUDY OF 3,871 PATIENTS

Sameer Dhalla1, Craig T. Tenner2, Ayse Aytaman3, Nilesh B. Shukla3, Gerald Villanueva4, Grace Punla5, Carlie Patterson4, JoAnn Comas3, Edmund J. Bini6

1Department of Medicine, NYU School of Medicine, New York, NY, USA. 2Division of General Internal Medicine, VA Medical Center & NYU School of Medicine, New York, NY, USA. 3Division of Gastroenterology, VA Medical Center, Brooklyn, NY, USA. 4Division of Gastroenterology, Bellevue Hospital Center & NYU School of Medicine, New York, NY, USA. 5Division of Gastroenterology, VA Medical Center & NYU School of Medicine, New York, NY, USA

#137
EVIDENCE OF INTERNATIONAL TRANSMISSION OF HCV IN PAN-EUROPEAN STUDY OF HIV-POSITIVE MEN WHO HAVE SEX WITH MEN (MSM)

Mark Danta1,2, Thijs van de Laar3, David Brown2, Olivier Pybus3, Sanjay Bhagani4, Martin Vogel5, Stefan Neifer6, Axel Baumgarten7, Helena Gotz7, Juergen Rockstoh5, Sylvia Bruisten8, Geofreym D. Dusheiko2

1St Vincent’s Clinical School, Sydney, NSW, Australia. 2Centre for Hepatology, University College and Royal Free Medical Schools, London, United Kingdom. 3Department of Zoology, University of Oxford, Oxford, United Kingdom. 4Department of HIV Medicine, Royal Free Hospital, London, United Kingdom. 5University of Bonn, Bonn, Germany. 6Practice Dupke, Carganico, Baumgarten, Berlin, Germany. 7Department of Infectious Diseases, Health Service, Rotterdam, Netherlands. 8Cluster of Infectious Disease, Health Service, Amsterdam, Netherlands

Hepatic Fibrogenesis: In Vivo Models and Clinical Studies

Monday, November 5

4:45 - 6:15 PM Hynes, Room 304/306

MODERATORS:
Wajahat Z. Mehal, MD
John P. Iredale, MD, FRCP

#138
ANGIOTENSIN-CONVERTING- ENZYME 2 IS A NEGATIVE REGULATOR OF CHRONIC LIVER INJURY

Christoph H. Oesterreicher1, Ekihiro Seki1, Samuele De Minicis1, KOJIRO TAURA1, Melitta Penz-Oesterreicher1, Johannes Kluwe2, David A. Brenner1

1Medicine, University of California, San Diego, CA, USA. 2Medicine, Columbia University, New York, NY, USA
5:00 PM

#140
REDUCED HEPATIC FIBROSIS IS ASSOCIATED WITH FEWER INTRAHEPATIC B CELLS IN FIBROBLAST ACTIVATION PROTEIN AND DIPEPTIDYL PEPTIDASE IV GENE KNOCKOUT MICE

Xin M. Wang\(^1\), Shaun Cordoba\(^1\), Didier Marguet\(^2\), Wolfgang Retting\(^2\), Andreas Schnapp\(^2\), Geoff W. McCaughan\(^1\), Mark D. Gorrell\(^1\)
\(^1\)Centenary Institute, Faculty of Medicine, University of Sydney, Sydney, NSW, Australia. \(^2\)Boehringer Ingelheim, Vienna, Austria.

5:15 PM

#141
GHRELIN ATTENUATES ACUTE AND CHRONIC HEPATIC INJURY IN RATS AND INFLUENCES FIBROSIS PROGRESSION IN PATIENTS WITH CHRONIC HEPATITIS C

Montserrat Moreno\(^1\), Javier F. Chaves\(^2\), Pau Sancho-Brú\(^1\), Fernando Ramalho\(^3\), Leandra N. Ramalho\(^1\), Josep Vidal\(^4\), Xavier Forns\(^1\), Montserrat Mari\(^3\), Maria L. Mansego\(^5\), Albert Morales\(^5\), Jordi Colmenero\(^1\), Marlene Dominguez\(^1\), Vicente Arroyo\(^1\), Juan Caballería\(^1\), Pere Gines\(^1\), Ramón Bataller\(^1\)
\(^1\)Liver Unit, IDIBAPS, CIBEK, Hospital Clinic de Barcelona, Barcelona, Spain. \(^2\)Laboratorio de Estudios Genéticos, Fundación de Investigación, Hospital Clínico Universitario de Valencia, Valencia, Spain. \(^3\)Consejo Superior de Investigaciones Científicas, Barcelona, Spain. \(^4\)Endocrinology Unit, Hospital Clinic de Barcelona, Barcelona, Spain.

5:30 PM

#142
COMPETITIVE INHIBITION OF LEPTIN SIGNALING BY A MUTAGENIC PEPTIDE RESULTS IN IMPROVEMENT OF HEPATIC FIBROSIS THROUGH INHIBITION OF HEPATIC STELLATE CELL ACTIVATION

Eran Elina\(^1\), Rafi Bruck\(^1\), Muhammed Ali\(^1\), Eli Brasowski\(^1\), Adam Phillips\(^1\), Zamir Halpern\(^1\), Arieh Gertler\(^2\)
\(^1\)Tel Aviv Sourasky Medical Center, Tel Aviv, Israel. \(^2\)Faculty of Agriculture, Hebrew University, Rehovot, Israel

5:45 PM

#143
INTEGRIN ALPHAV BETA 6 IS A UNIQUE PROGRESSION MARKER AND TARGET FOR ANTIFIBROTIC THERAPIES IN LIVER FIBROGENESIS

Yury Popov\(^1\), Jessica Zaks\(^1\), Killamangalam Bhaskar\(^1\), Eleonora Paterson\(^2\), Gerald Niedobitek\(^3\), Armin Kolb\(^4\), Helmut Friess\(^4\), Detlef Schuppan\(^1\)
\(^1\)Gastroenterology, Beth Israel Deaconess Medical Center, Boston, MA, USA. \(^2\)Clinical Pharmacology, University of Bern, Bern, Switzerland. \(^3\)Institute of Pathology, University of Erlangen, Erlangen, Germany. \(^4\)General Surgery, University of Heidelberg, Erlangen, Germany.

5:00 PM

#144
REGRESSION OF FIBROSIS AMONG LONG-TERM RESPONDERS TO ANTIVIRAL TREATMENT FOR CHRONIC VIRAL HEPATITIS

Cosimo Colletta\(^1\), Carlo Smirne\(^2\), Carlo Fabris\(^3\), Anna M. Foscolo\(^4\), Pierluigi Toniotti\(^5\), Rachele Rapetti\(^6\), Rosalba Minisini\(^7\), Lisa Sala\(^8\), Stefano Fangazio\(^9\), Mario Pirisi\(^10\)
\(^1\)Division of Medicine, COQ, Omegna, Italy. \(^2\)Dpt of Clinical & Experimental Medicine, University of Eastern Piedmont, Novara, Italy. \(^3\)DPMSC, University of Udine, Udine, Italy. \(^4\)Pathology, ASL 14, Verbania, Italy.

Innate and Adaptive Immunity in Liver Disease
Monday, November 5
4:45 - 6:15 PM Hynes, Room 311
MODERATORS: John M. Vierling, MD Pronati Mandrekar, PhD

4:45 PM

#145
CROSSPRESENTING LSEC PROMOTE LIVER-SPECIFIC NAÏVE CD8 T CELL RETENTION AND TOLERANCE IN VIVO

Nanette von Oppen\(^1\), Anna Schurich\(^1\), Rene Tolba\(^2\), Linda Diehl\(^1\), Percy Knolle\(^1\)
\(^1\)Molecular Medicine, University of Bonn, Bonn, Germany. \(^2\)House for Experimental Therapy, University of Bonn, Bonn, Germany.

5:00 PM

#146
CHRONIC HBV EVOLUTION IS ASSOCIATED WITH NUMERIC AND PHENOTYPIC CHANGES IN PERIPHERAL HBV-CORE EPITOPE SPECIFIC CD4+ T-CELLS: A STUDY USING A NOVEL HBV-CORE SPECIFIC HLA-DRB1*0101 TETRAMER FOR THE ANALYSIS OF ANTIVIRAL CD4+ T-CELL RESPONSES DURING ACUTE AND CHRONIC HEPATITIS B

Malte H. Heeg\(^1\), Axel Ulsenheimer\(^1\), Norbert H. Gruener\(^1\), Helmut M. Diepolder\(^1\), Winfried Schraut\(^2\), Martin Waechtler\(^3\), Reinhart Zachoval\(^1\), Gerd R. Pape\(^1,2\), Maria C. Jung\(^1,2\)
\(^1\)Dept. of Medicine II, LMU-Munich, Munich, Germany. \(^2\)Dept. of Immunology, LMU Munich, Munich, Germany. \(^3\)Hospital Munich-Schwabing, Munich, Germany.

5:15 PM

#147
A NOVEL THERAPEUTIC HBV VACCINE INDUCES POTENT SURFACE- AND CORE-SPECIFIC IMMUNOGENICITY IN MICE, Rhesus Macaques and HBV Transgenic Mice

Jason D. Marshall\(^1\), Marianne L. Gesner\(^1\), Darren S. Heeke\(^1\), Pascale Buchmann\(^2\), Eduardo Martins\(^1\), Brian D. Livingston\(^1\), Karl Melber\(^2\), Gary Van Nest\(^1\)
\(^1\)Dynavax Technologies, Berkeley, CA, USA. \(^2\)Dynavax Europe, Dusseldorf, Germany.
5:30 PM

#148

HDV-SPECIFIC IP-10 RESPONSES BUT NOT IP-10 SERUM LEVELS CORRELATE WITH RESPONSE TO PEG-IFNA-2A TREATMENT OF DELTA HEPATITIS: RESULTS FROM THE HEP-NET/INTERNATIONAL HIDIT-1 STUDY

Heiner Wedemeyer1, Cihan Yurdaydın2, Ayse Ciner1, Peter Hoffmann3, Peter Buggisch4, Nuray Aslan1, Kalliopi Zachou1, Stefan Zeuzem5, Michael P. Manns1

1Gastroenterology, Medizinische Hochschule Hannover, Hannover, Germany. 2Ankara University, Ankara, Turkey. 3University of Frankfurt, Frankfurt, Germany. 4University of Hamburg, Hamburg, Germany

5:45 PM

#149

A COMBINATION OF BETA-GLYCOLIPIDS OVERCOMES A CD1D DEPENDENT INHIBITION OF NKT LYMPHOCYTES: AN ALTERNATIVE FLOTILLIN-2 RAFT PROTEIN DEPENDENT REGULATORY CELL ACTIVATION PATHWAY

Gadi Lalazar, Ami Ben Ya’acov, Dan M. Livovsky, Sarah Preston, Yaron Ilan
Liver Unit, Hadassah Hebrew University Medical Center, Jerusalem, Israel

6:00 PM

#150

NATURAL KILLER DENDRITIC CELLS ARE A DISTINCT CLASS OF HYBRID DENDRITIC CELLS WITHIN THE MURINE LIVER NK1.1+ CELL POPULATION

Bryan M. Burt, George Plitas, Jennifer A. Stableford, Hoang M. Nguyen, Ronald P. DeMatteo
Hepatobiliary Surgery, Memorial Sloan-Kettering Cancer Center, New York, NY, USA

Signal Transduction and Nuclear Receptors
Monday, November 5

4:45 - 6:15 PM  Hynes, Room 309

MODERATORS:
Mark J. Czaja, MD
David Rudnick, MD, PhD

4:45 PM

#151

RAS/PI3-K/AKT PROMOTES CELLULAR GROWTH BY ENHANCING ALTERNATIVE SPLICING-MEDIATED INACTIVATION OF THE KLF6 TUMOR SUPPRESSOR IN HUMAN HEPATOCELLULAR CARCINOMA

Steven Yea, Goutham Narla, Xiao Zhao, Augusto Villanueva, John A. Martignetti, Josep M. Llovet, Scott I. Friedman
Mount Sinai School of Medicine, New York, NY, USA

5:00 PM

#152

THE TRANSCRIPTION FACTOR STAT5 CONFINES TGF-BETA LEVELS IN THE LIVER AND SERVES AS A PROTECTIVE AGENT IN LIVER FIBROSIS AND CANCER

Atsushi Hosui, Lothar Hennighausen
NIDDK, National Institute of Health, Bethesda, MD, USA

5:15 PM

#153

TGF-β PATHWAY MEMBERS SMAD3 AND ELF ARE KEY REGULATORS OF HEPATOCELLULAR CANCERS

Young Woo Kim1, Peter Kreishman2, Chohee Yun1, Wilma Jogonoori1, Eugene A. Volpe1, Tapas Saha1, Jonathan S. Mendelson1, Tiffany M. Blake1, Michael J. Pishvaian1, Yi Tang1, Bibhuti Mishra1, Lopa Mishra1,2
1Surgery, Georgetown University, Washington, MD, USA. 2DVAMC, Washington, DC, USA

5:30 PM

#154

REGULATION OF WNT/β-CATENIN PATHWAY BY CPLA2α-MEDIATED PPARδ ACTIVATION IN HUMAN CHOLANGIOCARCINOMA CELLS

Chang Han, Kyu Lim, LiHong Xu, Quiying Li, Tong Wu
Department of Pathology, University of Pittsburgh, School of Medicine, Pittsburgh, PA, USA

5:45 PM

#155

POTENTIATION OF PERIPHERAL INSULIN SENSITIVITY IN OB/OB MICE BY BETA-GLYCOLIPIDS IS MEDIATED BY IRS-1 MTOR SIGNALING PATHWAYS: A NOVEL THERAPEUTIC TARGET FOR NASH

Madi El-Haj1, Gadi Lalazar1, Ami Ben Ya’acov1, Mali Katznel-Gilad2, Gil Leibowitz2, Yaron Ilan1
1Liver Unit, Hadassah Hebrew University Medical Center, Jerusalem, Israel. 2Endocrinology and Metabolism, Hadassah Hebrew University Medical Center, Jerusalem, Israel

6:00 PM

#156

IMPACT OF HEPATOCYTE SPECIFIC LOSS OF C-MET SIGNALING ON LIVER REGENERATION

Valentina M. Factor, Pal Kaposi-Novak, Elizabeth A. Conner, Snorri S. Thorgeirsson
Laboratory of Experimental Carcinogenesis, National Cancer Institute, Bethesda, MD, USA
Steatosis: Experimental  
Monday, November 5  
4:45 - 6:15 PM  Hynes, Ballroom B  
MODERATORS:  Richard M. Green, MD  
Gyorgy Baffy, MD, PhD

4:45 PM  
#157  
DIFFERENTIAL EFFECTS OF JNK1 AND JNK2 ON THE DEVELOPMENT OF STEATOHEPATITIS  
Rajat Singh, Yongjun Wang, Youqing Xiang, Kathryn Tanaka, William A. Gaarde, Mark J. Czaja  
1Albert Einstein College of Medicine, Bronx, NY, USA. 2Isis Pharmaceuticals, Inc, Carlsbad, CA, USA

5:00 PM  
#158  
A PIVOTAL ROLE FOR CD154 IN LIVER STEATOSIS  
1Inserm U 889 and IFR 66, Bordeaux, France. 2Universite Victor Segalen Bordeaux 2, Bordeaux, France. 3EA 3842, Universite de Limoges, Limoges, France. 4Centre de Reference des Pathologies Plateaupaires, Centre Hospitalier et Universitaire and IF 4, Bordeaux, France

5:15 PM  
#159  
TRANSCRIPTION FACTOR NRF2 REGULATES HEPATIC LIPID METABOLISM THROUGH ORPHAN NUCLEAR RECEPTOR SHP  
Jiansheng Huang, Ningyi Xu, Noriko Esterly, Robert Nedved, Xilin Chen, Curtis Klaassen, Grace Guo, Li Wang  
1Medicine & Pharmacology, University of Kansas Medical Center, Kansas, KS, USA. 2Pharmacology, University of Kansas Medical Center, Kansas, KS, USA. 3AtheroGenics, Inc., Alpharetta, GA, USA

5:30 PM  
#160  
PROBIOTICS IMPROVE HIGH-FAT DIET INDUCED STEATOSIS AND INSULIN RESISTANCE THROUGH MODULATION OF HEPATIC NKT CELLS  
Xiong Ma, Jing Hua, Zhiping Li  
1Division of Gastroenterology, Johns Hopkins University, Baltimore, MD, USA. 2Gastroenterology, Renji Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai, China

5:45 PM  
#161  
RATS SELECTIVELY BREED FOR LOW AEROBIC CAPACITY DEVELOP NONALCOHOLIC FATTY LIVER DISEASE  
John Thyfault, R. Scott Rector, Grace Uptergrove, E. M. Morris, Yangzhong Wei, Matthew Laye, Suzanne Clark, L. G. Koch, S. L. Britton, Jamal A. Ibdah  
1Nutritional Science and Internal Medicine, University of Missouri, Columbia, MO, USA. 2Research, Harry S. Truman Memorial VA Hospital, Columbia, MO, USA. 3Physical Medical and Rehabilitation, University of Michigan, Ann Arbor, MO, USA. 4Molecular and Integrative Physiology, University of Michigan, Ann Arbor, MO, USA. 5Internal Medicine, University of Missouri, Columbia, MO, USA. 6Medical Pharmacology and Physiology, University of Missouri, Columbia, MO, USA

6:00 PM  
#162  
CB2 RECEPTOR ANTAGONISM REDUCES DIET-INDUCED OBESITY, INSULIN RESISTANCE AND HEPATIC STEATOSIS  
Vanessa Devaux, Yasukatsu Ichigotani, Fatima Teixeira-Clerc, Jeanne Tran-Van-Nhieu, Thomas Deacon, Melih Karzak, Andreas Zimmer, Ariane Mallat, Sophie Lotersztajn  
1INSERM, U841, Creteil, France. 2AP-HP, Groupe hospitalier Henri Mondor-Albert Chenevier, Service d’Hepatologie et de, Creteil, France. 3AP-HP, Groupe hospitalier Henri Mondor-Albert Chenevier, Dept de Pathologie, Creteil, France. 4University of Bonn, Department of Psychiatry,University of Bonn, Bonn, Germany
Focused Study Groups

### Monday, November 5
7:00 - 10:00 PM  Hynes, Room 302

**FSG-1: End Points and Study Design in Clinical Trials in Pediatric Hepatology**

**COURSE DIRECTOR:** Benjamin L. Shneider, MD

Special issues exist in the design of clinical trials involving pediatric participants. Currently there are four ongoing, NIH-funded multi-centered drug trials in pediatric hepatology. This session will review the study design in these trials and explore issues in designing future studies in pediatric hepatology.

**Goals and Objectives:**
- Understand special issues relating to the conduct of clinical trials in pediatric hepatology.
- Be aware of current drug trials in pediatric hepatology.
- Critically assess potential drug trial design for children with liver disease.

- **7:00 - 7:10 PM**  
  **Introduction**  
  Benjamin L. Shneider, MD

- **7:10 - 7:35 PM**  
  **Endpoints from the Perspective of the NIH and FDA**  
  Patricia R. Robuck, PhD

- **7:35 - 8:00 PM**  
  **Vitamin E or Metformin for Pediatric NAFLD**  
  Joel E. Lavine, MD, PhD

- **8:00 - 8:25 PM**  
  **Pegylated Interferon +/- Ribavirin for Hepatitis C**  
  Kathleen B. Schwarz, MD

- **8:25 - 8:50 PM**  
  **Perioperative Steroids for Biliary Atresia**  
  Jorge Bezerra, MD

- **8:50 - 9:15 PM**  
  **N-Acetylcysteine for Acute Liver Failure**  
  Robert H. Squires, MD

- **9:15 - 10:00 PM**  
  **Discussion**

### FSG-2: Portopulmonary Hypertension in the Era of Liver Transplantation
7:00 - 10:00 AM  Hynes, Room 304/306

**COURSE DIRECTORS:**  
Norman L. Sussman, MD  
Michael J. Krowka, MD

Moderate to severe portopulmonary hypertension (POPH) causes excess mortality in cirrhosis and is considered by many to be a contraindication to liver transplantation. Questions remain about a precise definition, criteria for treatment, and the role of either cadaveric or living-related liver transplantation. This workshop will propose a working definition of POPH, explore the pathophysiology of the condition and update the growing list of agents available for treatment. The benefits and dangers of pharmacotherapy in the context of liver transplantation and a suggested framework for future clinical trials will be discussed.

- **7:00 - 7:05 PM**  
  **Introduction and Welcome**  
  Norman L. Sussman, MD

- **7:05 - 7:30 PM**  
  **POPH: Historical Perspective to Current Knowledge**  
  Michael J. Krowka, MD

- **7:30 - 7:35 PM**  
  **Discussion**

- **7:35 - 7:55 PM**  
  **Pharmacotherapy - Pharmacogenetics: Proposed Mechanisms and Potential Drug Targets - Caveats**  
  Steven M. Kawut, MD

- **7:55 - 8:00 PM**  
  **Discussion**

- **8:00 - 8:20 PM**  
  **POPH Outcome Data to Date - Retrospective - Prospective Correlates**  
  Karen Swanson, DO

- **8:20 - 8:30 PM**  
  **Discussion and Break**

- **8:30 - 8:50 PM**  
  **Pretransplant Management of POPH**  
  Norman L. Sussman, MD

- **8:50 - 9:00 PM**  
  **Discussion**

- **9:00 - 9:20 PM**  
  **POPH Registry - Goals, Current Status, Future Research Directions & Animal Models**  
  Michael B. Fallon, MD

- **9:20 - 9:30 PM**  
  **Discussion**

- **9:30 - 9:55 PM**  
  **Panel Discussion: Issues to Be Addressed in Future Trials**  
  Norman L. Sussman, MD and Michael J. Krowka, MD

- **9:55 - 10:00 PM**  
  **Summary & Closing Remarks**  
  Norman L. Sussman, MD
Early Morning Workshops

**Tuesday, November 6**
6:45 - 7:45 AM  Refer to your luncheon ticket for meeting room location.

**Basic Research Workshops**

- **EM-30** Molecular Virology of Hepatitis C  
  T. Jake Liang, MD and Jean-Michel Pawlotsky, MD, PhD
- **EM-31** Cell Death  
  Mark J. Czaja, MD and Peter R. Galle, MD
- **EM-32** Experimental Therapies for Hepatitis C  
  Stefan Zeuzem, MD and David R. Nelson, MD
- **EM-33** Experimental Therapies for Hepatitis B  
  Jules L. Dienstag, MD and Mang M. Ma, MD
- **EM-34** Regeneration  
  Rebecca A. Taub, MD and Christian Trautwein, MD
- **EM-35** Mechanisms of Trafficking and Motility  
  Allan W. Wolkoff, MD and Hal F. Yee, MD, PhD

**Clinical Management Workshops**

- **EM-36** Viral Hepatitis in Children  
  Regino P. Gonzalez-Peralta, MD and Benjamin L. Schneider, MD
- **EM-37** Non-Invasive Diagnosis of NASH  
  Nezam H. Afzhal, MD and Ariel E. Feldstein, MD
- **EM-38** Emerging Therapies for HBV  
  Ira M. Jacobson, MD and Anna S. F. Lok, MD
- **EM-39** Current and Emerging Therapies of Hepatocellular Cancer  
  Adrian M. Di Bisceglie, MD and Morris Sherman, MD, PhD
- **EM-40** Nutrition in Liver Disease  
  Jaime Aranda-Michel, MD and Srinivasan Dasarathy, MD

**Poster Session 4**

**Tuesday, November 6, 2007**
8:00 AM – 12:30 PM
Hynes, Exhibit Hall C

Refer to page 202A for Poster Presentations
Plenary Sessions

Presidential Plenary III
Tuesday, November 6
8:00 - 9:30 AM  Hynes, Auditorium
MODERATORS:  Arun J. Sanyal, MD
              T. Jake Liang, MD

8:00 AM
#163
EXCELLENT LONG-TERM OUTCOME FOLLOWING DOWN-STAGING OF HEPATOCELLULAR CARCINOMA PRIOR TO LIVER TRANSPLANTATION: AN INTENTION-TO-TREAT ANALYSIS
Francis Y. Yao1,2, Robert Kerlan3, Timothy J. Davern1, Ryo Hirose2, Nathan M. Bass1, Sandy Feng2, Marion G. Peters1, Norah Terrault1, Chris Freise2, Nancy L. Ascher2, John P. Roberts2
1Medicine, University of California, San Francisco, San Francisco, CA, USA. 2Surgery, University of California, San Francisco, San Francisco, CA, USA. 3Radiology, University of California, San Francisco, San Francisco, CA, USA

8:15 AM
#164
HCV PATIENTS WITH GENOTYPE 2 OR 3 WHO DO NOT ACHIEVE A RAPID Virologic Response (RVR) WITH PEGINTERFERON ALFA-2A (40KD)(PEGASYS®) AND RIBAVIRIN (COPEGUS®) ARE NOT EASY TO TREAT: AN ANALYSIS OF NON-RVR PATIENTS FROM THE ACCELERATE STUDY
Mitchell L. Shiffman1, David R. Nelson2, Greg Hooper3, Diethelm Messinger4, Stefan Zeuzem5
1Hepatology Section, VCU Medical Center, Richmond, VA, USA. 2Department of Medicine, University of Florida, Florida, FL, USA. 3Roche, Welwyn, United Kingdom. 4IST GmbH, Mannheim, Germany. 5Department of Medicine I, J.W. Goethe University Hospital, Frankfurt, Germany

8:45 AM
#166
THE PLASMA LIPIDOMIC SIGNATURE OF NONALCOHOLIC STEATOHEPATITIS: DIFFERENTIAL LEVELS OF N-3 AND N-6 POLYUNSATURATED FATTY ACIDS AND THEIR LIPOXYGENASE PRODUCTS
Puneet Puri1, Michelle M. Wiest2, Meeta Patnaik2, Onpan Cheung1, Carol C. Sargeant1, Faridoddin Mirshahi1, Steven M. Watkins2, Arun J. Sanyal1
1Division of Gastroenterology, Hepatology and Nutrition, Virginia Commonwealth University, Richmond, VA, USA. 2Lipomics Technologies, Inc., West Sacramento, CA, USA
9:00 AM
#167
ROBUST SYNERGISTIC ANTIVIRAL EFFECT OF R1626 IN COMBINATION WITH PEGINTERFERON ALFA-2A (40KD), WITH OR WITHOUT RIBAVIRIN – INTERIM ANALYSIS RESULTS OF PHASE 2A STUDY
1Scripps Clinic, La Jolla, CA, USA. 2University of Florida, Gainesville, FL, USA. 3University Hepatitis Center, Bradenton, FL, USA. 4Fundacion de Investigacion de Diego, Santurce, PR, USA. 5University of Colorado Health Sciences Center, Denver, CO, USA. 6University of North Carolina at Chapel Hill, Chapel Hill, NC, USA. 7The Liver Institute at Dallas Methodist Hospital, Dallas, TX, USA. 8Brooke Army Medical Center, Houston, TX, USA. 9Kaiser Permanente Medical Center, San Diego, CA, USA. 10McGuire VA Medical Center, Richmond, VA, USA. 11Roche, Nutley, NJ, USA.

9:15 AM
#168
THE DIRECT TRIAL (DAILY-DOSE CONSENSUS INTERFERON AND RIBAVIRIN: EFFICACY OF COMBINED THERAPY): TREATMENT OF NON-RESPONDERS TO PREVIOUS PEGYLATED INTERFERON PLUS RIBAVIRIN: SUSTAINED VIROLOGIC RESPONSE DATA
Bruce Bacon 1, Arie Regev 2, Reem H. Ghalib 3, Giuseppe Morelli 4, Kenneth D. Rothstein 5, Mitchell L. Shiffman 6, Tarek Hassanein 7, Janet Hammond 8
1Saint Louis University Liver Center, Saint Louis University School of Medicine, St. Louis, MO, USA. 2University of Miami, Miami, FL, USA. 3Liver Institute at Methodist Hospital, Dallas, TX, USA. 4University of Florida, Gainesville, FL, USA. 5Albert Einstein Center for Liver Disease, Philadelphia, PA, USA. 6Virginia Commonwealth University, Richmond, VA, USA. 7University of California San Diego, San Diego, CA, USA. 8Valeant Pharmaceuticals North America, Aliso Viejo, CA, USA.

State-of-the-Art Lecture

Tuesday, November 6
9:30 - 10:00 AM Hynes, Auditorium
Leon Schiff State-of-the-Art Lecture

Primary Biliary Cirrhosis: Current Concepts, Emerging and Rational Therapies

Speaker: Raoul Poupon, MD
Moderator: Nicholas F. LaRusso, MD

Dr. Raoul Poupon is Professor of Hepatology and Gastroenterology at the Pierre et Marie Curie University and Head of the Department of Hepatology and Gastroenterology at Hospital Saint-Antoine in Paris. He is also Director of the National Reference Center dedicated to research and treatment of inflammatory biliary diseases in France and a member of the French National Academy of Medicine. Dr. Poupon received in 2006 the Adolph Windaus Prize for his major contribution in the field of bile acid research. He has been member of the Editorial Board of Hepatology for 15 years. Dr Poupon has written nearly 400 peer-reviewed research papers, review articles, editorials and book chapters in many areas of liver diseases. He has carried out many biological and clinical studies devoted to understanding the role of UDCA in PBC and cholestatic liver diseases. He has identified a syndrome referred to as “Low Phospholipid Associate Cholelithiasis and Cholestasis” and shown its association with defects in the MDR3 gene.

Goals and Objectives:

• To learn recent advances in the pathogenesis of this complex disease and, in particular, understand how genetic as well environmental factors and their stochastic interactions may explain susceptibility to and clinical diversity of PBC.
• To review state-of-art information on the nature and role of the pathobiological processes and mechanisms involved in liver injury in PBC.
• To learn recent advances in the clinical evaluation of PBC and to recognize the early signals of severity.
• To link mechanisms, biological and clinical aspects to current as well as emerging and rationale therapies.

The Leon Schiff State-of-the-Art Lecture honors Dr. Schiff and recognizes the work he did to elevate the study and practice of hepatology to the discipline it is today. The restricted fund supporting this lecture ensures that future hepatologists will have a distinct platform from which to provide their valuable insights at The Liver Meeting®. AASLD gratefully acknowledges the National Genetics Institute for its generous support of this fund.

AASLD BUSINESS MEETING (MEMBERS ONLY)
Tuesday, November 6
10:00 – 11:00 AM Hynes Convention Center Auditorium
Gregory J. Gores, MD, presiding
### Parallel Sessions

#### Advances in the Molecular and Cellular Biology of Bile Formation

**Tuesday, November 6**

**11:15 AM - 12:45 PM**  
**Hynes, Room 309**

**MODERATORS:**  
Michael Trauner, MD  
Shannon Glaser, PhD

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| 11:15 AM | #169      | CONFIRMATION OF THE GENES ENCODING THE BILIARY CHOLESTEROL TRANSPORTER ABCGS/ABCG8 AS GALLSTONE SUSCEPTIBILITY (LITH) GENES | Ulrike Kurtz, Daniel Teupser, Albrecht Hoffmeister, Joachim Mossner, Henning Wittenburg | Department of Medicine II, University of Leipzig, Leipzig, Germany.  
Institute of Laboratory Medicine, University of Leipzig, Leipzig, Germany. |
| 11:30 AM | #170      | A NOVEL ROLE OF TRANSFORMING GROWTH FACTOR β 1 IN TRANSCRIPTIONAL REPRESSION OF THE CHOLESTEROL 7 α-HYDROXYLASE GENE IN HUMAN HEPATOCYTES | Tiangang Li, John Chiang | Department of Biochemistry and Molecular Pathology, Northeastern Ohio University College of Medicine, Rootstown, OH, USA |
| 11:45 AM | #171      | MECHANICAL STRETCH STIMULATES CHOLANGIOCYTE PROLIFERATION AND PROFIBROTIC GENE EXPRESSION VIA THE ANGIOTENSIN TYPE I RECEPTOR: A NOVEL MECHANISM FOR DUCTAL PROLIFERATION DURING OBSTRUCTIVE CHOLESTASIS | Kristina Kelley, Yoshiyuki Uno, Michael McNeal, Benjamin F. Perry, Julie Venter, Shelley Vaculin, Sharon DeMorrow, Bradley Vaculin, Candace Wise, David Dostal, Shannon S. Glaser | Medicine, Scott & White Hospital and Texas A&M University System HSC, Temple, TX, USA.  
College of Medicine, Texas A&M University System Health Science Center, Temple, TX, USA.  
Medicine, Texas A&M University System Health Science Center, Temple, TX, USA.  
Research, Central Texas Veterans Health Care System, Temple, TX, USA.  
Division of Molecular Cardiology, Texas A&M University System Health Science Center, Temple, TX, USA.  
Division of Gastroenterology, Tohoku University Hospital, Aobaku, Japan.  
Research and Education, Scott & White Hospital, Temple, TX, USA |

### HCV: Clinical Development Strategies II

**Tuesday, November 6**

**11:15 AM - 12:45 PM**  
**Hynes, Auditorium**

**MODERATORS:**  
E. Jenny Heathcote, MD  
Keyur Patel, MD

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<th>Institutions</th>
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| 12:00 PM | #172      | PI3K-INDEPENDENT ACTIVATION OF P38MAPK IS REQUIRED FOR CAMP-STIMULATED MRP2 TRANSLATION IN HEPATOCYTES | Christopher Schonhoff, Cynthia R. Webster, Mohammed S. Anwer | Biomedical Sciences, Tufts Cummings School of Veterinary Medicine, North Grafton, MA, USA.  
Clinical Sciences, Tufts Cummings School of Veterinary Medicine, North Grafton, MA, USA |
| 12:15 PM | #173      | THE STEROID RECEPTOR CO-ACTIVATOR 2 (SRC2) REGULATES THE EXPRESSION OF THE FXR TARGET GENES, BILE SALT EXPORT PUMP (ABCB11) AND SMALL HETERODIMER PARTNER (SHP) | Martin J. Walsh, Meena Ananthanarayanan, Side Li, Natarajan Balasubramaniam, Frederick J. Suchy | Department of Pediatrics, Mount Sinai School of Medicine, New York, NY, USA |
| 12:30 PM | #174      | CHARACTERIZATION OF MICE NULL FOR LIVER-SPECIFIC UPTAKE TRANSPORTER ORGANIC ANION TRANSPORTING POLYPEPTIDE 1B2 (OATP1B2) | Hong Lu, Supratim Choudhuri, Kenichiro Ogura, Ivan Csanaky, Pei-zhen Song, Xiaohong Lei, Chuan Chen, Xingguo Cheng, Curtis Klaassen | Pharmacology, Toxicology & Therapeutics, University of Kansas Medical Center, Kansas City, KS, USA.  
Food and Drug Administration, College Park, MD, USA |

**12:00 PM**

**#172**

**PI3K-INDEPENDENT ACTIVATION OF P38MAPK IS REQUIRED FOR CAMP-STIMULATED MRP2 TRANSLATION IN HEPATOCYTES**

Christopher Schonhoff1, Cynthia R. Webster2, Mohammed S. Anwer1  
1Biomedical Sciences, Tufts Cummings School of Veterinary Medicine, North Grafton, MA, USA. 2Clinical Sciences, Tufts Cummings School of Veterinary Medicine, North Grafton, MA, USA

**12:15 PM**

**#173**

**THE STEROID RECEPTOR CO-ACTIVATOR 2 (SRC2) REGULATES THE EXPRESSION OF THE FXR TARGET GENES, BILE SALT EXPORT PUMP (ABCB11) AND SMALL HETERODIMER PARTNER (SHP)**

Martin J. Walsh, Meena Ananthanarayanan, Side Li, Natarajan Balasubramaniam, Frederick J. Suchy  
Department of Pediatrics, Mount Sinai School of Medicine, New York, NY, USA

**12:30 PM**

**#174**

**CHARACTERIZATION OF MICE NULL FOR LIVER-SPECIFIC UPTAKE TRANSPORTER ORGANIC ANION TRANSPORTING POLYPEPTIDE 1B2 (OATP1B2)**

Hong Lu1, Supratim Choudhuri2, Kenichiro Ogura1, Ivan Csanaky1, Pei-zhen Song1, Xiaohong Lei1, Chuan Chen1, Xingguo Cheng1, Curtis Klaassen1  
1Pharmacology, Toxicology & Therapeutics, University of Kansas Medical Center, Kansas City, KS, USA. 2Food and Drug Administration, College Park, MD, USA

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**11:15 AM**

**#175**

**FINAL RESULTS OF PATIENTS TREATED WITH PEG-INTERFERON-ALFA-2A (PEG-IFN) AND RIBAVIRIN (RBV) FOLLOW-ON THERAPY AFTER 28-DAY TREATMENT WITH THE HEPATITIS C PROTEASE INHIBITOR TELAPREVIR (VX-950), PEG-IFN AND RBV**

Maribel Rodriguez-Torres1, Eric J. Lawitz2, John G. McHutchison3  
1Fundacion de Investigacion de Diego, Santurce, PR, USA. 2Alamo Medical Research, San Antonio, TX, USA. 3Duke Clinical Research Institute & Division of Gastroenterology, Duke University, Durham, NC, USA
11:30 AM

#176
PRE-TREATMENT PD-1 EXPRESSION ON HCV-SPECIFIC CTLS PREDICTS EARLY AND LONG-TERM RESPONSE TO COMBINATION THERAPY IN AFRICAN AMERICANS BUT NOT CAUCASIAN AMERICANS

Jared Klarquist1, Lucy Golden-Mason1,2, Abdus S. Wahed3, Hugo R. Rosen1,2
1Division of Gastroenterology & Hepatology, Hepatitis C Center & Department of Medicine, University of Colorado Health Sciences Center, Denver, CO, USA. 2Integrated Program in Immunology, University of Colorado Health Sciences Center & National Jewish Hospital, Denver, CO, USA. 3Department of Biostatistics, Graduate School of Public Health, University of Pittsburgh, Denver, CO, USA

11:45 AM

#177
INTERIM ANALYSIS RESULTS FROM A PHASE 2 STUDY OF TELAPREVIR WITH PegINTERFERON ALFA-2A AND RIBAVIRIN IN TREATMENT-NAÏVE SUBJECTS WITH HEPATITIS C

Ira M. Jacobson1, Gregory T. Everson2, Stuart C. Gordon3, Robert Kauffman4, Lindsay McNair4, Andrew Muir5, John G. McHutchison6

1Division of Gastroenterology and Hepatology, Weill Cornell Medical College, New York, NY, USA. 2Division of Gastroenterology and Hepatology, University of Colorado Health Science Center, Denver, CO, USA. 3Division of Gastroenterology and Hepatology, Henry Ford Hospital, Detroit, MI, USA. 4Vertex Pharmaceuticals, Cambridge, MA, USA. 5Duke Clinical Research Institute, Duke University Medical Center, Durham, NC, USA

12:00 PM

#178
INTERIM DATA FROM A RANDOMIZED CONTROLLED TRIAL OF NITAZOXANIDE-PEGINTERFERON-RIBAVIRIN, NITAZOXANIDE-PEGINTERFERON AND PEGINTERFERON-RIBAVIRIN IN THE TREATMENT OF PATIENTS WITH CHRONIC HEPATITIS C GENOTYPE 4

Jean-Francois Rossignol1, Asem Elfert3, Yahia El-Gohary4, Emmet B. Keefe2, Jeffrey Glenn2

1The Romark Institute for Medical Research, Tampa, FL, USA. 2Division of Gastroenterology and Hepatology, Department of Medicine, Stanford University, School of Medicine, Stanford, CA, USA. 3Department of Gastroenterology and Hepatology, University of Tanta, Faculty of Medicine, Tanta, Egypt. 4Department of Tropical Medicine and Infectious Diseases, University of Alexandria, Faculty of Medicine, Alexandria, Egypt

12:15 PM

#179
IMPORTANCE OF A MINIMAL RESIDUAL VIREMIA FOR THE RELAPSE PREDICTION IN HCV TYP1 PATIENTS RECEIVING STANDARD OR INDIVIDUALIZED TREATMENT DURATION

Thomas Berg1, Viola Weich1, Gerlinde Teuber2, Hartwig Klinker3, Bernd Möller4, Jens Rasenack5, Holger Hirnichsen6, Tilman Gerlach7, Ulrich Spengler8, Peter Buggisch9, Heike Balk10, Myrra Zankel10, Christoph Sarrazin2, Stefan Zeuzem2

1Charite, Campus Virchow Klinikum, Berlin, Germany. 2Medizinische Universitätsklinik, Frankfurt, Germany. 3Klinikum der Universität Würzburg, Würzburg, Germany. 4Hepatologische Schwerpunktpraxis, Berlin, Germany. 5Medizinische Universitätssklinik, Freiburg, Germany. 6Christian-Albrecht-Universität, Kiel, Germany. 7Universitätsklinik, Zürich, Switzerland. 8Medizinische Universitätsklinik, Bonn, Germany. 9Universitätsklinik Eppendorf, Hamburg, Germany. 10essex GmbH, München, Germany

12:30 PM

#180
SUSTAINED VIROLOGIC RESPONSE RATES WITH ALBINTERFERON ALFA-2B PLUS RIBAVIRIN TREATMENT IN IFN-NAÏVE, CHRONIC HEPATITIS C GENOTYPE 1 PATIENTS

Stefan Zeuzem1, Eric M. Yoshida2, Yves Benhamou3, Vincent G. Bain4, Daniel Shouval5, Stephen Pianko6, Robert Flisiak7, Mircea Grigorescu8, Vratislav Rehak9, Kelly D. Kaita10, Patrick Cronin11, Erik Pulkstenis11, G M. Subramanian11, John G. McHutchison12

1J.W. Goethe-University Hospital, Frankfurt, Germany. 2University of British Columbia, Vancouver, BC, Canada. 3Hopital Pitie-Salpetriere, Paris, France. 4University of Alberta, Edmonton, AB, Canada. 5Hadassah University, Hadassah, Israel. 6Monash University, Melbourne, VIC, Australia. 7Medical University of Białystok, Białystok, Poland. 8Spatulul Clinic, Cluj-Napoca, Romania. 9Nuselská poliklinika, Prague, Czech Republic. 10University of Manitoba, Winnipeg, MB, Canada. 11Human Genome Sciences, Rockville, MD, USA. 12Duke Clinical Research Institute, Durham, NC, USA
Non-Invasive Assessment of Liver Fibrosis
Tuesday, November 6
11:15 AM - 12:45 PM  Hynes, Ballroom B
MODERATORS: John G. McHutchison, MD  Xavier Forns, MD

11:15 AM  #181
THE RATIO INTERQUARTILE RANGE / MEDIAN
VALUE OF LIVER STIFFNESS MEASUREMENT IS A
KEY FACTOR OF ACCURACY OF TRANSIENT
ELASTOGRAPHY (FIBROSCAN®) FOR THE
DIAGNOSIS OF LIVER FIBROSIS

Damien Lucidarme1, Juliette Foucher2, Brigitte Le Bail3, Laurent
Castera2, Sandrine Villars2, Gerard Forzy4, Bernard Filoche1, Patrice
Couzigou2, Victor de Ledinghen2,5
1Pathologie Digestive, groupe Hospitalier de l’Institut Catholique de
Lille, Lomme, France. 2Centre d’Investigation de la Fibrose Hépa-
tique, CHU de Bordeaux, Hôpital Haut-Lévêque, Bordeaux, France.
3Service d’Anatomie Pathologique, CHU de Bordeaux, Hôpital Pel-
legrin, Bordeaux, France. 4Laboratoire de Biologie, groupe Hospi-
talier de l’Institut Catholique de Lille, Lomme, France. 5INSERM
U889, Université Victor Segalen, Bordeaux, France

11:30 AM  #182
PERFORMANCE OF NON-INVASIVE METHODS IN
THE ASSESSMENT OF DISEASE SEVERITY IN
ROUTINE CLINICAL PRACTICE IN PATIENTS WITH
CHRONIC HEPATITIS C

Stella M. Martinez1, Marlene Dominguez1, Guillermo Fernandez-
Varo2, Patricia Gonzalez1, Ramón Bataller1, Ellen Sampson3, Wladimiro
Jimenez2, Xavier Forns1, José M. Sánchez-Tapias1
1Liver Unit, Hospital Clinic. IDIBAPS., Barcelona, Spain. 2Bio-
chemistry and Molecular Genetics, Hospital Clinic. IDIBAPS.,
Barcelona, Spain. 3Siemens Medical Solutions, Siemens, Tarry-
town, NY, USA

11:45 AM  #183
TRANSIENT ELASTOGRAPHY IN PATIENTS WITH
NONALCOHOLIC FATTY LIVER DISEASE (NAFLD)

Masato Yoneda1, Hironori Mawatari1, Takuma Higurashi2, Hiroshi
Iida1, Yuichi Nozaki1, Hiroki Endo1, Koji Fujita1, Hiroyuki
Kirikoshi1, Masaya Tamano2, Masashi Yoneda2, Hideyuki
Hiraishi2, Kensuke Kubota1, Satoru Saito1, Atsushi Nakajima1
1Division of Gastroenterology, Yokohama City University School of
Medicine, Yokohama, Japan. 2Division of Gastroenterology,
Dokkyo Medical School, Mibu, Japan

12:00 PM  #184
COMPARISON OF MR ELASTOGRAPHY,
ULTRASOUND ELASTOGRAPHY AND APRI FOR
THE NON-INVASIVE ASSESSMENT OF LIVER
FIBROSIS

Laurent Huwart, Christine Sempoux, Najat Salameh, Laurence
Annet, Yves Horsmans, Bernard Van Beers
Université Catholique de Louvain, Brussels, Belgium

12:15 PM  #185
FIBROTEST VERSUS LIVER BIOPSY: AN
INDEPENDENT MULTICENTER EVALUATION OF
PERFORMANCE

Maria Guido1, AlfredoAlberti2, Giorgio Bellati3, Guido Col-
loredo4, Antonio Craxi2, Vito Di Marco2, Stefano Fagiuoli5, Matteo
Fassan1, Luciano Giacomelli1, Alessandra Mangia1, Giada Sebas-
tian2, Massimo Rugge1
11. Department of Diagnostic Medical Sciences & Special Thera-
pies, University of Padova, Padova, Italy. 2Department of Clinical
and Experimental Medicine, University of Padova, Padova, Italy.
3S. Anna Hospital, Como, Italy. 4Pollicinico San Pietro, Bergamo,
Italy. 5Department of Gastroenterology, University of Palermo,
Palermo, Italy. 6Ospedali Riuniti, Bergamo, Italy. 7IRCSS Casa Sol-
lievo della Sofferenza, S. Giovanni Rotondo, Italy

12:30 PM  #186
PROSPECTIVE COMPARISON OF TWO
ALGORITHMS COMBINING NON INVASIVE TESTS
FOR STAGING OF LIVER FIBROSIS IN CHRONIC
HEPATITIS C

Laurent Castera1,2, Giada Sebastiani3, Brigitte Le Bail4, Victor de
Ledinghen1, Patrice Couzigou1, Alfredo Alberti3
1Hepatology, Hopital Haut Leveque, CHU Bordeaux, Pessac,
France. 2Hepatology, Hopital St Andre, CHU Bordeaux, Bordeaux,
France. 3Venetian Institute of Molecular Medicine, Padova, Italy.
4Pathology, Hopital Pellegrin, CHU Bordeaux, Bordeaux, France

Stem Cell Biology and Differentiation
Tuesday, November 6
11:15 AM - 12:45 PM  Hynes, Room 302
MODERATORS: Lopa Mishra, MD  Satdarshan P. Monga, MD

11:15 AM  #187
DIFFERENTIATION AND ENRICHMENT OF
HEPATOCELLS FROM HUMAN EMBRYONIC STEM
CELLS IN VITRO AND IN Vivo

Yuyou Duan1, Andreea M. Catana1, Ying Meng1, Naoki
Yamamoto1, Songqing He1, Sanjeev Gupta2, Sanjiv S. Gambhir3,
Mark A. Zern1
1Department of Internal Medicine, Transplant Research Program,
University of California, Davis Medical Center, Sacramento, CA,
USA. 2Medicine and Pathology, Albert Einstein College of Medi-
cine, Bronx, NY, USA. 3Molecular Imaging Program, Stanford Uni-
versity, Stanford, CA, USA

11:30 AM  #188
IDENTIFICATION OF ADULT HEPATIC
PROGENITOR/OVAL CELLS CAPABLE OF
REPOPULATING INJURED RAT LIVER

Mariana D. Dabeva1, Miladen I. Yovchev1, Petar N. Grozdanov1,
Hongchao Zhou2, Chandan Guha2
1Department of Medicine, Albert Einstein College of Medicine,
Bronx, NY, USA. 2Department of Radiation Oncology, Albert Ein-
stein College of Medicine, Bronx, NY, USA
11:45 AM

#189
ISOLATION AND EXPANSION OF EPCAM POSITIVE PROGENITOR CELLS FROM HUMAN FETAL LIVER

Yock Young Dan¹,², Lidyana Amer², Lin Lin Su³, Peng Cheang Wong¹, Seng Gee Lim¹,²
¹Gastroenterology and Hepatology, National University Hospital, Singapore, Singapore. ²Dept of Medicine, Yong Loo Lin School of Medicine, National University Singapore, Singapore, Singapore. ³Dept of Obstetrics and Gynecology, Yong Loo Lin School of Medicine, National University Singapore, Singapore, Singapore

12:00 PM

#190
SALL4 REGULATES DIFFERENTIATION AND PROLIFERATION IN HEPATIC STEM/PROGENITOR CELLS

Tsunekazu Oikawa¹, Akihide Kamiya¹, Sei Kakinuma¹, Ryuichi Nishinakamura², Hiromitsu Nakauchi¹
¹Laboratory of Stem Cell Therapy, Center for Experimental Medicine, University of Tokyo, Tokyo, Japan. ²Division of Integrative Cell Biology, Institute of Molecular Embryology and Genetics, Kumamoto University, Kumamoto, Japan

12:15 PM

#191
MICRORNA ANALYSIS OF HEPATOCYTIC DIFFERENTIATION OF LIVER STEM CELLS REVEALS UP REGULATION OF MIR23B, 27B, 24 AND DOWN REGULATION OF SMAD3 AND SMAD5 TARGETS IN THE TGFβ/BMP SIGNALING PATHWAY

Leslie E. Rogler, Tatayana Tchaikovskaya, Lauretta LeVoci, Raquel Norel, Charles E. Rogler
Hepatology Division Medicine, Albert Einstein College of Medicine, Bronx, NY, USA

12:30 PM

#192
PROSTAGLANDIN E2 MODULATES WNT-MEDIATED CONTROL OF LIVER DEVELOPMENT AND REGENERATION IN ZEBRAFISH

Wolfram Goessling¹,², Trista E. North², Allegra M. Lord², Sang Lee³, Mark Puder³, Randall T. Moon⁴, Leonard I. Zon²
¹Gastrointestinal Unit, Massachusetts General Hospital, Boston, MA, USA. ²Stem Cell Program, HHMI, Children’s Hospital, Boston, MA, USA. ³Department of Surgery, Children’s Hospital, Boston, MA, USA. ⁴Department of Pharmacology, HHMI, University of Washington, Seattle, WA, USA
Poster Sessions

Poster Session 1
Saturday, November 3

POSTER VIEWING: 2:00 - 8:00 PM
Hynes, Exhibit Hall C

Presenters in attendance:
5:30 - 7:00 PM

Those posters identified as AASLD Presidential Poster of Distinction by a ribbon icon have received review scores that place them within the top 10 percent of all posters. We encourage you to make them a priority as you visit the poster sessions.

Alcohol: Clinical and Experimental

#193
TUMOR NECROSIS FACTOR RECEPTOR 1 AND 2 DEFICIENCY OR ACIDIC SPHINGOMYELINASE ABLEATION AMELIORATES ETHANOL-INDUCED LIVER DAMAGE

Anna Fernandez, Anna Colell, Francisco Caballero, Carmen Garcia-Ruiz, Jose C. Fernandez-Checa
Liver Unit, Hospital Clinic, Barcelona, Spain

#194
AN ESSENTIAL ROLE OF EARLY GROWTH RESPONSE-1 TRANSCRIPTION FACTOR IN STEATOSIS AFTER ACUTE ETHANOL ADMINISTRATION

Terrence M. Donohue1,3, Sandra Todero1, John Davis2, Ronda L. White3, Dahn L. Clemens1,3, Tiana Curry-McCoy3,1, Natalia A. Osna1,3
1Research, VA Medical Center, Omaha, NE, USA. 2Ob/GYN, Univ Nebraska Med. Center, Omaha, NE, USA. 3Medicine and Pathology, Univ Nebraska Med Center, Omaha, NE, USA

#195
ETHANOL-INDUCED HISTONE ACETYLATION: A NOVEL MECHANISM FOR ENHANCEMENT OF INFLAMMATION IN ALCOHOLIC HEPATITIS?

Stuart Kendrick, Graeme O’Boyle, Jelena Mann, David E. Jones, Christopher P. Day
Institute of Cellular Medicine, Newcastle University, Newcastle upon Tyne, United Kingdom

#196
ZEBRAFISH EMBRYOS DEVELOP SIGNS OF LIVER DISEASE FOLLOWING SHORT-TERM EXPOSURE TO ALCOHOL

Michael Passeri, Ayca Cinaroglu, Kirsten C. Sadler
Division of Liver Diseases and Molecular Cell and Dev. Biology, Mt. Sinai School of Medicine, New York, NY, USA

PKCε PLAYS A CAUSAL ROLE IN ETHANOL-INDUCED STEATOSIS

J. Phillip Kaiser1, Claudia von Montfort1, Juliane I. Beier1, Luping Guo1, Yuting Zheng2, Aruni Bhatnagar1,2, Gavin E. Arteel1,3
1Pharmacology and Toxicology, University of Louisville, Louisville, KY, USA. 2Division of Cardiology, University of Louisville, Louisville, KY, USA. 3James Graham Brown-Cancer Center, University of Louisville, Louisville, KY, USA

DOSE-DEPENDENT EFFECTS OF ETHANOL ON ADIPOCYTE, HEPATOCYTE AND CARDIAC MYOCYTE FATTY ACID UPTAKE AND TRIGLYCERIDE ACCUMULATION IN C57BL/6J MICE

Shengli Zhou1, Sandy Twaddell2, Elektra Carras1, Jay H. Lefkowitch3, Paul D. Berk1
1Medicine, Columbia University Medical Center, New York, NY, USA. 2Pathology, Columbia University Medical Center, New York, NY, USA

CONTRIBUTION OF THE SYMPATHETIC HORMONE EPINEPHRINE TO THE SENSITIZING EFFECT OF ETHANOL ON LIPOPOLYSACCHARIDE-INDUCED LIVER DAMAGE

Claudia von Montfort1, Juliane I. Beier1, Luping Guo1, Gavin E. Arteel1,2
1Pharmacology & Toxicology, University of Louisville, Louisville, KY, USA. 2James Graham Brown Cancer Center, University of Louisville, Louisville, KY, USA

ENHANCEMENT OF FIBRIN DEPOSITION CONTRIBUTES TO THE SYNERGISTIC EFFECT OF ETHANOL ON LPS-INDUCED LIVER INJURY

Juliane I. Beier1, Luping Guo1, James P. Luyendyk2, Claudia von Montfort1, Gavin E. Arteel1,3
1Pharmacology and Toxicology, University of Louisville Health Science Center, Louisville, KY, USA. 2Pharmacology, Toxicology & Therapeutics, The University of Kansas, Kansas City, KS, USA. 3James Graham Brown Cancer Center, University of Louisville, Louisville, KY, USA

PROSPECTIVE SCREENING OF INFECTION IN PATIENTS WITH SEVERE ALCOHOLIC HEPATITIS TREATED WITH STEROIDS: EARLY RESPONSE TO THERAPY IS THE KEY FACTOR

Alexandre Louvet1,2, Sébastien Dharamy1,2, Jeanne Boitard1, Faustine Wartel1, Valérie Canva1, Pierre Deltenre1, Philippe Mathurin1,2
1Hepatology, Hôpital Huriez, Lille, France. 2INSERM U 795, Lille, France

Denotes AASLD Presidential Poster of Distinction
#202  TOLERANCE AND EFFICACY OF THE MARS SYSTEM IN PATIENTS WITH SEVERE ALCOHOLIC HEPATITIS NON-RESPONSER TO STEROIDS: A PILOT STUDY

Jeanne Boitard, Alexandre Louvet, Benjamin Bismuth, Sébastien Dharancy, Faustine Wattel, Valérie Canva, Pierre Deltenre, Brigitte Jude, François Fourrier, Philippe Mathurin. 1Hepatology, Hôpital Huriez, Lille, France. 2INSERM U 795, Lille, France.

#203  EFFECT OF CHRONIC ALCOHOL CONSUMPTION ON NUCLEAR GENE REGULATORS OF MITOCHONDRIAL FUNCTION

Charles S. Lieber, Maria A. Leo, Xiaolei Wang, Anatoly Ponomarenko, Leonore M. DeCarli. 1Research, James J. Peters VA Medical Center, Bronx, NY, USA. 2Mount Sinai School of Medicine, New York, NY, USA.

#204  ALBUMIN AND OTHER ENDOTOXIN REMOVAL METHODS RESTORE NEUTROPHIL FUNCTION EX VIVO IN PATIENTS WITH SEVERE ALCOHOLIC HEPATITIS


#205  DIAGNOSTIC ACCURACY OF CARBOHYDRATE DEFICIENT TRANSFERRIN IN PATIENTS WITH CHRONIC LIVER DISEASE. ALCOHOL CONSUMPTION AND LIVER DISEASE SEVERITY EFFECTS

Armand Abergel, Corinne Bonny, Karine Randl, Carine Nicolas, Sylvie Massoulier, Bernardette Cruz, Brigitte Chanteranne, Laurence Roszyk, Pierre Jouanel, Gilles Bommelaer, Vincent Sapin. 1Hepato-gastroenterology, CHRU Clermont-Ferrand, Clermont-Ferrand, France. 2Biochemistry, CHRU Clermont-Ferrand, Clermont-Ferrand, France.

#206  PLASMA FROM PATIENTS WITH SEVERE ALCOHOLIC HEPATITIS INDUCES A FUNCTIONAL DEFECT POSSIBLY THROUGH EXPRESSION OF TOLL-LIKE-RECEPTORS 2 AND 9 BUT NOT 4


#207  NUTRITIONAL AND METABOLIC SIMILARITIES BETWEEN ALCOHOLIC AND NON-ALCOHOLIC STEATOHEPATITIS: A GENERAL POPULATION ASSESSMENT

Steven L. Condon, Matthew J. Gurka, Abdullah M. Al-Osaimi, Stephen H. Caldwell, Patrick G. Northrup. 1Gastroenterology and Hepatology, University of Virginia Health System, Charlottesville, VA, USA. 2Public Health Sciences, University of Virginia Health System, Charlottesville, VA, USA.

Biliary Physiology, Transport, Cholangiocyte Biology, Experimental Cholestasis

#208  MEMBRANE TRAFFICKING OF THE HUMAN ORGANIC ANION-TRANSPORTING POLYPEPTIDE C (HOATP-C)

An-Qiang Sun, Vijaya M. Ponomgi, James L. Boyer, Frederick J. Suchy. 1Pediatrics, Box 1664, Mt Sinai Sch Med, New York, NY, USA. 2Yale Liver Center, Yale University School of Medicine, New Haven, CT, USA.

#209  CHEMICAL CHAPERONES PARTIALLY REVERSE THE MISPROCESSING OF A BRIC2 MUTANT OF THE BILE SALT EXPORT PUMP, ABCB11

Ping Lam, Carol J. Soroka, James L. Boyer. Internal Med., Yale University, New Haven, CT, USA.

#210  5’ UNTRANSLATED REGIONS (UTRS) OF HUMAN MULTIDRUG RESISTANCE PROTEIN 2 (MRP2) GENE REGULATE IN VITRO TRANSLATION

Yuan-yuan Zhang, Wei Li, Mary Vore. 1Graduate Center for Toxicology, University of Kentucky, Lexington, KY, USA. 2Hematology Oncology, VA Hospital, Lexington, KY, USA.

#211  FOXA2 REGULATES BILE ACID METABOLISM AND PREVENTS ER STRESS IN THE LIVER

Irina Bochkis, Nir Rubins, Peter White, Klaus Kaestner. Genetics, University of Pennsylvania, Philadelphia, PA, USA.

#212  THE MEMBRANE-BOUND BILE SALT RECEPTOR TGR5 IS EXPRESSED IN NON-PARENCHYMAL CELLS OF RAT LIVER

Verena Keitel, Dieter Häussinger, Ralf Kubitz. Clinic for Gastroenterology, Hepatology and Infectious Diseases, Heinrich-Heine-University, Duesseldorf, Germany.
#213
EXENDIN-4 PROTECTS CHOLANGIOCYTES FROM APOPTOSIS, BOTH IN VITRO AND IN VIVO
Marco Marzioni1, Gianfranco Alpini1, Stefania Saccomanno1, Giammaramo Fava1, Cinzia Candelaresi1, Chiara Rychlicki1, Heather L. Francis2, Luciano Trozzi1, Julie Venter2, Antonio Benedetti1
1Department of Gastroenterology, Università Politecnica delle Marche, Ancona, Italy. 2Division Research & Education,, Scott & White Hospital and The Texas A & M University System Health Science Center College of Medicine, Temple, TX, USA. 3Division of Medicine and Research, Scott & White Hospital and The Texas A & M University System Health Science Center College of Medicine, Temple, TX, USA.

#214
EXPRESSION OF SOLUTE TRANSPORTERS AND WATER CHANNELS IN ARPKD: IMPLICATIONS FOR HEPATIC CYSTOGENESIS
Jesus M. Banales1,2, Pamela S. Tietz1, Seung-Ok Lee1, Bing Q. Huang1, Tatiana V. Masyuk1, Angela J. Stroope1, Sergio A. Gradilone1, Anatoly I. Masyuk1, Juan F. Medina2, Nicholas F. LaRusso1
1Internal Medicine, Mayo Clinic College of Medicine, Rochester, MN, USA. 2Laboratory of Molecular Genetics, University of Navarra School of Medicine, Clinica Universitaria and CIMA, Pamplona, Spain.

#215
NM23-H2 INHIBITS CHOLANGIOCARCINOMA GROWTH BY BINDING TO AND DOWNREGULATING PPARδ
Xuefeng Xia, Wenzheng Zhang, Yuhua Xiao, Dongbing Gao, Gene D. LeSage
Internal Medicine, University of Texas Medical School at Houston, Houston, TX, USA.

#216
SMALL CHOLANGIOCYTES PROLIFERATE IN RESPONSE TO H1 HISTAMINE RECEPTOR STIMULATION VIA ACTIVATION OF THE IP3/CA2+/CAMK/CREB-DEPENDENT PATHWAY
Heather Francis2, Sharon DeMorrow3, Shannon Glaser2, Julie Venter4, Eugenia Gaudio6, Yoshiyuki Ueno7, Paolo Onori6, Antonio Franchitto5, Bradley Vaculin4, Shelley Vaculin2, Giammaramo Fava9, Antonio Benedetti8, Cristina Kelley1, Gianfranco Alpini1
1Department of Medicine and Systems Biology and Translational Medicine, Central Texas Veterans Health Care System, Scott & White and Texas A & M HSC, Temple, TX, USA. 2Research, Central Texas Veterans Health Care System, Temple, TX, USA. 3R&E, Scott & White, Temple, TX, USA. 4Medicine, Texas A&M HSC COM, Temple, TX, USA. 5Medicine and R&E, Scott & White and Texas A & M HSC COM, Temple, TX, USA. 6Gastroenterology, Medicine, Tohoku University School of Medicine, Sendai, Japan. 7Medicine, Texas A&M HSC COM, Temple, TX, USA. 8Gastroenterology, Medicine, Universita' di Ancona, Ancona, Italy. 9Obstetrics and Gynecology, Scott & White and Texas A & M HSC COM, Temple, TX, USA.

#217
PROGESTERONE REGULATES CHOLANGIOCYTE PROLIFERATION DURING CHOLESTASIS BY AUTOCRINE SIGNALING MECHANISMS
Shannon Glaser, Sharon DeMorrow, Heather Francis, Julie Venter, Eugenia Gaudio, Yoshiyuki Ueno, Paolo Onori, Antonio Franchitto, Bradley Vaculin, Shelley Vaculin, Giammaramo Fava, Antonio Benedetti, Cristina Kelley, Gianfranco Alpini
1Department of Medicine and Systems Biology and Translational Medicine, Central Texas Veterans Health Care System, Scott & White and Texas A & M HSC, Temple, TX, USA. 2Research, Central Texas Veterans Health Care System, Temple, TX, USA. 3R&E, Scott & White, Temple, TX, USA. 4Medicine, Texas A&M HSC COM, Temple, TX, USA. 5Medicine and R&E, Scott & White and Texas A & M HSC COM, Temple, TX, USA. 6Gastroenterology, Medicine, Tohoku University School of Medicine, Sendai, Japan. 7Medicine, Texas A&M HSC COM, Temple, TX, USA. 8Gastroenterology, Medicine, Universita’ di Ancona, Ancona, Italy. 9Obstetrics and Gynecology, Scott & White and Texas A & M HSC COM, Temple, TX, USA.

#218
CONTRIBUTION OF COMBINATORIAL LIGAND LIBRARIES TO THE HUMAN BILE PROTEOME: NEW IDENTIFICATIONS AND PROMINENCE OF BINDING PROTEINS
Luc Guerrier1, Stephane Claverol2, Laetitia Finzi3, Frederic Fortis1, Egisto Boschetti1, Chantal Housset3
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Marc S. Elias, Lee-Arng Chang, Richard M. Green
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**HCV: Treatment**

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**AN OPEN LABEL, COMPARATIVE, MULTICENTER STUDY OF PEGINTERFERON ALFA-2A PLUS RIBAVIRIN IN THE TREATMENT OF PATIENTS WITH CHRONIC HEPATITIS C/HEPATITIS B CO-INFECTION VERSUS THOSE WITH CHRONIC HEPATITIS C MONOINFECTION**

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**EARLY DISCONTINUATION OF RIBAVIRIN IN HCV-2 AND HCV-3 PATIENTS RESPONDING TO PEG-INTERFERON ALFA-2A AND RIBAVIRIN**

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**RANDOMIZED, DOUBLE-BLIND, PLACEBO-CONTROLLED TRIAL OF PEGINTERFERON ALFA-2A (40KD) AND RIBAVIRIN WITH AND WITHOUT 400 MG AMANTADINE-SULPHATE FOR 48 WEEKS IN TREATMENT NAIVE HCV GENOTYPE 1-INFECTED PATIENTS**

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**RITUXIMAB COMBINED WITH PEG-INTERFERON-RIBAVIRIN IN REFRACTORY HCV-ASSOCIATED CRYOGLOBULINEMIA VASCULITIS**

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**SUSTAINED VIROLOGICAL RESPONSE IS ASSOCIATED WITH ERADICATION OF HEPATITIS C VIRUS AND DECREASE IN ANTI-HCV TITER IN PATIENTS TREATED FOR CHRONIC HEPATITIS C WITH INTERFERON ALPHA 2B OR PEGYLATED INTERFERON ALPHA-2B+RIBAVIRIN**

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**PREEMPTIVE TREATMENT OF HCV AFTER LIVER TRANSPLANTATION IS UNJUSTIFIED EXCEPT FOR GENOTYPE 3A**

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ASSOCIATION BETWEEN GENE POLYMORPHISMS AND PSYCHIATRIC SYMPTOMS DURING PEGINTERFERON TREATMENT FOR CHRONIC HEPATITIS C

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GENE EXPRESSION OF CYTOPLASMIC VIRAL SENSORS AND REGULATORS INVOLVING INNATE IMMUNITY AND RESISTANCE TO PEG-INTERFERON ALFA-2B PLUS RIBAVIRIN TREATMENT IN CHRONIC HEPATITIS C

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LIVER GENE EXPRESSION SIGNATURE TO PREDICT RESPONSE TO PEGYLATED INTERFERON PLUS RIBAVIRIN COMBINATION THERAPY IN PATIENTS WITH CHRONIC HEPATITIS C

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POSITIVE IMPACT OF ANTIVIRAL THERAPY ON THE LONG TERM OUTCOME OF CHRONIC HEPATITIS C PATIENTS WITH CIRRHOSIS

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LONG-TERM PROGNOSIS OF ELDERLY PATIENTS WITH HEPATITIS C VIRUS-RELATED CHRONIC LIVER DISEASE — A COHORT STUDY OF 2,379 JAPANESE PATIENTS

Kenji Ikeda, Yasuji Arase, Yusuke Kawamura, Hiromi Yatsuji, Hitomi Sezaki, Tetsuya Hosaka, Norio Akuta, Masahiro Kobayashi, Satoshi Saitoh, Fumitaka Suzuki, Yoshiyuki Suzuki, Hiromitsu Kumada
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EFFICACY AND SAFETY OF ESCITALOPRAM FOR THE PREVENTION OF DEPRESSIVE EPISODES INDUCED BY PEG-INTERFERON ALFA2A AND RIBAVIRIN IN CHRONIC HEPATITIS C PATIENTS. A DOUBLE-BLIND, RANDOMIZED, PLACEBO-CONTROLLED TRIAL

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RESPONSE TO PEGINTERFERON ALFA-2B + RIBAVIRIN COMBINATION THERAPY IN GENOTYPE 2 AND 3 PATIENTS WITH POOR BASELINE PROGNOSTIC FACTORS: RESULTS OF THE CANADIAN POWER PROGRAM

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CHANGES IN ANTRODUODENAL MOTILITY DURING INTERFERON TREATMENT FOR CHRONIC HEPATITIS C

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VACCINATION WITH HCV MRNA TRANSFECTED DENDRITIC CELLS IN HCV TRIMERA MICE

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PRETREATMENT INSULIN SENSITIVITY CONTRIBUTES TO THE TREATMENT RESPONSE TO PEGINTERFERON PLUS RIBAVIRIN COMBINATION THERAPY FOR PATIENTS WITH CHRONIC HEPATITIS C

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HEPATITIS C VIRAL KINETICS IN PLASMA AND PERIPHERAL BLOOD MONONUCLEAR CELLS DURING TREATMENT WITH PEGYLATED INTERFERON ALFA-2A PLUS RIBAVIRIN

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RESPONSE TO HIGH RIBAVIRIN DOSE IN COMBINATION WITH PEG-INF ALFA-2A FOR TREATMENT OF HCV GENOTYPE 1 PREVIOUS NON-RESPONDERS

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SEQUENCE VARIATION OF INTERFERON/RIBAVIRIN RESISTANCE-DETERMINING REGION (IRDRR) OF HCV NS5A IS A PREDICTIVE MARKER FOR SUSTAINED Virological RESPONSE UPON COMBINATION THERAPY WITH PEGYLATED INTERFERON AND RIBAVIRIN

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OPIOID MAINTENANCE THERAPY IS NOT ASSOCIATED WITH TREATMENT FAILURE TO HEPATITIS C THERAPY IN A LARGE GERMAN MULTICENTRE COHORT

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FINAL RESULTS OF THE CANADIAN POWER (PEGINTERFERON ALFA-2B PROSPECTIVE OPTIMAL WEIGHT-BASED DOSING RESPONSE) PROGRAM. SUSTAINED VIROLOGIC RESPONSE (SVR) TO WEIGHT-BASED PEGINTERFERON ALFA-2B + RIBAVIRIN IN A LARGE, MIXED COMMUNITY AND ACADEMIC OBSERVATIONAL STUDY

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INFLUENCE OF NK CELL RECEPTOR AND HLA-C LIGANDS GENES ON THE RESPONSE TO ANTIVIRAL THERAPY OF CHRONIC HEPATITIS C INFECTED PATIENTS

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HEPATIC EXPRESSION OF INNATE IMMUNE MOLECULES WITH RESPECT TO INTRAHEPATIC HCV REPLICATION AND OUTCOMES OF PEGINTERFERON PLUS RIBAVIRIN COMBINATION TREATMENT

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EARLY HEPATITIS C VIRUS DECAY WITH WEIGHT-BASED RIBAVIRIN PLUS EITHER PEGIFNα-2A OR PEGIFNα-2B IN HIV/HCV COINFECTED PATIENTS

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ASSESSMENT OF THE IMPACT OF PSYCHIATRIC DISORDERS ON SAFETY, COMPLIANCE, AND SUSTAINED VIROLOGICAL RESPONSE AFTER HEPATITIS C TREATMENT (CHEOBS)

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DANAZOL INCREASES THE PLATELETS COUNT IN THROMBO-CYTOPENIC PATIENTS WITH CHRONIC HEPATITIS C AND LIVER CIRRHOSIS TREATED WITH PEG-INFERNALPHA 2A AND RIBAVIRIN

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RELAPSE RATES AMONG HCV GENOTYPE 1 EARLY VIROLOGICAL RESPONDERS IN A RETROSPECTIVE COMMUNITY-BASED COHORT OF PATIENTS TREATED WITH PEGETRON® IN BRITISH COLUMBIA, CANADA

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VIRAL AND STAT KINETICS IN PATIENTS WITH CHRONIC HEPATITIS C TREATED WITH PEGYLATED INTERFERON A-2B PLUS RIBAVIRIN

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MORPHOLOGICAL ANALYSIS AND ASSESSMENT OF HCV-RNA AND RIBAVIRIN CONCENTRATION IN SEMINAL FLUID OF CHRONIC HEPATITIS C PATIENTS UNDERGOING ANTIVIRAL COMBINATION THERAPY

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#265
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#266
GENE EXPRESSION BIOMARKERS PREDICTING RESPONSE TO PEGYLATED INTERFERON ALPHA (PEG-INF) AND RIBAVIRIN (RBV) IN TREATMENT-NAÏVE PATIENTS WITH CHRONIC HEPATITIS C (CH-C)
Zobair M. Younossi1, Rochelle Collantes1, Ancha Baranova1, Anita Bakshi1, Maria Stepanova1, Ganiri June Manyam1, Chris Santini2, Chris Sigua2, Joanne Chan2, Ayuko Iverson2, Sheng-Yung Chang2
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#268
TREATMENT OF RECENTLY ACQUIRED HEPATITIS C INFECTION IN INJECTING DRUG USERS: PRELIMINARY RESULTS FROM THE AUSTRALIAN TRIAL IN ACUTE HEPATITIS C (ATAHC)
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SHORT-TERM PROLONGATION OF PEGINTERFERON PLUS RIBAVIRIN COMBINATION THERAPY IS A SAFE AND EFFECTIVE TREATMENT STRATEGY FOR GENOTYPE 1B CHRONIC HEPATITIS C PATIENTS
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#271
PREDICTORS OF RESPONSE TO PEGYLATED INTERFERON-α2A AND RIBAVIRIN IN A Cohort of Patients Infected with the Same Strain of HCV: The O’Brian Project
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#272

**TIME TO HCV RNA NEGATIVATION IN HEPATITIS C VIRUS (HCV) TYPE 1-INFECTION DURING PEG-INTERFERON-ALPHA-2B PLUS RIBAVIRIN THERAPY: DIFFERENCES IN RELATION TO THE ASSAY SENSITIVITY (INDIV-1 STUDY GROUP)**

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#273

**IMPACT OF ANTIVIRAL THERAPY AND RESPONSE TO TREATMENT ON LONG-TERM OUTCOME OF CHRONIC HEPATITIS C (CHC): A PROPENSITY SCORE ANALYSIS IN A POPULATION-BASED COHORT OF 1159 PATIENTS**

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**ARTIFICIAL INTELLIGENCE PLATFORM FOR CHRONIC HEPATITIS C (CHC): PREDICTION OF CLINICAL OUTCOME AND MORE EFFICIENT TREATMENT WITH PEGYLATED INTERFERON PLUS RIBAVIRIN**

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#275

**INSULIN RESISTANCE (IR) DEFINED BY THE HOMEOSTASIS MODEL OF ASSESSMENT INSULIN RESISTANCE (HOMA-IR) INDEX HAS A DIRECT EFFECT ON EARLY VIRAL KINETICS DURING PEGYLATED-INTERFERON THERAPY FOR CHRONIC HEPATITIS C**

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#276

**TREATMENT OF CHRONIC HEPATITIS C WITH PEGINTERFERON ALFA-2A (40KD) (PEG) AND RIBAVIRIN (RBV) IN NAÏVE PATIENTS WITH HIV-HCV CO-INFECTION IN THE REAL-LIFE SETTING IN GERMANY**

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**DOES PROLONGED THERAPY IN HCV-GENOTYPE 3 PATIENTS WITH HIGH VIRAL LOAD IMPROVE SUSTAINED VIRAL RESPONSE RATES?**

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#278
SILYMARIN DOWN-REGULATES HCV CORE AND UP-REGULATES HEME OXYGENASE-1 GENE EXPRESSION IN THE CNS3 REPLICON LINE OF HUMAN LIVER CELLS

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#279
TREATMENT OF HEMODIALYSIS (HD) PATIENTS WITH CHRONIC HEPATITIS C (CHC) USING AN ESCALATING DOSE REGIMEN OF PEGYLATED INTERFERON (PEG-IFN) ALFA-2B

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#280
IFN SENSITIVITY DETERMINING REGION IN NSSA OF HEPATITIS C VIRUS CORRELATES WITH THE RESPONSE TO PEG-IFN ALFA-2B PLUS RIBAVIRIN TREATMENT

Michihito Murao, Kyoko Kobayashi, Nariumi Komura, Hiroaki Shimazaki, Takui Nakano, Yoshifumi Nitta, Masao Harata, Naoto Kawabe, Senju Hashimoto, Kentaro Yoshioka
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#281
REAL-LIFE RATES OF TREATMENT COMPLETION FOR HCV

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#282
CLINICAL RELEVANCE OF RAPID VIROLOGICAL RESPONSE (RVR) IN DECOMPENSATED HCV-RELATED CIRRHOSIS TREATED WITH PEG-INTERFERON AND RIBAVIRIN

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#283
ANALYSIS OF GENE EXPRESSION DURING THE FIRST 10 WEEKS FOLLOWING PEG-INTERFERON-ALFA2B/RIBAVIRIN TREATMENT OF A GROUP OF HEPATITIS C PATIENTS

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#284
IRON DEPLETION AND RESPONSE TO INTERFERON IN CHRONIC HEPATITIS C (CHC): A META-ANALYSIS

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EFFICACY OF PEGINTERFERON ALFA-2A AND RIBAVIRIN IN 2101 PATIENTS WITH HCV INFECTION IN REAL-LIFE CLINICAL PRACTICE: RESULTS OF THE FRENCH HEPATYS STUDY

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#286
DYNAMICS OF LIVER STIFFNESS DURING PEGINTERFERON ALPHA-RIBAVIRIN TREATMENT IN PATIENTS WITH CHRONIC HEPATITIS C

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INHIBITION OF REPLICATION AND EXPRESSION OF HCV NSSA PROTEIN BY SMALL INTERFERING RNA

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**VIRAL KINETICS OF HCV GENOTYPE 5 IN SOUTH AFRICAN PATIENTS TREATED WITH PEGYLATED-INTERFERON-ALFA AND RIBAVIRIN**

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**HEPATITIS C VIRUS GENOTYPE 5: EPIDEMIOLOGICAL DATA AND RESPONSE TO TREATMENT**

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**PREDICTORS OF SUSTAINED VIROLOGICAL RESPONSE TO INTERFERON-BASED TREATMENT IN HEMODIALYSIS PATIENTS WITH CHRONIC HEPATITIS C VIRUS INFECTION: A PATIENT LEVEL META-ANALYSIS**

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**BEZAFIBRATE TREATMENT FOR CHRONIC HEPATITIS C AFTER FAILURE OF PREVIOUS COMBINATION THERAPY WITH INTERFERON AND RIBAVIRIN**

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#292

**IMPACT OF ANTIVIRAL TREATMENT ON NON-INVASIVE PREDICTORS OF LIVER FIBROSIS IN PATIENTS WITH CHRONIC HEPATITIS C**

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**EARLY REDUCTION OF RIBAVIRIN LEADS TO RETARDATION OF VIRAL CLEARANCE AND AFFECTS SVR**

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**INTRACELLULAR TARGETING OF HEPATITIS C VIRUS CORE PROTEIN WITH A SINGLE CHAIN ANTIBODY**

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#295

**TREATMENT UPTAKE AND OUTCOMES AMONG CURRENT AND FORMER INJECTION DRUG USERS (IDUS) RECEIVING DIRECTLY OBSERVED THERAPY WITHIN A MULTIDISCIPLINARY GROUP MODEL FOR THE TREATMENT OF HEPATITIS C VIRUS (HCV) INFECTION**

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#296

**INFREQUENT HEPATITIS C VIRUS (HCV) RE-INFECTION AFTER SUSTAINED VIROLOGICAL RESPONSE (SVR) AMONG CURRENT AND FORMER INJECTION DRUG USERS (IDUS) HAVING RECEIVED TREATMENT FOR HCV INFECTION**

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**PEGYLATED INTERFERON ALFA-2B PLUS RIBAVIRIN REDUCES INSULIN RESISTANCE AND IMPROVES GLUCOSE METABOLISM IN PATIENTS WITH CHRONIC HEPATITIS C**

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**#298**

**Whole-body, not only liver, insulin sensitivity is strongly associated with an early and sustained virologic response to peginterferon plus ribavirin treatment in patients with chronic hepatitis C genotype 1b and high viral load**

Toshihiko Mizuta, Yuichiro Eguchi, Yasunori Kawaguchi, Keisuke Ario, Hirokazu Takahashi, Shinji Iwane, Noriko Oza, Iwata Ozaki Internal Medicine, Saga Medical School, Saga, Japan

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**#299**

**Evaluation of a multidisciplinary support program in hepatitis C treatment**

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**#300**

**The TGF-β codon 19 T/C and 25 G/C polymorphisms affects response to interferon-therapy in acutely HCV-infected HIV-positive patients**

Jacob Nattermann, Martin Vogel, Hans Dieter Nischalke, Golo Ahlenstiel, Monika Schulz, Tilman Sauerbruch, Jürgen K. Rockstroh, Ulrich Spengler Internal Medicine I, University of Bonn, Bonn, Germany

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**#301**

**The impact of steatosis and steatohepatitis on the response to treatment in patients with chronic hepatitis C infection**

Timothy J. Cross, Alberto Quaglia, Jonathan Nolan, Ian Fletcher, Kosh Agarwal, Philip M. Harrison Institute of liver studies, Kings college hospital, London, United Kingdom

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**#302**

**Consistency of sustained virologic response (SVR) across weight categories: results from the Canadian power (PEGinterferon alfa-2b prospective optimal weight-based dosing response) program**

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**#303**

**Rapid virological response at week 4 is the best predictor of treatment outcome in patients with chronic hepatitis C: a multivariate analysis**

Michelle Martinot-Peignoux, Sarah Maylin, Rami Moucari, Marie Pierre Ripaillé, Nathalie Boyer, Nathalie Giuily, Corinne Castelnau, Tarick Asselah, Patrick Marcellin 1INSERM U773-CRB3 Université paris VII, Hospital Beaujon, Clichy, France. 2Service d’Hépatologie, Hospital Beaujon, Clichy, France

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**#304**

**Assessment of both virological response at week 4 and at week 12 optimizes prediction of treatment outcome in patients with chronic hepatitis C treated with peginterferon alfa-2b plus ribavirin**

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**#305**

**Predictability of response: positive and negative predictive values of rapid and early virologic responses to peginterferon alfa-2b and ribavirin in the treatment of chronic hepatitis C**

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**#306**

**Impact on sustained response and predictive factors of the different patterns of early virological response during therapy in naive patients with chronic hepatitis C (CHC)**

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**#307**

**Similar viral kinetics of hepatitis C virus genotypes 1 and 4**

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#308
HIGH PREDICTIVE VALUE OF EARLY VIRAL KINETICS IN PEGINTERFERON PLUS RIBAVIRIN COMBINATION THERAPY OF GENOTYPE 1 INFECTED PATIENTS WITH CHRONIC HEPATITIS C

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#309
IMPACT OF REDUCING PEGINTERFERON ALFA-2B AND RIBAVIRIN ON EARLY VIRAL RESPONSE IN GENOTYPE 1 INFECTED PATIENTS WITH CHRONIC HEPATITIS C

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#310
EFFECTS OF SYSTEMATIC NURSE-PROVIDED THERAPEUTIC EDUCATION ON ADHERENCE AND EFFICIENCY OF PEG-INTERFERON-A2A(PEGASYS®)-RIBAVIRIN TREATMENT IN CHRONIC HEPATITIS C (PEGOS PROTOCOL)

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#311
CYTOKINES-CHEMOKINES NETWORK IS MODULATED BY PEGYLATED INTERFERON MONOTHERAPY IN CHRONIC HEPATITIS C PATIENTS

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#312
VIRAL KINETICS CAN QUICKLY PREDICT SUSTAINED VIROLOGICAL RESPONSE IN HCV PATIENTS WITH NORMAL ALT TREATED WITH PEGYLATED IFN α2B AND RIBAVIRIN: A PROSPECTIVE STUDY

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#313
COMPARISON OF APOPTOSIS, ALT, AST AND GGT DYNAMICS IN PATIENTS WITH CHRONIC HEPATITIS C DURING ANTIVIRAL THERAPY

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#314
**B-CELL ACTIVATING FACTOR (BAFF/BLYS) AND RESPONSE TO INTERFERON BASED THERAPY IN CHRONIC HEPATITIS C VIRUS INFECTION**

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#315
**HEPATITIS C TREATMENT OUTCOMES IN THE RHODE ISLAND DEPARTMENT OF CORRECTIONS**

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#316
**THE CEREBRAL METABOLIC AND COGNITIVE EFFECTS OF PEGYLATED INTERFERON (PIFN) AND HEPATITIS C VIRAL (HCV) CLEARANCE**

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#317
**ACROLEIN, A DERIVATIVE OF ENDOGENOUS LIPID PEROXIDATION AND A COMMON ENVIRONMENTAL POLLUTANT, INHIBITS INTERFERON-ALPHA MEDIATED ANTIVIRAL SIGNALING: IMPLICATIONS FOR HCV THERAPY**

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#318
**THE LEVEL OF PRETREATMENT HCV CORE ANTIGEN IS A NEW AND USEFUL PREDICTOR FOR THE EFFICACY OF PEGINTERFERON ALFA-2B AND RIBAVIRIN IN PATIENTS WITH CHRONIC HEPATITIS C**

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**HEPATOCELLULAR CARCINOMA IN LONG-TERM SUSTAINED VIROLOGIC RESPONDERS TO ANTIVIRAL TREATMENT FOR CHRONIC HEPATITIS C**

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#320
**SYSTEMIC FACTORS ASSOCIATED WITH VIROLOGIC NONRESPONSE TO PEGINTERFERON/RIBAVIRIN RETREATMENT OF CHRONIC HEPATITIS C**

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#321
**HIGH RATES OF SUSTAINED VIROLOGICAL RESPONSE (SVR) IN PATIENTS >50 YEARS INFECTED WITH HCV GENOTYPE 1 WITH POSITIVE PROGNOSTIC FACTORS TREATED WITH PEGINTERFERON ALFA-2A (40KD) (PEGASYS®) AND RIBAVIRIN (COPEGUS®)**

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#322
**LOW DOSE PEGINTERFERON ALFA-2A AND RIBAVIRIN FOR CHRONIC HEPATITIS C, GENOTYPE 2 & 3: VIRAL KINETICS, EFFICACY AND SAFETY**

Yaron Rotman¹, Brian B. Borg¹, Alejandro Soza¹, Rohit Loomba¹, Apurva A. Modi¹, Jordan J. Feild¹, Elenita Rivera¹, Edward Doo¹, Theo Heller¹, Marc Ghany¹, Avidan U. Neumann², T. Jake Liang¹, Jay H. Hoofnagle¹

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#323
**IMPACT OF PEGYLATED INTERFERON α AND RIBAVIRIN THERAPY OF CHRONIC HEPATITIS C ON MAJOR SUBSETS OF PERIPHERAL BLOOD AND INTRAHEPATIC LYMPHOCYTES**

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#324
PREVALENCE OF BIPOLAR AFFECTIVE DISORDER IN PATIENTS WITH A POSITIVE DEPRESSION SCREEN AT THE INITIATION OF INTERFERON THERAPY FOR CHRONIC HEPATITIS C
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#325
PATIENT EDUCATION IMPROVES ADHERENCE TO PEGINTERFERON α-2B AND RIBAVIRIN IN CHRONIC GENOTYPE 2 OR 3 HEPATITIS C VIRUS INFECTION: A PROSPECTIVE, REAL-LIFE STUDY (CHEOSB STUDY)
Patrice Cacoub1, Denis Ouzan2, Thierry Fontanges3, Jean-Philippe Lang4, Pascal Melin5, Michel Riotly6, Marina Varaste7, Patrick Marcillai8, Michel Chousterman9
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#326
PEGINTERFERON ALFA-2B AND RIBAVIRIN TREATMENT OF PATIENTS WITH CHRONIC HEPATITIS C AND NORMAL VERSUS ELEVATED AMINOTRANSFERASE LEVELS – FINAL RESULTS OF A PROSPECTIVE OPEN TRIAL
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#327
COMPARISON OF EFFICACY OF TREATMENT WITH PEGINTERFERON ALFA-2A PLUS RIBAVIRIN VS PEGINTERFERON ALFA-2B PLUS RIBAVIRIN AMONG PATIENTS CHRONICALLY INFECTED WITH NON 2/3 HCV GENOTYPES WITH LOW AND HIGH PRETREATMENT VIRAL LOAD
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#328
PEG INFERENCE ALFA-2A MONOTHERAPY IN DIALYSIS PATIENTS INFECTED WITH HEPATITIS C VIRUS
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#329
PEGINTERFERON ALPHA-2B VS PEGINTERFERON ALFA-2A IN THE TREATMENT OF CHRONIC HEPATITIS C INFECTION
Ihab Hammoud, Mary Ann H. Sherbondy, Dilip Moonka, Stuart C. Gordon, Rodolfo Guevara, Kimberly Brown gastro, Henry Ford Hospital, Detroit, MI, USA

#330
GM-CSF MAY MODULATE THE RESPONSE TO THERAPEUTIC IFN-α IN CHRONIC HEPATITIS C VIRUS (HCV) INFECTION
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#331
HEPATITIS C ASSOCIATED SYSTEMIC CRYOGLOBULINEMIA: SUCCESSFUL TREATMENT WITH PLASMA EXCHANGE
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#332
COMPARISON BETWEEN THE TWO PEGINTERFERONS ALFA IN THE TREATMENT OF CHRONIC HEPATITIS C
Pham T. Thuy, Ho Tan Dat
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#333
IMPAIRED SENSITIVITY IN NON-RESPONDERS TO PEGYLATED INTERFERON PLUS RIBAVIRIN THERAPY ASSESSED BY 2’-5’OLIGOADENYLYLATE SYNTHETASE ACTIVITY

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#334
EIGHT-WEEK ORAL ADMINISTRATION OF MELOXICAM, A COX-II SPECIFIC NON-Steroidal ANTI-INFLAMMATORY DRUG, PREVENTS DOSE REDUCTION OF PEGYLATED INTERFERON ALFA-2A IN THE TREATMENT OF CHRONIC HEPATITIS C

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#335
GENE EXPRESSION BIOMARKERS PREDICTING RESPONSE TO PEGYLATED INTERFERON ALPHA (PEG-IFN) AND RIBAVIRIN (RBV) IN THE PERIPHERAL BLOOD OF PATIENTS WITH CHRONIC HEPATITIS C (CH-C), NON-RESPONDER (NR) TO PREVIOUS TREATMENT

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#336
TREATMENT AND OUTCOME OF GENOTYPE 4 CHRONIC HEPATITIS C PATIENTS WITH PEGIFN ALFA 2B AND RIBAVIRIN IN THE CLINICAL SETTING IN GERMANY

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#337
PEG-IFN ALFA-2A PLUS RIBAVIRIN IS SUPERIOR COMPARED TO HIGH DOSE CONSENSUS INTERFERON (CIFN) AND RIBAVIRIN IN THE TREATMENT OF PATIENTS WITH CHRONIC HEPATITIS C

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#338
BONE MINERAL DENSITY AND METABOLISM IN NON-CIRRHOTIC PATIENTS WITH CHRONIC HEPATITIS C BEFORE AND AFTER ANTIVIRAL THERAPY WITH PEGYLATED INTERFERON α. AND RIBAVIRIN: A PROSPECTIVE CONTROLLED STUDY

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#339
EFFICACY OF INTERFERON-BASED ANTIVIRAL THERAPY IN PATIENTS WITH CHRONIC HEPATITIS C INFECTED WITH HEPATITIS C VIRUS GENOTYPE 5: A META-ANALYSIS OF TWO LARGE PROSPECTIVE BELGIAN CLINICAL TRIALS

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#340
FACTORS IMPACTING SVR IN HCV GENOTYPE 1 PATIENTS WITH EVR AND WEEK 24 NEGATIVITY

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#341
SILYMARIN PHARMACOKINETICS (PK) IS ALTERED IN PATIENTS WITH CHRONIC HEPATITIS C VIRUS (HCV) AND NONALCOHOLIC FATTY LIVER DISEASE (NAFLD) AND CORRELATES WITH CASPASE-3/7 ACTIVITY

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NEGATIVE IMPACT OF ABACAVIR ON RESPONSE TO PEGIFN PLUS RBV IN HIV/HCV COINFECTED PATIENTS

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SUSTAINED VIROLOGIC RESPONSE (SVR) TO PEG-INTERFERON PLUS RIBAVIRIN IN GENOTYPE-4 HCV-HIV COINFECTED PATIENTS

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DETERMINING SUITABLE INTERFERON TREATMENT PHASE USING ACUTE HEPATITIS C CELL CULTURE MODEL

Meng Sheng-Xi, Kata Naoya, Shao Run-Xuan, Muroyama Ryosuke, Chang Jin-Hai, Kawabe Takao, Omata Masao Gastroenterology, University of Tokyo, Tokyo, Japan

RE-TREATMENT WITH PEGYLATED INTERFERON PLUS WEIGHT-ADJUSTED RIBAVIRIN IN HIV+ PATIENTS WITH CHRONIC HEPATITIS C: THE PILOT-NR STUDY

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FEASIBILITY OF COMBINATION THERAPY FOR CHRONIC HEPATITIS C IN IVDU IN THE FRAMEWORK OF AN HERION DETOXIFICATION PROTOCOL

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DESCRIPTION OF PATIENTS WITH VIRAL BREAKTHROUGH DURING PEG IFN AND RIBAVIRIN TREATMENT FOR CHRONIC HCV GENOTYPE 1

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EXTENDING ANTIVIRAL TREATMENT FOR 12 MONTHS AFTER HCV-RNA CLEARANCE IS ASSOCIATED WITH A HIGH RESPONSE RATE IN DIFFICULT-TO-TREAT HCV PATIENTS

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ULTRA RAPID VIROLOGIC RESPONSE PREDICTS SUSTAINED VIROLOGIC RESPONSE IN HCV INFECTED PATIENTS WITH GENOTYPE 3 AND HIGH VIRAL LOAD: THE GET-C STUDY

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CLINICAL RELEVANCE OF THE INTERFERON-ACTIVATED 2′-5′-OLIGOADENYLATE SYNTHETASE (OAS)/RNASE L SYSTEM FOR TREATMENT SUCCESS IN CHRONIC HEPATITIS C

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INCIDENCE AND MANAGEMENT OF PSYCHIATRIC DISTURBANCES IN HEPATITIS C PATIENTS TREATED OUTSIDE OF SPONSORED RESEARCH PROTOCOLS WITH PEGINTERFERON AND RIBAVIRIN

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James Garner, Justus Homburg
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Mohamed Amer, Mohammed Youssi, Fatma Barakat, Yehia Naga, Fatma Barakat, Yoko Kono, Lisa M. Richards, Elliot Alpert, Claude B. Sirlin, Rose Steven, Marquis Hart, Aijal Khanna, Tarek Hassanein
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Chang-Min Cho, Hyun-Cheol Lee, Min-Kyu Jung, Seong-Woo Jeon, Won-Young Tak, Young-Oh Kweon
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Masatoshi Kudo, Kinuyo Hatanaka, Yasunori Minami, Hiroshi Tei, Kiyoshi Maekawa
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Keisuke Kohga1, Tetsuo Takehara1, Hayata Hikita1, Akira Sasakawa1, Akio Uemura1, Ryotaro Sakamori1, Shinjiro Yamaguchi1, Tomohide Tatsumi1, Kazuyoshi Okawa1, Tatsuya Kanto1, Naoki Hiramatsu1, Kazuhiro Katayama2, Michio Katoh1, Norio Hayashi1
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Hikaru Fujioka, Koichi Nonaka, Atsumasa Komori, Seigo Abiru, Shinya Onizuka, Kiyoshi Migita, Masahiro Ito, Hiroshi Yatsuhashi, Hiromi Ishibashi
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Akiko Saito1, Michiyo Chiba1, Tomoko Komiya1, Yuki Yoneda1, Keiko Shiratori1, Shinichi Arizumi1, Satoshi Katagiri1, Hideo Katuragawa1, Masakazu Yamamoto1, Ken Takasaki1, Toshio Morizane2
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Gian Ludovico Rapaccini, Laura Riccardi, Manuela Nestola, Fulvia Elia, Nicoletta de Matthaes, Antonio Grieco, Giovanni Gasbarri, Maurizio Pompili
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Junichi Yamanaka, Shinichi Saito, Yuji limuro, Tadamichi Hirano, Nobukazu Kuroda, Toshihiro Okada, Takaaki Sugimoto, Yasukane Asano, Naoki Uyama, Jiro Fujimoto
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Viral Hepatitis: Pathobiology

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Donna Douglas, Gordon Broderick, Jamie Lewis, Yutaka Yasui, David Bond, Norman Kneteman
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Bettina Langhans1, Ingrid Braunschweiger2, Wibke Schulte1, Judith Satoguina2, Achim Hoerauf2, Tilman Sauerbruch1, Ulrich Spengler1
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Andrew Kim, Shigenobu Kawai, Waihong Chung, Eun Kim, Jack R. Wands, Jisu Li
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Hideki Miyatake1, Tatsuya Kanto1, Michiyu Inoue1, Mitsu Sakakibara1, Ichiro Itoh1, Masanori Miyazaki1, Naruyasu Kikita1, Naoki Hiramoto1, Tetsu Takehara1, Akinori Kasahara2, Norio Hayashi1
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Amany Zekry1,2, Nicole Walley2, Michael Giguère1, Pierre Charneau5, John G. McHutchison3
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Kei Fujiwara, Richard Y. Wang, Robert D. Allison, Cathy Schechterly, Francesco M. Marincola, Harvey Alter
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Lucy Golden-Mason1,2, Brent Palmer3, Jared Klarquist1, John A. Mengshol1, Nicole Castelblanco1, Hugo R. Rosen1,2
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#457
HEPATITIS C VIRUS-INDUCED REACTIVE OXYGEN SPECIES CAUSE IRON ACCUMULATION IN MICE BY REDUCING HEPcidin TRANSCRIPTION
Sojhi Nishina1, Keisuke Hino2, Masaaki Korenaga1, Antonello Pietrangelo2, Yoichi Mizukami3, Takakazu Furutani1, Michiari Okuda1, Isao Hidaka1, Kiwamu Okita4, Isao Sakaida1
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#458
NATURAL HCV CORE VARIANTS REDUCE CELL GROWTH INHIBITION AND FACILITATE INDUCTION OF EPITHELIAL TO MESENCHYMAL TRANSITION (EMT) MEDIATED BY TGF-BETA
Serena Battagia1, Soizic Nobilet1, Olivier Bregerie1, Michelle Giguère1, Pierre Charneau1, Christian Bréchot5, Marie F. Bourgeade1
1CHB, INSERM, Villejuif, France. 2Virology, Institut Pasteur, Paris, France
#459
A CIRRHOSIS RISK SCORE IDENTIFIES THOSE CHRONIC HEPATITIS C INFECTED PATIENTS PRESENTING WITH NO LIVER FIBROSIS THAT ARE AT HIGH RISK FOR FIBROSIS PROGRESSION
Bradford Young1, Martina Gerotto2, Moira Marcolongo2, Francesca Dal Pero2, Robert Logier1, Charles Rowland1, Giada Sebasti2, Alfredo Alberti2.1
1Celera, Alameda, CA, USA. 2Venetian Institute of Molecular Medicine, Padova, Italy. 3Department of Clinical and Experimental Medicine, University of Padova, Padova, Italy

#460
EFFECTS OF HEME OXYGENASE-1 (HO-1) OVEREXPRESSION ON HEPATITIS C VIRUS (HCV) REPLICATION AND CELLULAR INJURY IN VITRO
Zhaowen Zhu1,2, Meleah Mathahs2,1, Feng Wen1,2, Kyle Brown2,1, Bruce A. Luxon1,2, Warren N. Schmidt1,2
1Division of GI & Hepatology, Department of Internal Medicine, University of Iowa, Iowa City, IA, USA. 2Research Services, Veterans Administration Medical Center, Iowa City, IA, USA

#461
COMPREHENSIVE GENE EXPRESSION ANALYSIS OF IRON METABOLISM-RELATED GENES IN PATIENTS WITH CHRONIC VIRAL HEPATITIS AND HEPATOCELLULAR CARCINOMA
Masao Honda, Teruyuki Ueda, Taro Yamashita, Ryuhei Nishino, Hajime Takatori, Shuichi Kaneko
Gastroenterology, Graduate School of Medical Science, Kanazawa University, Kanazawa, Japan

#462
AN ASSESSMENT OF THE ROLE OF ENDOPLASMIC RETICULUM (ER) STRESS IN THE PATHOGENESIS OF HCV
Stuart McPherson1,2, Elizabeth E. Powell1,2, Helen D. Barrie2, Andrew D. Clouston2, Julie R. Jansson2
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#463
PROTECTION BY HLA-B27 IN HCV INFECTION: ROLE OF VIROLOGICAL AND IMMUNOLOGICAL FACTORS
Christoph Neumann-Haefelin1, Eva Dazert2, Susan McKiernan3, Damien Kelleher4, Hubert E. Blum1, Ralf Bartenschlager2, Robert Thimme1
1Department of Medicine II, University Hospital Freiburg, Freiburg, Germany. 2Department of Molecular Virology, University of Heidelberg, Heidelberg, Germany. 3St. James Hospital, Dublin, Ireland

#464
IMPAIRED TLR/RIG-I-MEDIATED INNATE IMMUNITY IN MYELOID DENDRITIC CELLS IN HCV-INFECTED INDIVIDUALS
Masanori Miyazaki, Tatsuya Kanto, Naruyasu Kakita, Michiyoshi Inoue, Ichiro Itose, Hideki Miyatake, Mitsuaki Sakakibara, Takayuki Yakushijin, Naoki Hiramoto, Tetsuo Takehara, Akinori Kasahara, Norio Hayashi
Osaka University Graduate School of Medicine, Suita, Japan

#465
ERADICATION OF HCV BY INTERFERON TREATMENT LEADS TO IMPROVEMENTS IN WHOLE-BODY INSULIN SENSITIVITY
Yasunori Kawaguchi, Toshihiko Mizuta, Yuichiro Eguchi, Tsutomu Yasutake, Keisuke Ario, Hirokazu Takahashi, Shinya Iwane, Iwata Ozaki
Internal Medicine, Saga Medical School, Saga, Japan

#466
INTRAHEPATIC ANGIPOIETIN-2 PROTEIN EXPRESSION MODULATION BY HEPATITIS C VIRUS: MAPK, PI3K AND REACTIVE OXYGEN SPECIES (ROS) IMPLICATION
Paloma Sanz-Cameno1, Samuel Martin-Vilchez1, Yolanda Rodriguez-Munoz1, Maria Jesus Borque1, Jose A. Moreno-Montagudo1, Pedro L. Majano2, Francisca Molina-Jimenez2, Manuel Lopez-Cabrera2, Ricardo Moreno-Otero1
1Liver Unit, Hospital universitario “La Princesa”, Madrid, Spain. 2Molecular Biology Unit, Hospital universitario “La Princesa”, Madrid, Spain

#467
LACK OF FUNCTIONAL RESTORATION BY PD1/PD-L1 BLOCKADE IN INTRAHEPATIC HCV-SPECIFIC CD8 T CELLS FROM CHRONICALLY HCV-INFECTED PATIENTS
 Nobuhiro Nakamoto1,2, David Kaplan1,2, Yun Li1,2, Jennifer Coleclough1,2, Abraham Shaked1, Mary Kaminski1, David A Price5, E. John Wherry3, Gordon Freeman4, Kyong-Mi Chang1,2
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#468
CRYoglobulinemia in Chronic Hepatitis C: A New Marker of Advanced Fibrosis and Severe Steatosis
Rami Moucar1,2, Pierre Bedossa3, Michel Vidaud4, Anna-Carolina Cardoso1, Marie-Pierre Ripault1, Nathalie Boyer1, Michèle Martin-Peignoux2, Marie Hélène Nicolas-Chaoune5, Dominique Valla1,2, Patrick Marcellin1,2, Tarik Asselah1,2
1Hepatology, Beaujon Hospital, Clichy, France. 2INSERM U773-CRB8, Beaujon Hospital, Clichy, France. 3Pathology, Beaujon Hospital, Clichy, France. 4Biochemistry, Beaujon Hospital, Clichy, France. 5Microbiology, Beaujon Hospital, Clichy, France.
#469
HIV AND GP120 ENHANCE HCV REPLICATION AND UPREGULATE TGF-β1
Wenyu Lin, Ethan Weinberg, Kyung Ah Kim, Lee F. Peng, Sun Suk Kim, Mark Brockman, Hernan Lopez-Morra, Carolina Bastos De Sao Borges, Guibenson Hypolite, Run-Xuan Shao, Raymond T. Chung
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#470
INCREASED IRF-1 AND IFN-α GENE EXPRESSION IN CHRONIC HEPATITIS C G-300A IRF-1 PROMOTER POLYMORPHISM A ALLELE CARRIERS
Parwez Aidery, Giuliano Ramadori, Sabine Mihm
Gastroenterology, Georg-August-Universität, Göttingen, Germany

#471
IMPAIRMENT OF CHEMOKINE RECEPTOR CXCR4- EXPRESSION IN HCV INFECTION AS POTENTIAL IMMUNE ESCAPE MECHANISM
Kerstin Herzer1, Christoph C. Schimanski1, Regina Bager1, Sandra Weyer1, Stefan Biesterfeld2, Gerd G. Otto3, Stephan Kanzler1, Peter R. Galle1
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#472
CONTROLLING B-CELL ACTIVATING FACTOR (BAFF/BLYS) LEVELS IN CHRONIC HEPATITIS C VIRUS INFECTION
Gerond Lake-Bakaar, Peter Tsang
Medicine, Weill Cornell University Medical Center, New York, NY, USA

#473
INVOLVEMENT OF REGULATORY T CELL DYNAMICS IN THE ACHIEVEMENT OF BIOCHEMICAL RESPONSE IN 48-WEEK PEG-IFNα2B AND RIBAVIRIN COMBINATION THERAPY FOR CHRONIC HEPATITIS C PATIENTS
Ichiro Ito1, Tatsuya Kanto1, Michiyo Inoue1, Naruyasu Kakita1, Masanori Miyazaki1, Hideki Miyatake1, Mitsuru Sakakibara1, Takayuki Yakushijin1, Naoki Hiramatsu1, Tetsuo Takehara1, Akinori Kasahara2, Norio Hayashi1
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#474
ADIPONECTIN: A NEW INDEPENDENT PREDICTOR OF LIVER STEATOSIS AND RESPONSE TO IFN-A TREATMENT IN CHRONIC HEPATITIS C?
Theodoros A. Zagoras1, Christos Liaskas1, Eirini I. Rigopoulou1, Elias Togouidis1, Konstantinos Makaritsis1, Anastasios Gemenis1, George N. Dolekos1
1Dept. of Medicine, Academic Liver Unit and Research Lab of Internal Medicine, University of Thessaly, Medical School, Larissa, Greece. 2Laboratory of Immunology and Histocompatibility, University of Thessaly, Medical School, Larissa, Greece

#475
ABERRATIONS OF CELL CYCLE MACHINERY IN CHRONIC HEPATITIS C INFECTION
Medicine, Aga Khan University, Karachi, Pakistan

#476
SELECTION, EXPANSION AND FUNCTIONAL RESTORATION OF NS3-SPECIFIC CD4 T CELLS FROM HCV-INFECTED PATIENTS
Marta Bes1, Silvia Saulea1, Josep Quer2, Maria Cubero2, Jaume Guardia2, Lluís Puig1, Rafael Esteban2, Juan I. Esteban2
1Transfusion Safety Lab, Banc de Sang i Teixits, Barcelona, Spain. 2Liver Unit, Hospital Vall d’Hebron, Barcelona, Spain

#477
HCV CORE PROTEINS FROM PATIENTS WITH CHRONIC HEPATITIS C WITH OR WITHOUT STEATOSIS INDUCE GENOTYPE-DEPENDENT MORPHOLOGICAL CHANGES OF INTRACELLULAR LIPID DROPLETS
Aurélié Piodi, Philippe Chouteau, Hervé Lerat, Christophe Hezode, Jean-Michel Pawlotsky
Henri Mondor Hospital, University of Paris 12, Creteil, France

Viral Hepatitis: Virology

#478
MOLECULAR DETERMINANTS OF E6AP-DEPENDENT DEGRADATION OF HEPATITIS C VIRUS CORE PROTEIN
Ikuo Shoji, Kyoko Murakami, Kouichiou Fukuda, Motonao Osaki, Tetsuro Suzuki, Tatsu Miyamura, Takaji Wakita
Department of Virology II, National Institute of Infectious Diseases, Tokyo, Japan

#479
HUMAN MONOCLONAL ANTIBODIES RECOGNIZING A CONSERVED HCV E1 DOMAIN EFFECTIVELY NEUTRALIZE HCV IN THE CELL CULTURE (HCVCC) SYSTEM
Ann Union1, Erik Depla1, Jean-Christophe Meunier2, Vera Goossens1, Suzanne U. Emerson2, Sofie Priem1, Robert H. Purcell2, Geert Maertens1
1Therapeutics, Innogenetics NV, Zwijnaarde, Belgium. 2Laboratory of Infectious Diseases, NIAID, Bethesda, MD, USA

#480
CELL CULTURE-PRODUCED HEPATITIS C VIRUS IMPAIRS PLASMCYTOID DENDRITIC CELL MATURATION AND FUNCTION VIA DIRECT INTERACTION, BUT NOT INFECTION
Masaaki Shiina, Barbara Rehermann
Immunology Section, Liver Diseases Branch, NIDDK, National Institutes of Health, Bethesda, MD, USA


#481

HEPATITIS C P7 PROTEIN ALTERS THE PROTON CONDUCTANCE OF INTRACELLULAR MEMBRANE VESICLES

Ann L. Wozniak1, Stephen Griggen2, David Rawlands2, Mark Harris2, Steven A. Weinman1

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#482

HEPATITIS C VIRUS NS5A BINDING TO NUCLEOSOME ASSEMBLY PROTEIN 1 (NAP 1) ACCELERATED HCV REPLICATION

Run-Xuan Shao1,2, Naoya Kato1, Motoyuki Otsuka1, Naoyuki Yamada3, Jin-Hai Chang3, Ryoosuke Muroyama1, Takao Kawabe1, Masao Omata1

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#483

PRODUCTION OF INFECTIOUS GENOTYPE 1B HEPATITIS C VIRUS IN HUMAN HEPATOMA CELLS

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#484

THE CHOLESTEROL AND SPHINGOLIPIDS OF HEPATITIS C VIRUS PARTICLES PLAY CRITICAL ROLES IN THE VIRAL INFECTIVITY

Hideki Aizaki1, Masagi Fukasawa2, Hideki Tani3, Yoshiharu Mat-suura3,Kentaro Hanada3, Michael M. Lai4, Tatsuo Miyamura1, Takaji Wakita1, Tetsuro Suzuki1

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#485

NEUTRALIZING HOST RESPONSES IN HEPATITIS C VIRUS INFECTION TARGET VIRAL ENTRY AT POST-BINDING STEPS AND MEMBRANE FUSION

Anita Haberstroh1,2, Eva K. Schnober1,2, Patric Carol1,2, Heidi Barth1, Mirjam B. Zeis1,2, Hubert E. Blum1, Marlene Dreux4,5, George Koutsoudakis6, Ralf Hartenschlager6, Arvind Patel7, Catherine Schuster1,2, Françoise Stoll-Keller1,2, Eric Deplaa8, Michel Doffae9, François-Loïc Coisset10, Thomas F. Baumert11,2,9

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#486

HLA CLASS I ASSOCIATED SEQUENCE POLYMORPHISMS IN HCV REVEAL REPRODUCIBLE PATTERNS OF IMMUNE ESCAPE – TOWARDS A COMPREHENSIVE MAP OF KEY RESIDUES FOR VACCINE DESIGN

Thomas Kuntzen1, Andrew Berical1, Niall Lennon2, David Hecker-man3, Joerg Timm4, Sharon Adams5, Julian C. Schulze zur Wiesch6, Jonathan Carlson7, Sarah Young2, Arthur Y. Kim1, Carl Kades1, Georg M. Lauer1, Francesco M. Marincola2, Raymond T. Chung7, Florian K. Bihi8, Andreas Cerny8, Christian Brander1, Ulrich Spengler9, Bruce W. Birren2, Bruce D. Walker1, Gerold Lake-Bakaar10, Eric S. Daar12, Ira M. Jacobson11, Edward D. Gomperts11, Brian R. Edlin11, Sharyne M. Donfield14, Andrew Talal11, Tony N. Marion10, Matthew R. Henn9, Todd M. Allen1

1Partners AIDS Research Center, Department of Infectious Diseases, Massachusetts General Hospital, Harvard Medical School, Charlestown, MA, USA. 2Broad Institute of Massachusetts Institute of Technology & Harvard, Cambridge, MA, USA. 3Microsoft Research, Redmond, WA, USA. 4Department of Virology, Essen University Hospital, Essen, Germany. 5Clinical Center, HLA Laboratory, National Institutes of Health, Bethesda, MD, USA. 6Medizinische Klinik I, Universitätsklinikum Hamburg Eppendorf, Hamburg, Germany. 7Gastroenterology Unit, Massachusetts General Hospital, Harvard Medical School, Boston, MA, USA. 8The Swiss HCV Cohort Study, Clinica Moncucco, Lugano, Switzerland. 9Department of Internal Medicine I, Bonn University Hospital, Bonn, Germany. 10Hepatitis C Cooperative, University of Tennessee Health Science Center, Memphis, TN, USA. 11Center for the Study of Hepatitis C, Weill Medical College of Cornell University, New York, NY, USA. 12Divisions of HIV Medicine and Infectious Diseases, Department of Medicine, Los Angeles Biomedical Research Institute at Harbor, UCLA Medical Center, David Geffen School of Medicine at UCLA, Los Angeles, CA, USA. 13The Saban Research Institute, Childrens Hospital Los Angeles, Los Angeles, CA, USA. 14Department of Biostatistics, Rho, Inc., Chapel Hill, NC, USA

#487

HEPATITIS C RESOLUTION CORRELATES WITH PATTERNED KIR RECEPTOR EXPRESSION ON KILLER LYMPHOCYTES

James C. Ryan1,2, Alexander Monto1,2, Mandeepeh Lehli, Michael Kim1,2, Daniel Tracy1,2, Sally George2, Alan P. Kennedy2, Teresa L. Wright1,3, Sue Currie1,2

1Medicine, GI Division, University of California, San Francisco, San Francisco, CA, USA. 2Division of Gastroenterology, San Francisco Veterans Affairs Medical Center, San Francisco, CA, USA.
#489
GENOMIC ANALYSIS OF HCV BREAKTHROUGHS OCCURRING DURING THE HALT-C TRIAL LEAD-IN TREATMENT PHASE
Hejun Yuan1, Mamta K. Jain1, Michael Gale2, William M. Lee1
1Internal Medicine, UTSW Medical Center at Dallas, Dallas, TX, USA. 2Microbiology, UTSW Medical Center at Dallas, Dallas, TX, USA

#490
INTERACTIONS OF CXCL-8 WITH INNATE ANTIVIRAL DEFENSES DURING HCV INFECTION
Jessica Wagoner1, Anne M. Wertheimer3, Hugo R. Rosen2, Stephen J. Polyak1
1Laboratory Medicine, University of Washington, Seattle, WA, USA. 2Gastroenterology, University of Colorado Health Sciences Center, Denver, CO, USA. 3Vaccine and Gene Therapy Institute, Beaverton, OR, USA

#491
A NOVEL IRES IN THE CORE-ENCODING REGION STIMULATES PRODUCTION OF MINI-CORE, A SMALL PROTEIN COMPRISED OF THE C-TERMINAL PORTION OF THE CORE PROTEIN
Francis J. Eng1, Laura K. McMullan2, Arielle L. Klepper1, Matthew J. Evans3, Charles M. Rice1, Suresh M. Desai3, Andrea D. Branch1
1Division of Liver Diseases, Mount Sinai School of Medicine, New York, NY, USA. 2Center for the Study of Hepatitis C, Rockefeller University, New York, NY, USA. 3Infectious Diseases Research and Development, Abbot Laboratories, Abbot Park, IL, USA

#492
DEVELOPMENT OF JFH1-BASED INTERGENOTYPIC CELL CULTURE SYSTEMS OF HEPATITIS C VIRUS GENOTYPES 1-5 AND THEIR USE IN STUDIES OF VIRAL ENTRY AND NEUTRALIZATION
Judith M. Gottwein1, Troels Scheel1, Tanja Bertelsen Jensen1, Jan-nick Prentel1, Maria L. Knudsen1, Jasper Eugen-Olsen1, Jens Buch1,2
1Department of Infectious Diseases and Clinical Research Unit, Copenhagen University Hospital, Hvidovre, Denmark. 2Laboratory of Infectious Diseases, National Institutes of Health, Bethesda, MD, USA

#493
PLAQUE-FORMING ASSAYS FOR HEPATITIS C VIRUS AND ISOLATION OF HCV-JFH1 MUTANTS WITH ENHANCED CYTOPATHOGENICITY AND REPLICATION CAPACITY
Naoya Sakamoto1,2, Yuko Sekine-Osajima1, Kako Mishima1, Mina Nakagawa1,2, Megumi Tasaka1, Yuki Nishimura-Sakurai1, Yasuhiro Itsu1, Takaji Wakita3, Mamoru Watanabe1
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#494
ANTI-MALARIAL DRUG CHLOROQUINE SUPPRESSES THE REPLICATION OF HCV REPLICON VIA PKR-INDEPENDENT MECHANISMS
Tomokazu Mizui1, Shunhei Yamashina1, Yoshiyuki Takei1, Kenichi Ikejima1, Tsuneo Kitamura1, Takashi Ueno2, Eiki Kominami2, Sumio Watanabe1
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#495
CROSS-LINKING OF CD81 WITH HCV E2 REGULATES MIGRATION OF NATURAL KILLER (NK) CELLS IN A ROCK-DEPENDENT FASHION
Jacob Nattermann, Benjamin Krämer, Ludger Leifeld, Hans Dieter Nischalke, Tilman Sauerbruch, Ulrich Spengler
Internal Medicine I, University of Bonn, Bonn, Germany

#496
SCAVENGER RECEPTOR BI IS REQUIRED FOR AN ENTRY STEP CLOSELY LINKED TO CD81
Mirjam B. Zeisel1,2, George Koutsoudakis3, Eva K. Schnober1,4, Anita Haberstroh1,4, Hubert E. Blum4, François-Loïc Cosset1,2, Takaji Wakita5, Daniel Jaeck6, Michel Doffao7, Cathy Royer1,2, Eric Soulier1,2, Evelyne Schvoirer1,2, Catherine Schuster1,2, Françoise Stoll-Keller1,2, Ralf Bartenschlager2, Thomas Pletschmann2, Heidi Barth4, Thomas F. Baumert1,9
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#497
EVIDENCE FOR HIV AND HCV REPLICATION IN THE PERITONEAL MACROPHAGE
John J. Garber1, Arielle L. Klepper2, Julio A. Gutierrez2, Theresa L. Chang1, Aprilre Rapista3, Viktoriya Khaitova4, Douglas T. Dieterich2, Thomas D. Schiano3, Andrea D. Branch2
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#498
ESTABLISHMENT OF INFECTIOUS GENOTYPE 1B HEPATITIS C VIRUS CLONE TO HUMAN HEPATOCYTE CHIMERIC MOUSE
Takashi Kimura1, Michio Imamura1, Kiyomi Toyota1, Nobuhiko Hira1, Chiemi Naguchi1, Masataka Tsuchi1, Shoichi Takahashi1, Keiko Arakita2, Kazuaki Chayama1
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#499

**HLA-E RESTRICTED RECOGNITION OF HEPATITIS C VIRUS BY A SUBSET OF IFN-γ SECRETING CD8+ T CELLS**

Daniela Schulte¹, Agathe Iwan¹, Monika Schulz¹, Claudia Zwank¹, Hans Dieter Nischalke¹, Ludger Leifeld¹, Elisabeth Weiss², Tilman Sauerbruch¹, Ulrich Spengler¹, Jacob Nattermann¹

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#500

**HYPERPROLACTINEMIA ASSOCIATED WITH CHRONIC HEPATITIS C AND INDUCTION OF PROLACTIN EXPRESSION IN HUMAN PERIPHERAL BLOOD MONONUCLEAR CELLS STIMULATED BY HEPATITIS C VIRUS**

Rika Ishii¹, Takafumi Saito¹, Shao Li², Kazuo Okumoto¹, Jun-itsu Ito¹, Kazuhiko Sugahara¹, Koji Saito¹, Hitoshi Togashi¹, Sumio Kawata¹

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#501

**ANTIVIRAL EFFECTS OF INTERFERON-INDUCED PROTEINS, GBP-1, IFI-6-16 AND IFI-27 AND THEIR INTERACTIONS WITH HEPATITIS C VIRUS NONSTRUCTURAL PROTEINS**

Yasuhiro Itsui¹, Naoya Sakamoto¹, Mina Nakagawa¹, Yuki Sekine-Osajima¹, Yuki Nishimura-Sakurai¹, Megumi Tasaka¹, Mamoru Watanabe¹

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#502

**HCV-RELATED PROTEINS INDUCE PROINFLAMMATORY CYTOKINE PRODUCTION BY THE HUMAN KUPFFER CELL VIA THE MYD88-DEPENDENT SIGNALING CASCADE**

Hiroshi Kono, Kenichi Ishii, Naohiro Hosomura, Nobuyuki Tanaka, Masanori Matsuda, Hideki Fujii

First Department of Surgy, University of Yamanashi, Chuo, Japan

#503

**FULL-LENGTH SEQUENCE ANALYSIS OF HCV GENOTYPE-3A REVEALS NOVEL REGIONS OF HYPERVARIABILITY**

Isla Humphreys¹, Larry Park², Katja Pfafferott², Silvana Gaudieri², Paul Kleereman¹, Eleanor Barnes¹

¹Peter Medawar building, Nuffield department of medicine, Oxford, United Kingdom. ²Royal Perth Hospital, Centre for Clinical immunology and Biomedical statistics, Perth, WA, Australia
Poster Session 2
Sunday, November 4
POSTER VIEWING: 8:00 AM - 5:30 PM
Hynes, Exhibit Hall C

Presenters in attendance:
1:00 - 2:30 PM

Those posters identified as AASLD Presidential Poster of Distinction by a ribbon icon have received review scores that place them within the top 10 percent of all posters. We encourage you to make them a priority as you visit the poster sessions.

Cell Death and Hepatoxicity

#504
LOSS OF AUTOPHAGY PREVENTS APOPTOTIC CELL DEATH DURING ISCHEMIA-REPERFUSION IN THE MOUSE LIVER
Shunhei Yamashina1, Yoshiyuki Takei2, Tomokazu Mizui1, Kenichi Ikejima1, Masaaki Komatsu3, Takashi Ueno1, Eiki Kominami3, Sumio Watanabe1
1Dept of Gastroenterology, Juntendo university, Tokyo, Japan. 2Dept of Gastroenterology, Mie University, Tsu-City, Japan. 3Dept of Biochemistry, Juntendo University, Tokyo, Japan

#505
GP130, THE SIGNAL-TRANSUCING IL-6 RECEPTOR SUBUNIT, IS A SUBSTRATE OF CASPASES
Dirk Graf, Katrin Haslow, Ivo Münks, Johannes Bode, Dieter Häussinger
Clinic of Gastroenterology and Hepatology, Düsseldorf, Germany

#506
ENDOTOXIN INDUCES ER STRESS IN RAT PRIMARY HEPATOCYTES VIA STELLATE CELLS: ROLE OF JNK MAPK ACTIVATION
SeonHee Oh1, Noor Mohamed Jameel1, Donna B. Stolz2, Chandrashekhar R. Gandhi1
1Surgery, University of Pittsburgh, Pittsburgh, PA, USA. 2Cell Biology, University of Pittsburgh, Pittsburgh, PA, USA

#507
ARSENIC AT SUBHEPATOTOXIC DOSES SYNERGISTICALLY ENHANCES LIPOPOLYSACCHARIDE-INDUCED LIVER INJURY IN MICE
Gavin E. Arteel1,2, Heather L. Miller1, Theresa S. Chen1, Luping Guo1, Thomas J. Schlierf1, Juliane I. Beier1, J. Phillip Kaiser1, J. Christopher States1
1Dept of Pharmacology/Toxicology, University of Louisville, School of Medicine, Louisville, KY, USA. 2The James Graham Brown Cancer Center, University of Louisville, Louisville, KY, USA

#508
METFORMIN PROTECTS RAT HEPATOCYTES AGAINST APOPTOSIS VIA THE PI3-KINASE/AKT SURVIVAL PATHWAY, BUT HAS NO EFFECT ON NF-kB SIGNALING
Titia E. Vrenken, Laura Conde de la Rosa, Manon Buist-Homan, Klaas Nico Faber, Han Moshage
Gastroenterology and Hepatology, University Medical Center Groningen, Groningen, Netherlands

#509
ZINC SUPPLEMENTATION STIMULATES HEPATIC REGENERATION BY PRESERVING HEPATOCYTE NUCLEAR FACTOR-4α IN MICE SUBJECTED TO A LONG-TERM ETHANOL ADMINISTRATION
Xinjin Kang, Zhenyuan Song, Craig J. McClain, Y. James Kang, Zhanxiang Zhou
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#510
QUALIFICATION OF FOUR SERUM BIOMARKERS OF HEPATOTOXICITY BY THE PREDICTIVE SAFETY TESTING CONSORTIUM (PSTC)
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#511
OXIDATIVE STRESS MODULATES THE EXPRESSION OF KRÜPPEL-LIKE FACTOR-6 AND ITS SPLICE-VARIANTS
Raquel Urtasun, Francisco Javier Cubero, Steven Yea, Scott L. Friedman, Natalia Nieto
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#512
P38- AND ERK- MAPK MEDIATE SUPEROXIDE-INDUCED APOPTOSIS OF ACTIVATED RAT HEPATIC STELLATE CELLS, WHICH IS REVERSED BY RETINOIC ACID
Noor Mohamed Jameel1, Chinnasamy Thirunavukkarasu1, Simon C. Watkins1, Tong Wu3, Chandrashekhar R. Gandhi1
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#513
A PROTEOMIC APPROACH TO NUCLEOSIDE ANALOGUE ASSOCIATED MITOCHONDRIAL TOXICITY
Matthias Banasch1, Sven Liffers2, Wolfgang E. Schmidt1, Helmut E. Meyer2, Kai Stühler2
1Department of Internal Medicine 1, St. Josef-Hospital, University of Bochum, Bochum, Germany. 2Medical Proteome-Center, University of Bochum, Bochum, Germany
**SUPPRESSIVE EFFECTS OF RETINOIDS ON IRON-INDUCED OXIDATIVE STRESS IN LIVER BY DOWNREGULATION OF HEMOJUVELIN**

Hiroyuki Tsuchiya, Tomohiko Sakabe, Toshiko Hoshikawa, Goshi Shiota  
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**HEPATO主旨 GROWTH FACTOR PROTECTS AGAINST OXIDATIVE INJURY INDUCED BY ETHANOL METABOLISM**

Argelia Valdes-Arzate, Luis E. Gómez-Quiroz, Cynthia Licona, Leticia Bucio, Veronica Souza, Elizabeth Hernandez, David Kushner,  
Tottori University, Yonago, Japan

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**HEPATIC MITOCHONDRIAL α-TOCOPHEROL AND CARDIOLIPIN IN YOUNG RATS MAY AFFORD PROTECTION AGAINST BILE ACID-INDUCED MITOCHONDRIAL PATHWAYS OF CELL DEATH**

Ronald J. Sokol, Michael W. Devereaux, Genevieve C. Sparagna, Scott W. Leonard, Maret G. Traber, Eric Gumpricht  
University of Colorado School of Medicine, Denver, CO, USA. Oregon State University, Corvallis, OR, USA

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**INHIBITION OF MITOCHONDRIAL PROTEIN OXIDATION FOLLOWING OXIDATIVE STRESS DOES NOT ALTER CELL FATE**

Jorge Allina, Bin Hu, Jingxiang Bai, Joseph Odin  
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**COSUPPLEMENTATION WITH VITAMIN E AND COENZYME Q(10) REDUCES IRON-OVERLOADED INDUCED HEPATIC STEATOSIS IN TRANSGENIC MICE EXPRESSING THE HEPATITIS C VIRUS POLYPROTEIN**

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**CORRELATION OF LIVER “COLLAGEN PROPORTIONATE AREA” BY COMPUTER ASSISTED IMAGE ANALYSIS AND HEPATIC VENOUS PRESSURE GRADIENT IN PATIENTS WITH RECURRENT HCV INFECTION AFTER LIVER TRANSPLANTATION**

Vincenza Calvaruso, Richard Standish, Pinelopi Manousou, Sergio Mainone, David Patch, James O’Beirne, Elias Xirochakis, Alexandros Sigalas, Alice Corbani, Amar P. Dhillon, Andrew K. Burroughs  
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**EVALUATION OF WEEK 12 RESPONSE ON SVR IN HCV POST TRANSPLANT PATIENTS UNDERGOING PEG/RBV THERAPY**

Reem H. Ghalib, Cheryl Levine, Blaine Hollinger, Rise Stribling, Terry Box, William R. Hutson, Anthony B. Post, Shobha N. Joshi, Anisha Steephen, Jeffrey Weinstein, Alejandro Mejia, Stephen Cheng  
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**CHRONIC HEPATITIS E : A NEW ENTITY IN ORGAN TRANSPLANT PATIENTS**

Nassim Kamar, Jean-Marie Péron, Leila Ouezzani, Jean-Michel Mansuy, Jannick Selves, Joelle Gutierrez, Jacques Izopet, Dominique Durand, Jean-Pierre Vinel, Lionel Rosetaing  
Service d’Hépato-Gastro-Entérologie, CHU PURPAN, Toulouse, France. Service de Néphrologie et Transplantation d’Organe, CHU RANGUEIL, Toulouse, France. Laboratoire de Virologie, CHU PURPAN, Toulouse, France. Service d’Anatoomo-Pathologie, CHU PURPAN, Toulouse, France

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**POST-LIVER TRANSPLANT SURVIVAL IN HEPATITIS C PATIENTS IS IMPROVING, NOT DECLINING**

Jacqueline G. O’Leary, Lafaine M. Grant, Henry Randall, Nicholas Onaca, Linda Jennings, Goran Klintmalm, Gary L. Davis  
Internal Medicine, Baylor University Medical Center, Dallas, TX, USA. Surgery, Baylor University Medical Center, Dallas, TX, USA
#523
INTRAHEPATIC COVALENTLY CLOSED CIRCULAR DNA (CCC DNA) DETECTION IN PATIENTS TRANSPLANTED FOR HBV-RELATED CIRRHOSIS: A TOOL TO JUDGE FOR HBIG PROPHYLAXIS WITHDRAWAL IN LOW-RISK TRANSPLANT RECIPIENTS?
Ilaria Lenci1, Giuseppe Tisone1, Marco Ciotti2, Daniele Di Paolo1, Laura Taricciotti1, Fabio Marcuccilli3, Carlo F. Perno2, Mario Angelico1
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#524
HBV GENOTYPE C IS ASSOCIATED WITH HIGHER RATES OF HCC AND POST-LIVER TRANSPLANT (OLT) MORTALITY COMPARED TO GENOTYPES A, B AND D
Paul J. Gaglio2, Sundeep Singh1, Bulent Degertekin1, Michael B. Ishitan3, Munira Hussain1, Robert P. Perrillo4, Anna S. Lok1, Study Group NIH HBV Olt1
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#525
EVALUATION OF THE INTRAHEPATIC EXPRESSION OF TWO INTERFERON-INDUCIBLE PROTEINS, MXA AND IFI16, DURING ACUTE REJECTION AND VIRAL REINFECTION OF LIVER ALLOGRAFTS
Carlo Smirne1, Cinzia Borgona1, Carlo Fabris2, Barbara Azimonti1, Cecilia Marconi1, Elena Mossi1, Michela Burlone1, Pierluigi Tonitutto2, Rosalba Minisini1, Marisa Gariglia1, Mario Pirisi1
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#526
SUSTAINED VIROLOGICAL RESPONSE IN PATIENTS SUCCESSFULLY TREATED FOR RECURRENT HEPATITIS C FOLLOWING LIVER TRANSPLANTATION IS HIGHLY DURABLE
Les Lilly, Nigel Girgrah, George Therapondos
Toronto General Hospital, Toronto, ON, Canada.

#527
COMPARISON BETWEEN HEPATIC ELASTICITY MEASUREMENTS (FIBROSCAN®) AND LIVER BIOPSY IN PATIENTS TRANSPLANTED FOR HCV CIRRHOSIS
Flávio Feitosa1,2, Sylvie Radenne2, Tierry Bizollon2, Argemiro d’Oliveira1, Pierre Pradat2, Raymundo Parana1, Christian Trepo2
1Gastro-Hepatology, Hospital Prof Edgar Santos, Salvador, Brazil. 2Gastroenterologie et Hepatologie, Hopital de l’Hotel-Dieu, Lyon, France.

#528
HYPERIMMUNE ANTI-HBS PLASMA FOR THE PREVENTION OF HBV RECURRENCE AFTER LIVER TRANSPLANTATION: EFFICACY, SAFETY, KINETICS AND ECONOMICS IN 26 PATIENTS DURING 14-YEAR EXPERIENCE OF TWO CENTERS
Florian K. Bihl1, Vanina Gurtner2, Stefan Russman3, Loriana Di Giannmarino4, Claudia Steineman5, Andreas Cerny5, Antoine Hadengue1, Pietro Majno6, Emiliano Giastra1, Damiano Castelli2, Gilles Menth6
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#529
CAUCASIAN AMERICAN HEPATITIS B LIVER TRANSPLANT PATIENTS HAVE HIGHER RATES OF WAITLIST MORTALITY AND HBV RECURRENT THAN ASIAN AMERICANS
Nathalie H. Bzowej2, Emmet B. Keeffe3, Tram Tran4, Steven Han4, Sukru Emre6, Bulent Degertekin1, Anna S. Lok1, Study Group NIH HBV Olt1
1University of Michigan, Ann Arbor, MI, USA. 2California Pacific Medical Center, San Francisco, CA, USA. 3Stanford University, Palo Alto, CA, USA. 4Cedars Sinai Med Ctr, Los Angeles, CA, USA. 5UCLA, Los Angeles, CA, USA. 6Mt Sinai Med Ctr, New York, NY, USA.

#530
METABOLIC SYNDROME IS AN INDEPENDENT PREDICTOR OF FIBROSIS PROGRESSION IN PATIENTS WITH RECURRENT HEPATITIS C (HCV) AFTER LIVER TRANSPLANTATION (LT) USING SERIAL BIOPSY SPECIMENS
Ibrahim A. Hanouneh1, Charles M. Miller2, Arthur J. McCullough3, Rocio Lopez4, Federico Aucejo5, Ariel E. Feldstein5, Nizar N. Zein3
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#531
MULTICENTER RANDOMIZED TRIAL IN HCV-INFECTED PATIENTS TREATED WITH INTERFERON ALFA-2A AND RIBAVIRIN FOLLOWED BY RIBAVIRIN ALONE AFTER LIVER TRANSPLANTATION: FINAL REPORT

Yvon Calmus1, Christophe Duvoux2, Georges-Philippe Pageaux3, Michel Messner4, Philippe Wolfl, Lionel Rostaing5, Claire Vanlemmens2, Danielle Botta-Fridlund8, Sebastien Dharyancy9, Jean Gugenhheim10, Francois Durand11, Martine Neau-Cransac12, Olivier Boilor13, Olivier Chazouilleres14, Isabelle Lonjon-Domanec15, Laurence Samelson15, Didier Samuel16
1Hôpital Cochin, Paris, France. 2Hôpital Henri Mondor, Créteil, France. 3Hôpital Saint Eloi, Montpellier, France. 4Hôpital Pontchaillou, Rennes, France. 5Hôpital Hautepierre, Strasbourg, France. 6Hôpital Rangueil, Toulouse, France. 7Hôpital Minjoz, Besançon, France. 8Hôpital La Conception, Marseille, France. 9Hôpital Huriez, Lille, France. 10Hôpital L’Arche, Nice, France. 11Hôpital Beaujon, Clichy, France. 12Hôpital Pellegrin, Bordeaux, France. 13Hôpital Edouard Herriot, Lyon, France. 14Hôpital St Antoine, Paris, France. 15Roche, Neuilly sur Seine, France. 16Hôpital Paul Brousse, Villejuif, France

#532
HEPATITIS C VIRUS (HCV) INFECTION IS A PROTECTIVE FACTOR FOR HEPATITIS B VIRUS REACTIVATION (HBV) AFTER RECEIVING HEPATITIS B CORE POSITIVE (HBCAB+) DONORS FOR LIVER TRANSPLANTATION (LT)

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#533
HIGH PREVALENCE OF GLOMERULONEPHRITIS IN PATIENTS WITH END-STAGE HCV-INDUCED CIRRHOSIS

Brendan McGuire1, Bruce A. Julian1, Jan Novak2, Steve Bynon3, William J. Cook4, Stacy Eddleman1, Devin E. Eckhoff3
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#534
RAPID FIBROSIS IN HCV-PATIENTS AFTER LIVER TRANSPLANTATION IS ASSOCIATED WITH AN UPREGULATION OF COLLAGEN TYPE I, MMP-9 AND TIMP-1

UlF Neumann1, Martin Ruehl2, Tarkan Dagdelen2, Friedmar Delissen2, Walburga Dieterich2, Maximilian Schmeding1, Martin Zeitz2, Peter Neuhaus1, Rajan Somasundaram2
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#535
ANTI-HCV IMMUNE RESPONSES MODULATE RECURRENT OF HCV INFECTION AND SEVERITY OF LIVER HISTOLOGY AFTER LIVER TRANSPLANTATION IN HIV/HCV CO-INFECTED PATIENTS

A. Samri1,2, Anne-Marie Roque-Afonso3, Anne Beran1,4, C. Fera2,5, E. Dussaux6,7, Didier Samuel1,7, B. Autran2,3, Jean-Charles Duclos-Vallée1,6
1Centre Hépato-Biliaire, AP-HP Hôpital Paul Brousse, Villejuif, France. 2Unité 543 Cell Immunology, Inserm, Paris F-75013, France. 3UMR-S 543, AP-HP Hôpital Pitié Salpêtrière, Paris F-75013, France. 4UMR-S 543, Université Pierre et Marie Curie Paris VI, Paris F-75013, France. 5Département de Virologie, AP-HP Hôpital Paul Brousse, Villejuif F-94804, France. 6U 785, Inserm, Villejuif F-94804, France. 7UMR-S 785, Univ Paris-Sud, Villejuif F-94804, France. 8Département de Microbiologie, AP-HP Hôpital Paul Brousse, Villejuif F-94804, France

#536
SERUM LEVELS OF FIBROSIS PROGRESSION BIOMARKERS MEASURED EARLY AFTER LT ARE ASSOCIATED TO SEVERE HCV RECURRENCE

Dariela Micheloud1, Magdalena Salcedo2, Salvador Resino1, Diego Rincon2, Emilio Alvarez3, Gerardo Clemente4, Maria Vega Catalina2, Judith Gomez-Camarena2, Raquel Lorente1, Maria Angeles Muñoz-Fernandez1, Rafael Baraeraes2
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#537
HLA-DRW LOCUS DISPARITY IS ASSOCIATED WITH WORSE OUTCOME IN HCV TRANSPLANT RECIPIENTS

Alexandros Sigalas1, Pinelopi Manousou1, Elias Xiouchakis1, Vincenza Calvaruso1, Alice Corbani1, Graham Shirley3, Dimitrios N. Samonakis1, James O’Beirne1, David Patch1, Nancy Rolando1, Keith Rolles4, Brian Davidson4, Federica Grillo5, Amar P. Dhillon2, Andrew K. Burroughs1
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#538
TRANSIENT ELASTOGRAPHY (TE) IS AN ACCURATE TOOL IN MONITORING LIVER TRANSPLANTED (LT) PATIENTS WITH RECURRENT HEPATITIS C

Cristina Rigamonti1, Maria Francesca Donato1, Francesca Agnelli1,2, Mirella Fraquelli2, Giovanni Casazza3, Giorgio Rossi4, Massimo Colombo1
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#539

**PATIENTS WITH HCV-INDUCED CIRRHOSIS WITH IMMUNE-COMPLEX GLOMERULONEPHRITIS HAVE PROGRESSIVE PROTEINURIA AFTER LIVER TRANSPLANTATION**

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#540

**FIBROSIS PROGRESSION ON PROTOCOL LIVER BIOPSIES IN PATIENTS WITH RECURRENT HCV FOLLOWING LIVER TRANSPLANTATION**

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#541

**CONSENSUS INTERFERON THERAPY FOR HEPATITIS C RECURRENT AFTER PEGYLATED INTERFERON FAILURE POST LIVER TRANSPLANT**

Marwan Ghabril¹, Rolland C. Dickson¹, Jennifer Lucas², Jaime Aranda-Michel¹, Andrew Keaveny¹, Barry Rosser¹, Denise M. Harnois, Rolland C. Dickson, Raj Satyanarayana, Daniel Yip, Andrew Keaveny

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#542

**A PROSPECTIVE STUDY ON THE SAFETY AND EFFICACY OF LAMIVUDINE AND ADEFOVIR DIPIVOXIL PROPHYLAXIS IN HBsAG POSITIVE LIVER TRANSPLANTATION CANDIDATES**

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#543

**WHAT IS THE RISK OF HBV TRANSMISSION IN RECIPIENTS OF LIVER GRAFT FROM HBC POSITIVE ANTIBODY, HBS NEGATIVE ANTIGEN DONORS? A SINGLE CENTER EXPERIENCE OVER 10 YEARS**

Bruno Roche¹,³, Anne-Marie Roque-Afonso²,³, Teresa Antonini¹, Michelle Gigou³,⁴, Denis Castaing¹,⁴, Didier Samuel¹,⁴

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#544

**RESPONSE OF FIBROSING CHOLESTATIC RECURRENT HEPATITIS C FOLLOWING LIVER TRANSPLANTATION TO COMBINATION INTERFERON/RIBAVIRIN THERAPY**

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#545

**PREDICTING CARDIOVASCULAR EVENTS AFTER LIVER TRANSPLANTATION**

Michael Herman, Surakit Pungpapong, Jaime Aranda-Michel, Barry Rosser, Denise M. Harnois, Rolland C. Dickson, Raj Satyanarayana, Daniel Yip, Andrew Keaveny

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#546

**TRANSPLANTATION TRENDS IN RECIPIENTS OVER THE AGE OF 65**

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#547

**THE EFFECT OF DISEASE RECURRENT ON GRAFT SURVIVAL FOLLOWING ORTHOTOPIC LIVER TRANSPLANTATION**

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#548

**NATURAL HISTORY AND PROGNOSTIC INDICATORS IN CIRRHOTIC PATIENTS WITH PULMONARY HYPERTENSION**

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#549

**EXCELLENT OUTCOME OF PATIENTS WITH ACUTE ALCOHOLIC HEPATITIS AFTER LIVER TRANSPLANTATION**

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**#550**

**EVALUATION OF THE BENEFIT ON RENAL FUNCTION OF A DELAYED INTRODUCTION OF TACROLIMUS IN LIVER TRANSPLANT RECIPIENTS AT 13 FRENCH CENTERS**

Yvon Calmus1, Lionel Rostaing2, Jean Gugenheim3, Christophe Duvoux4, Christian Ducerf5, Philippe Wolf6, Didier Samuel7, Claire Vanlemmens8, Martine Neau-Cransac9, Ephrem Salamé10, Olivier Chazouillères11, Nicole Declerc12, Georges-Philippe Pageaux13

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**#551**

**THE ISCHEMIC PRECONDITIONING (IPC) PARADOX IN LIVER TRANSPLANTATION (LT) – EVIDENCE FROM A PROSPECTIVE RANDOMIZED TRIAL**

Kunj Desai, Asif Shareef, George Dikdan, Kenneth Klein, Andrew delaTorre, Meelie Debroy, Adrian Fisher, Dorian Wilson, Bo Peng, Arun Samanta, Baburao Koneru

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**#552**

**MELD-NA IS SUPERIOR TO OTHER ORGAN ALLOCATION SYSTEMS**

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**#553**

**LONG TERM RESULTS OF A RANDOMIZED TRIAL OF TACROLIMUS MONOTHERAPY VERSUS TRIPLE THERAPY IN HCV CIRRHOSIS LIVER TRANSPLANT RECIPIENTS**

Pinelopi Manousou1, Dimitris Samonakis1, Alice Corsbani1, E. Cholongitas1, Alexandros Sigalas1, Elias Xiouchakis1, Vincenza Callvaruso1, Federica Grillo2, David Patch1, James O’Beirne1, Amor P. Dhillon2, Keith Rolles2, Brian Davidson3, Andrew K. Burroughs1

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**#554**

**GRAFT SURVIVAL AFTER LIVING DONOR OR CADAVERIC LIVER TRANSPLANTATION: AN ANALYSIS STRATIFIED BY DONOR RISK**

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**#555**

**SERUM SODIUM LEVELS ARE NOT INDEPENDENT OF FLUID STATUS AND DISEASE SEVERITY IN LIVER TRANSPLANT WAITING LIST CANDIDATES**

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**#556**

**SAFETY AND EFFICACY OF THYMoglobulin FOR STEROID RESISTANT ACUTE CELLULAR REJECTION FOLLOWING LIVER TRANSPLANTATION**

Ajay K. Sahaipal, Vishnu Madhok, J. E. Hay, Michael R. Charlton, C. B. Rosen, R. Wiesner, K. V. Menon

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**#557**

**MYCOPHENOLATE MOFETIL PLUS LOW DOSE CALCINEURIN INHIBITOR FOR RENAL DYSFUNCTION IN LIVER TRANSPLANT: A 24-MONTH CONTROLLED CLINICAL TRIAL**

Maurizio Biselli1, Giovanni Vitale1, Annaguiulia Gramenzi1, Anna Rili1, Sonia Berardi1, Francesca Dazzani1, Carlo Cammà2, Alessandra Scuteri1, Maria Cristina Morelli1, Claudia Sama1, Antonio Daniele Pinna3, Mauro Bernardi1, Pietro Andreone1

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**#558**

**SMOKING RELATED MORBIDITY AND MORTALITY POST LIVER TRANSPLANTATION**

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**#559**

**MULTICENTER, RANDOMIZED TRIAL OF CONVERSION TO EVEROLIMUS WITH CALCINEURIN INHIBITOR MINIMIZATION OR DISCONTINUATION IN LIVER TRANSPLANT PATIENTS WITH RENAL IMPAIRMENT**

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LIVER TRANSPLANTATION FOR BUDD-CHIARI SYNDROME: A 20-YEAR NATIONAL REGISTRY ANALYSIS
Dorry L. Segev¹, Geoffrey C. Nguyen², Jayme E. Locke¹, Christopher E. Simpkins¹, Robert A. Montgomery¹, Warren R. Maley¹, Paul J. Thuluvath²
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COMBINED LIVER HEART TRANSPLANTATION IN THE UNITED STATES
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AMYLOID CARDIOMYOPATHY FOLLOWING LIVER TRANSPLANTATION FOR FAMILIAL AMYLOID POLYNEUROPATHY ATTR MET30 (PORTUGUESE TYPE): RESULTS FROM THE COLLABORATIVE BRITISH-SWEDISH STUDY
Arie J. Stangou¹, Mark Monaghan², John G. O’Grady¹, Chris Bachman³, Thodore Kyriakides⁴, Mohamed Rela¹, Bo-Goran Ericzon², Nigel Heaton³, Ole Suhr⁴
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Rohit Satoskar, Andrew Aronsohn, Helen S. Te, Smruti R. Mohanty, Kapuluru G. Reddy, Nancy Reau, Donald M. Jensen
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Xavier Xiol¹, Pere Gines², Lluís Castells³, Alba Ribalta⁴, Jorge Twose⁴, Clara Ventura⁵, Joan Carles Reverter², Xavier Fuentes¹, Roser Deulofeu⁴
¹Hospital de Bellvitge. Idibell, L’Hospitalet, Spain. ²Hospital Clinic, Idiabas, Barcelona, Spain. ³Hospital Vall d’Hebrón, Barcelona, Spain. ⁴OCATT, Barcelona, Spain

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DECEPTION DURING PSYCHOLOGICAL EVALUATION FOR LIVER TRANSPLANT IN ALCOHOLIC CIRRHOSIS: A DUI CORROBORATION STUDY
Jasmin D. Baajali¹, Muhammad Hafeezullah¹, Andrea Thompson², Kia Saedian¹, Jose Franco¹, Rebecca C. Anderson²
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Sheila L. Esward, Elizabeth R. Lyden, Timothy M. McCashland
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Laura M. Kulik, Basset Atassi, Robert Lewandowski, Mary Mulcahy, Michael Abecassiss, Riad Salem
Northwestern University, Chicago, IL, USA

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Isabel Aguilera¹, Jose Manuel Sousa², Ingeborg Wichmann¹, Francisco Gavilan³, Angel Bernardo⁴, Antonio Nuñez-Roldán¹
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#569
NEGATIVE IMPACT OF OBESITY ON LONG-TERM OUTCOMES OF ORTHOTOPIC LIVER TRANSPLANTATION (OLT)
Joel A. Rodriguez¹, John M. Vierling², Thomas A. Aloia¹, Natasha S. Becker¹, Christine A. O’Mahony¹, John A. Goss¹
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#570
HAVE U.S. ORTHOTOPIC LIVER TRANSPLANT (OLT) OUTCOMES FOR ACUTE LIVER FAILURE (ALF) IMPROVED IN THE LAST DECADE?
Baylor College of Medicine, Houston, TX, USA
#571
PRETRANSPLANT RISK FACTORS FOR ADVANCED CHRONIC KIDNEY DISEASE IN LIVER TRANSPLANTATION

Santiago Tome1,2, Julio Pascual2, Milagros Samaniego2, Adnan Said2, Luis Fernandez2, Tony D’Alessandro2, Aji Djamali2, Michael R. Lucey2, John Pirsch2, Hans Sollinger2, Stuart J. Knechtle2
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THE EFFECT OF HEPATIC ENCEPHALOPATHY ON POST LIVER TRANSPLANTATION (LT) MORBIDITY AND MORTALITY D. BRANDMAN, S.W. BIGGINS, J.P. ROBERTS, N.A. TERRAULT

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#573
LONG-TERM OUTCOME WITH RATG INDUCTION AND STEROID-FREE IMMunosUPPRESSION IN PEDIATRIC LIVER TRANSPLANTATION (PLTX)

George V. Mazariegos, Zurab Machaidze, Kyle Solty, Geoffrey Bond, Robert Squires, Rakesh Sindhi
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USE OF MULTIVISCERAL TRANSPLANTATION IN THE MANAGEMENT OF CIRRHOTIC PATIENTS WITH DIFFUSE PORTOMESENTERIC THROMBOSIS

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LONG-TERM OUTCOMES FOR PATIENTS TRANSPLANTED FOR ACETAMINOPHEN INDUCED ACUTE LIVER FAILURE; NO WORSE THAN FOR OTHER ETIOLOGIES

Constantine J. Karvellas, Julia Wendon, Niloufar Safinia, Georg Auzinger, Elizabeth Sizer, Andrew J. Portal, Nigel Heaton, Mohammed Rela, Paolo Muiian, Michael A. Heneghan, John G. O’Grady, William Bernal
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#576
SEXUAL DYSFUNCTION BEFORE AND AFTER LIVER TRANSPLANTATION (LT)

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IMPLICATIONS OF PREOPERATIVE PULMONARY FUNCTION TESTING FOR POST LIVER-TRANSPLANT OUTCOMES

Peter Ghali1, Marc Deschenes1, Jeffrey Barkun2, Phillip Wong1, Nir Hilzenrat1, Peter Metrakos2, Jean Tchervenkov2, Marcelo Cantarovich1, Kevin Schwartzman1
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Derek Dubay, Leslie Lilly, Robert Smith, Gary A. Levy, George Therapondos
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#579
THE IMPACT OF ISCHAEMIC PRECONDITIONING ON GENE EXPRESSION IN HUMAN LIVER TRANSPLANTATION

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#580
A MULTI-INSTITUTIONAL ANALYSIS OF 235 PATIENTS UNDERGOING OLT FOR HEPATOPULMONARY SYNDROME

Natasha S. Becker, Jessica Suarez, Thomas A. Aloia, Joel A. Rodriguez, Rise Stirling, Norman L. Sussman, John M. Vierling, Christine A. O’Mahony, John A. Goss
Michael E. DeBakey Department of Surgery, Baylor College of Medicine, Houston, TX, USA

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ABUNDANCE OF PD-1 AND PD-L1 EXPRESSING CD8 T-CELLS IN THE LIVER MAY BE KEY TO HEPATIC TOLEROCENICITY

Pengyun Wang, Wayel Jassem, Maria Serena Longhi, Nigel Heaton, Giorgina Mieli-Vergani, Diego Vergani, Mohamed Rela, Yun Ma
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STERoid AVOIDANCE IN LIVER TRANSPLANTATION: META-ANALYSIS AND META-REGRESSION OF RANDOMIZED TRIALS

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Monika Hurtova1, Alexis Laurent2, Dora Bachir3, Pablo Bartolucci3, Daniel Cherqui2, Christophe Duvoux1
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HIGH CIRCULATING REGULATORY T CELL LEVELS IN LONG-TERM STABLE LIVER TRANSPLANTS TREATED WITH CYCLOSPORINE COMPARED TO TACLORIMUS
Hyun Young Woo, Jong Young Choi, Chan Ran You, Si Hyun Bae, Seung Kew Yoon, Jin Mo Yang, Sang Wook Choi, Young Seok Lee, Chang Don Lee, Kyu Won Chung
Internal Medicine, The Catholic University of Korea, College of Medicine, Seoul, South Korea

#585
TRENDS IN LIVER TRANSPLANTATION FOR HEPATOCELLULAR CARCINOMA IN THE UNITED STATES
Benjamin H. Leach, Jonathan Prather, David Scott, Douglas Norman, Jonathan M. Schwartz
Oregon Health and Sciences University, Portland, OR, USA

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HOMA-IR AND MICROALBUMINURIA ARE ASSOCIATED WITH MICROVASCULAR COMPLICATIONS IN PRE-LIVER TRANSPLANT PATIENTS - A CASE-CONTROL STUDY
Karen L. Krok, Farida Millwala, Anurag Maheshwari, Paul J. Thuluvath
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#587
LIVER BIOPSY TO PREDICT MORTALITY IN FULMINANT HEPATIC FAILURE
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RECURRENT AUTOIMMUNE HEPATITIS AFTER LIVER TRANSPLANTATION AT A CENTER PRACTICING CORTICOSTEROID MINIMIZATION
Jeffrey Campsen, Michael Zimmerman, James Trotter, Thomas Bak, Michael Wachs, Tracy Steinberg, Maria Kaplan, Igal Kam
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#589
PRE-TRANSPLANT VARIABLES PREDICT QUALITY OF LIFE IN LIVER TRANSPLANT RECIPIENTS
Sammy Saab, Bijal Surti, Ayman Ibrahim, Francisco A. Durazo, Steven-Huy B. Han, Hasan Yerisz, Douglas Farmer, R M. Ghobrial, Leonard Goldstein, Myron J. Tong, Ronald Busuttil
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#590
MICROVASCULAR INVASION DOES NOT IMPACT SURVIVAL IN PATIENTS UNDERGOING ORTHOTOPIC LIVER TRANSPLANTATION (OLT) FOR HEPATOCELLULAR CARCINOMA (HCC) WHO HAD PRE-OLT LOCAL THERAPY
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GB VIRUS-C INFECTION IS ASSOCIATED WITH BETTER 10-YEAR-SURVIVAL OF LIVER TRANSPLANT RECIPIENTS
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Natasha S. Becker, Thomas A. Aloia, Joel A. Rodriguez, Rise Stirling, John M. Vierling, Norman L. Sussman, Christine A. O’Mahony, John A. Goss
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RENAL FAILURE FOLLOWING LIVER TRANSPLANTATION
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NODULAR REGENERATIVE HYPERPLASIA POST SOLID ORGAN (NON-LIVER) TRANSPLANT
Shahid M. Malik, David A. Sass, Jaideep Behari, Rajagopal Chadalavada, A Jake Demetris, Jawad Ahmad
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EVALUATION OF PATIENTS WITH ALCOHOLIC LIVER DISEASE FOR LIVER TRANSPLANTATION
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#598
HEPATOCELLULAR CARCINOMA (HCC) IN PATIENTS WITH HCV VERSUS NON-HCV CIRRHOSIS: GRAFT AND PATIENT SURVIVAL AFTER LIVER TRANSPLANTATION (LT) IN PRE-MELD AND MELD ERA
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EFFECTIVENESS OF PERCUTANEOUS LASER ABLATION AS BRIDGE TREATMENT TO LIVER TRANSPLANTATION IN CIRROTIC PATIENTS WITH HEPATOCELLULAR CARCINOMA
Maurizio Pompili1, Erica Nicolardi1, Giampiero Francica2, Alessandra Petrolati3, Mario Angelico4, Giuseppe Tison4, Giuseppe Santeusanio5, Paolo Crabosleda6, Bonaventura Marina6, Zaccaria Rossi7, Sara Pacella8, Gian Ludovico Rapacchini1, Giovanni Gasbarrini1, Claudio M. Pacella9
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#600
FOLLOW-UP OF PATIENTS AFTER LIVER TRANSPLANTATION USING DOMINO GRAFTS
Ana P. Barreiros1, Rosa Walz1,2, Christian Geber1, Frank Birklein3, Cathrin Theis4, Christian Weiss4, Felix Post4, Karl F. Kreitner5, Michael Heise2, Marcus Schuchmann1, Peter R. Galle1, Gerd Otto1
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#602
THE READABILITY OF HEALTH EDUCATION MATERIALS FOR LIVER TRANSPLANT PATIENTS
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#603
LONG TERM OUTCOMES OF LIVER TRANSPLANTATION IN A HIGH-MELD COHORT
Jason Lee1,2, Maximilian Lee1, Lynn Shapiro1, Alice L. Yang1, Alex S. Lapasaran1, Sanah Parvez1, Ahmad Kamal1,2, Emmet B. Keeffe1, Carlos O. Esquivel1, Aijaz Ahmed1
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CORONARY ARTERY DISEASE SCREENING IN LIVER TRANSPLANTATION CANDIDATES

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EARLY HEPATOCYTE C4D EXPRESSION AND FAILURE TO RESTORE BASAL HEPATIC TOTAL PROTEIN CONTENT AFTER ISCHEMIA REPERFUSION INJURY ARE ASSOCIATED WITH ONE YEAR GRAFT LOSS IN HUMAN LIVER TRANSPLANTATION

Paola Barsotti 1, Maria Siciliano 2, Rosanna De Marco 2, Antonio Molinaro 2, Andrea Onetti Muda 1, Novella Gualtieri 1, Manuela Merli 2, Adolfo Francesco Attili 2, Pasquale Berlolo 3, Massimo Rossi 1, Stefano Ginani Corradini 2

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PATIENTS ON CHRONIC DIALYSIS HAVE LOWER LIVER TRANSPLANTATION WAITING LIST MORTALITY THAN THOSE WITH ACUTE RENAL FAILURE

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RESECTION OF HEPATOCELLULAR CARCINOMA: LONG-TERM OUTCOME

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MINIMISING BILIARY COMPLICATIONS AFTER TRANSPLANTATION OF LIVERS FROM DONORS AFTER CARDIAC DEATH

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ALLOGRAFT SURVIVAL IS DECREASED IN PATIENTS WITH RECURRENT PRIMARY SCLerosING CHOLANGITIS

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NATIVE RENAL FUNCTION ONE YEAR FOLLOWING COMBINED LIVER-KIDNEY TRANSPLANT

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DEVELOPMENT OF A UK SCORE FOR PATIENTS WITH END-STAGE LIVER DISEASE

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PROPOSAL OF NEW SELECTION CRITERIA FOR LIVING DONOR LIVER TRANSPLANTATION CANDIDATES FOR HEPATOCELLULAR CARCINOMA

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C4D IMMUNOHISTOCHEMICAL (IHC) REACTIVITY AS AN ADJUNCT IN DISTINGUISHING BETWEEN ACUTE CELLULAR REJECTION (ACR) AND RECURRENT HEPATITIS C VIRAL (RHCV) INFECTION IN LIVER TRANSPLANTS

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PULMONARY AND BLOOD STREAM INFECTIONS IN ADULT LIVER TRANSPLANT RECIPIENTS

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OUTCOME DIFFERENCES AFTER LIVING DONOR LIVER TRANSPLANTATION (LDLT) IN ADULTS WITH AUTOIMMUNE AND CHOLESTATIC LIVER DISEASES: SRTR ANALYSIS

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TRANSPLANT COSTS OF ADULT LIVING DONOR LIVER TRANSPLANTATION AT AN EXPERIENCED TRANSPLANT CENTER ARE SIMILAR TO DECEASED DONOR LIVER TRANSPLANTATION DESPITE INCREASED SURGICAL COSTS

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SUCCESSFUL ALGORITHM FOR SELECTIVE LIVER BIOPSY IN THE RIGHT LOBE LIVER DONOR (RLD)

Mary Ann Simpson, Jennifer E. Verbesey, Urmila Khettry, Fredric Gordon, James J. Pomposelli, Roger L. Jenkins, Elizabeth A. Pomfret Liver transplantation, Lahey Clinic, Burlington, MA, USA

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HIGH MELD SCORES DO NOT PREDICT ONE-YEAR SURVIVAL RATE OF PATIENTS WITH A SMALL-FOR-SIZE GRAFT IN ADULT LIVING DONOR LIVER TRANSPLANTATION

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IMPACT OF ISCHEMIC PRECONDITIONING IN GRAFT FUNCTION AND INFLAMMATORY MEDIATORS IN ORTHOTOPIC LIVER TRANSPLANTATION

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Denotes AASLD Presidential Poster of Distinction
#623
ASSESSMENT OF REPRODUCIBILITY OF CREATININE MEASUREMENT AND MELD SCORING IN FOUR LIVER TRANSPLANT UNITS IN THE UK

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#624
DOES HEALTH RELATED QUALITY OF LIFE CORRELATE WITH THE MODEL FOR END STAGE LIVER DISEASE SCORE BEFORE LIVER TRANSPLANTATION?

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#625
SHOULD DONOR ORGANS AND RECIPIENTS BE MATCHED IN LIVER TRANSPLANTATION? AN ANALYSIS OF THE UNITED KINGDOM AND IRELAND LIVER TRANSPLANT DATABASE

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#626
LIVER TRANSPLANTATION FOR HEPATOCELLULAR CARCINOMA: VALIDATION OF A NEW PROGNOSTIC SCORE PREDICTING OVERALL SURVIVAL

Thomas Decaens1, Françoise Roudot-Thoraval2, Hanana Badran1, Carole Meyer1, François Durand3, Rene Adam3, Olivier Boillot1, Claire Vanlenens10, Jean Gugenheim18, Sebastien Dharancy9, Pierre-Henri Bernard15, Philippe Campagnon6, Yvon Calmus5, Jean Hardwigsen13, Christian Ducet14, Georges-Philippe Pageaux11, Marie Noëlle Hillement16, Olivier Chazouillères6, Daniel Cherqui17, Christophe Duvoix1
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#627
HEPATECTOMY FOR HEPATOCELLULAR CARCINOMA AT LIVER TRANSPLANT CENTERS IS ASSOCIATED WITH REDUCED IN-HOSPITAL MORTALITY

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#628
TREATMENT OUTCOMES OF TRANSCATHETER ARTERIAL CHEMOINFUSION (TACI) IN PATIENTS WITH UNRESECTABLE HEPATOCELLULAR CARCINOMA (HCC) PRIOR TO ORTHOTROPIC LIVER TRANSPLANTATION

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Experimental Hepatocarcinogenesis

#629
INOS AND COX-2 SYNERGISTICALLY ENHANCE PGE2 PRODUCTION THROUGH S-NITROSYLATION OF CYTOSOLIC PHOSPHOLIPASE A2α IN HUMAN CHOLANGIOCARCINOMA CELLS

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#630
ROLE OF IKKβ/NF-κB ACTIVATION FOR DEVELOPMENT OF LIVER METASTASIS

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#631
A PROTECTIVE ROLE FOR ADIPONECTIN IN HUMAN HEPATOCELLULAR CARCINOMA: ACTIVATION OF AMPK INHIBITS HEPG2 PROLIFERATION IN VITRO AND STUNTS TUMOR GROWTH IN HEPG2-NUDE MICE XENOGRAFTS

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#632
CANCER TARGETED AND TISSUE SPECIFIC GENE THERAPY OF IN VIVO HEPATOCELLULAR CARCINOMA MODEL BY HTERT-TARGETING TRANS-SPlicing RIBOZYME AND LIVER SPECIFIC PROMOTER
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#633
DELETION OF THE GNMT GENE PREDISPOSES TO LIVER INJURY AND DEVELOPMENT OF HEPATOCELLULAR CARCINOMA
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#634
RELEVANCE OF IGF SIGNALING PATHWAY IN HUMAN HEPATOCELLULAR CARCINOMA, AND PRE-CLINICAL ASSESSMENT OF NOVEL TARGETED THERAPIES
Victoria Tovar1, Clara Alsinet1, Manel Solé1, Augusto Villanueva2, Derek Chiang3, Laia Cabellos1, Adriana Lázaro1, Myron Schwartz2, Vincenzo Mazzaferraro4, Jordi Bruix1, Josep M. Llovet1,2
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#635
PREVENTION OF INTRAHEPATIC METASTASIS OF HEPATOCELLULAR CARCINOMA BY COMBINATION OF SUICIDE GENE THERAPY AND MONOCYTE CHEMOATTRACTANT PROTEIN-1 DELIVERY IN MICE
Kaheita Kakinoki1, Yasunari Nakamoto1, Takashi Kagaya1, Tomoya Tsuchiyama1, Yoshio Sakai1, Naofumi Mukaida2, Shuichi Kaneko1
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#636
IFNγ IS CRITICALLY REQUIRED FOR IL-12-MEDIATED NK CELL ACTIVATION AND ANTI-TUMOR EFFECT IN THE LIVER
Akio Uemura, Tetsuo Takehara, Hayato Hikita, Akira Sasakawa, Keisuke Kohga, Ryuotaro Sakamori, Shinjiro Yamaguchi, Takuya Miyagi, Tomohide Tatsumi, Kazuyoshi Ohkawa, Tatsuya Kanto, Naoki Hiramatsu, Norio Hayashi
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#637
LIBRARY OF GENETIC ASSOCIATIONS: CONNECTING COMPLEX LIVER DISEASES AND GENETICS
Stefan Buchkremer, Jasmin Hendel, Markus Krupp, Arndt Weinmann, Kai Schlamp, Peter R. Galle, Andreas Teufel
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#638
INHIBITION OF HEPATIC TUMOR ANGIogenesis WITH MET INHIBITORS
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#639
PEGYLATED INTERFERON ATTENUATES WNT/β-CATENIN SIGNALING IN HEPATOCELLULAR CANCER
Michael D. Thompson, Gang Zeng, Ben Cieply, Satdarshan P. Monga
University of Pittsburgh, Pittsburgh, PA, USA

#640
EXPLOITING THE MULTIPLE MECHANISMS OF SORAFENIB: TUMOR GROWTH INHIBITION WITH A NOVEL COMBINATION OF SORAFENIB AND RAPAMYCIN TARGETING BOTH RAS AND MTOR PATHWAYS
Pippa Newell1, Augusto Villanueva1, Judit Peix1, Yingbei Chen1, Analisa Di Feo1, Stijn Van Laarhoven1, Eric R. Lemmer1, Maria Isabel Fiel1, Swan N. Thung1, Steven Yeo1, Susan Roayaie1, Myron Schwartz1, John A. Martignetti1, Scott L. Friedman1, Josep M. Llovet1,2
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#641

**ACTIVATION OF THE WNT/B-CATENIN PATHWAY IN HEPATOCELLULAR CARCINOMA, AND IN VITRO GROWTH INHIBITION WITH A NOVEL SMALL MOLECULE ICG-001**

Pippa Newell1, Augusto Villanueva1, Derek Chiang4, Judit Peix1, Clara Alsina1, Stijn Van Laerhoven1, Swan N. Thung1, Maria Isabel Fiel1, Steven Yea1, Sasan Roayaie1, Myron Schwartz1, Michael Kahn3, Scott L. Friedman1, Josep M. Llovet1,2

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**CLOSE RELATIONSHIP BETWEEN CYTOKERATIN 19 EXPRESSION AND SIDE POPULATION PHENOTYPE DURING HUMAN HEPATOCELLULAR CARCINOGENESIS**

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#643

**THE INTRONIC P53 BINDING SITE IS ESSENTIAL FOR ACTIVATION OF THE CD95 GENE BY THE P53 FAMILY NETWORK**

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#644

**EXPRESSION OF MULTIDRUG RESISTANCE-ASSOCIATED PROTEIN 1 IN HEPATOCELLULAR CARCINOMA IS ASSOCIATED WITH AGGRESSIVE TUMOR PHENOTYPE AND CAN REFLECT PROGENITOR CELL ORIGIN**

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#645

**SELECTIVE HOMING OF ENDOTHELIAL PROGENITOR CELLS WITH CYTOSINE DEAMINASE CDN TO TUMOR TISSUES SUPPRESSES GROWTH OF HEPATOCELLULAR CARCINOMA BY 5'-FLUOROURACIL SECRETION**

Takiji Torimura1,2, Takato Ueno2,1, Kinya Inoue1,2, Eitaro Taniguchi1,3, Toru Nakamura1,2, Osamu Hashimoto1,2, Hironori Koga1,2, Hirohisa Yano1,2, Masamichi Kajiro1,2, Michio Sata1,2

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**MAPK7 IDENTIFIED AS A PROBABLE TARGET FOR AMPLIFICATION AT 17P11 IN HEPATOCELLULAR CARCINOMA**

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**NEIGHBOR OF PUNC E11 (NOPE): A NOVEL MARKER FOR MURINE HEPATOCELLULAR CARCINOMA**

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**HSP90 INHIBITION ABROGATES HUMAN HCC GROWTH THROUGH CDC2-MEDIATED G2/M CELL CYCLE ARREST**

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**LIVER SPECIFIC LDB1 DELETION RESULTS IN ENHANCED LIVER CANCER DEVELOPMENT**

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LIVER AND SPLEEN DENDRITIC CELL FUNCTION MODIFICATION DURING A MURINE MODEL OF HEPATOCARCINOGENESIS
Antonino Castellaneta, Nicola De Tullio, Francesca I. Gagliardi, Doriana Francioso, Michele Barone, Alfredo Di Leo, Antonio Francavilla
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#651
TARGETING HISTONE DEACETYLATION FOR TREATMENT OF HEPATOCellular CARCINOMA
Yun-Han Lee1, Hyun Goo Woo1, Itzhak Avital1,2, Adam D. Judge3, Marian Durkin1, Anita Toni1, Ju-Seog Lee4, Elizabeth A. Conner1, Valentina Factor1, Ian MacLachlan3, Snorri S. Thorgeirsson1
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#652
ACTIVATION STATUS OF PI3K/AKT/MTOR PATHWAY IN HEPATOCellular CARCINOMA (HCC): ONCOGENIC ROLE AND CLINICAL IMPLICATIONS RICTOR (MTOR COMPLEX 2)
Augusto Villanueva1, Derek Y. Chiang2, Philippa Newell1, Judit Peix1, Yujin Hoshida2, Si Jin Van Laarhoven1, Swan N. Thung3, Sasar Roayaie4, Clara Alsinet5, Victoria Tovar5, M. Isabel Fiel3, Myron Schwartz4, Samuel Waxman1, Jordi Bruix5, Vincenzo Mazzaferro6, Todd R. Golub2, Scott L. Friedman1, Matthew Meyerson2, Josep M. Llovet1,5
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#653
DECREASED INTRAHEPATIC CD8 TRAPPING IS ASSOCIATED WITH SUPPRESSION OF HEPATOCellular CARCINOMA: A ROLE FOR THE BETA GLYCOLIPID ACYL CHAIN LENGTH
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#654
SUPPRESSION OF ALPHA-1,6 FUCOSYLTRANSFERASE INHIBITS INVASIVENESS OF HUMAN HEPATOCellular CARCINOMA CELLS
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#655
INDUCTION OF HEPATOMA-SPECIFIC IMMUNITY BY SUICIDE GENE THERAPY IN CC CHEMOKINE RECEPTOR (CCR) 1- OR CCR5-DEPENDENT MANNER
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#656
MOLECULAR COMBINED THERAPY IN HEPATOCellular CARCINOMA (HCC) WITH AEE788 (EGFR/HER2/VEGFR INHIBITOR) AND RAD001, EVEROLIMUS (MTOR INHIBITOR): ANTINEOPLASTIC ACTIVITY IN A XENOGRAFT MODEL OF HCC
Augusto Villanueva3, Philippa Newell1, Judit Peix1, Analisa Di Feo3, Si Jin Van Laarhoven1, Swan Thung3, Fiel Isabel1, Steven Yea1, Sasar Roayaie4, Myron Schwartz3, John Martignetti2, Scott L. Friedman1, Josep M. Llovet1,5
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INTEGRATIVE GENOMIC CLASSIFICATION OF HEPATITIS C VIRUS POSITIVE HEPATOCellular CARCINOMAS
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EXPRESSION AND ROLE OF STEAROYL COENZYMIE A DESATURASE (SCD) IN HUMAN HCC
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#659
KNOCKDOWN OF MIRNAS ENCODED BY THE POLYCIESTRON, Mir-17-92, CAUSES A PARTIAL REVERSION OF THE MALIGNANT PHENOTYPE OF HEPG2 CELLS
Erin Connolly, Tatjana Tchaikovskaya, Leslie E. Rogler, Charles E. Rogler
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#660
ABROGATION OF CONSTITUTIVE STAT3 ACTIVITY SENSITIZES HUMAN HEPATOMA CELLS TO TRAIL-MEDIATED APOPTOSIS
Kazuhiro Nakao, Mariko Kusaba, Tatsuki Ichikawa, Hisamitsu Miyaki, Yusuke Motoyoshi, Hitota Shibata, Katsumi Eguchi
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Experimental Liver Transplantation and Liver Surgery

#661
HEPATOCYTE PROLIFERATIVE RESPONSE TO PARTIAL HEPATECTOMY IS MARKEDLY IMPAIRED IN TIRAP KNOCKOUT MICE
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#662
INTRACELLULAR INTERLEUKIN-2 IN CYTOTOXIC CD8+ T-LYMPHOCYTES CORRELATES TO THE BANFF SCORE DURING ACUTE ORGAN REJECTION IN LIVER TRANSPLANT RECIPIENTS
Bora Akoglu1,2, Susanne Kriener3, Stefan Zeuzem1, Dominik Faust2,1
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#663
INCREASED MORTALITY IN MMP-9 KNOCKOUT MICE FOLLOWING 75-PERCENT PARTIAL HEPATECTOMY
Norifumi Ohashi, Naoki Shimojima, Justin H. Nguyen
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#664
DOWN-REGULATION BY 1,25 DlHYDROXYVITAMIN D3 OF CD40L-INDUCED IMMUNOREGULATORY CYTOKINES PRODUCTION AND CO-STIMULATORY ACTIVITY IN MONOCYTE-MACROPHAGES FROM LIVER TRANSPLANT RECIPIENTS
Cristiana Almerighi1, Alberto Bergamini2, Raffaella Lionetti1, Anna Sinistro2, Simona Francioso1, Giuseppe Tisone3, Mario Angelico1
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#665
PERIPHERAL BLOOD MONONUCLEAR CELLS GZB POSITIVE INCREASE AFTER THE FIRST WEEK FROM LIVER TX IN PATIENTS EXPERIENCING HCV RECURRENT
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#666
HEPATITIS C VIRUS COULD BE ERADICATED WITH STRONG IMMUNE RESPONSES AFTER WITHDRAWING INTERFERON TREATMENT POST LIVING DONOR LIVER TRANSPLANTATION
Akinobu Takaki1, Takahito Yagi2, Yoshiaki Iwasaki1, Hiroshi Sadamori2, Kazuko Koike1, Masashi Tatsukawa1, Haruhiko Kobashi1, Kohsaku Sakaguchi1
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#667
ISCHEMIC PRECONDITIONING INCREASES Mn-SUPEROXIDE DISMUTASE AND PREVENTS FREE RADICAL-DEPENDENT MITOCHONDRIAL DEPOLARIZATION IN SMALL-FOR-SIZE LIVER GRAFTS
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#668
ADENOVIRAL GENE DELIVERY OF INTERLEUKIN-10 REDUCES HEPATIC ISCHEMIA-REPERFUSION INJURY IN RATS THRU INHIBITION OF KUPFFER CELL ACTIVATION
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#669

**URIDINE-5’-TRIPHOSPHATE (UTP) PROTECTS AGAINST HEPATIC ISCHEMIC INJURY IN MICE**

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#670

**INTRAHEPATIC COMPLEMENT ACTIVATION, SINUSOIDAL ENDOTHELIAL INJURY AND LACTIC ACIDOSIS IS ASSOCIATED WITH INITIAL POOR FUNCTION OF THE LIVER POST TRANSPLANTATION**

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#671

**INTERMITTENT REPERFUSION STRESS ACCELERATES LIVER REGENERATION BY INDUCING ENTRY AND PROGRESSION IN CELL CYCLE OF HEPATOCYTES**

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#672

**OXIDATIVE DAMAGE TO MITOCHONDRIAL DNA AND PROTEINS IN SMALL-FOR-SIZE LIVER GRAFTS**

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**ALTERNATIVE PRECONDITIONING WITH DEATH LIGANDS TNF-α & FASL PROTECTS THE CIRRHOTIC MOUSE LIVER AGAINST ISCHEMIC INJURY**

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**P66SHC, AN AGEING PROTEIN, PLAYS A PIVOTAL ROLE IN POST-HEPATECTOMIZED LIVER REGENERATION IN AGED MICE**

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**METHOXYPOLYETHYLENE GLYCOL MODIFIED-ALBUMIN (PEG-ALB) ENHANCED THE COLD PRESERVATION PROPERTIES OF UW SOLUTION IN RAT LIVER GRAFTS**

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**HUMAN HEPATIC NRF2 CORRELATES WITH POST-TRANSPLANT IL-8 AND TRANSAMINASES INDICATING A NOVEL PROTECTIVE MECHANISM**

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**MURINE ADIPOSE TISSUE DERIVED STROMAL CELLS TRANS-DIFFERENTIATE INTO HEPATIC LINEAGE CELLS BY BASIC FIBROBLAST GROWTH FACTOR**

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DO INJECTED AUTOLOGOUS BONE MARROW CELLS WORK IN PATIENTS WITH ADVANCED LIVER CIRRHOSIS?
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N-ACETYLCYSTEINE IMPROVES THE METABOLIC AND SYNTHETIC FUNCTION OF HUMAN HEPATOCYTES ISOLATED FROM SEVERELY STEATOTIC DONOR LIVERS
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PURIFICATION OF A SPECIFIC CELL POPULATION CONTAINING ALL OF THE REPOPULATION POTENTIAL OF FETAL LIVER STEM CELLS FOR THE NORMAL ADULT RAT LIVER
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A CRITICAL ROLE OF FAS (CD95) IN ALLOREJECTION OF TRANSPLANTED HEPATOCYTES
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#682
MECHANISMS REGULATING SURVIVAL OF DONOR CELLS IN THE NON-HEART BEATING RAT LIVER
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#683
HUMAN HEPATOCYTE GROWTH FACTOR EXPRESSION IN ENDOTHELIAL PROGENITOR CELLS ENHANCES REGENERATIVE PROPERTIES IN RAT CIRRHOTIC LIVER
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#684
INITIAL CLEARANCE OF TRANSPLANTED CELLS FROM THE LIVER IS REGULATED IN PART BY COX-1-MEDIATED INFLAMMATORY MECHANISMS AND COX-INHIBITION IMPROVES CELL ENGRAFTMENT
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#686
PRIMARY SCLEROSING CHOLANGITIS IS AN INDEPENDENT PREDICTOR OF EARLY HEPATIC ARTERY THROMBOSIS FOLLOWING PRIMARY LIVER TRANSPLANTATION: A COHORT MULTI-CENTRE STUDY
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IMPAIRMENT OF THE INDOLEAMINE 2,3-DIOXYGENASE PATHWAY IN PRIMARY BILIARY CIRRHOsis
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INDUCTION OF PRIMARY BILIARY CIRRHOSIS IN GUINEA PIGS USING CHEMICAL XENOBIOTIC IMMUNIZATION: IMPLICATIONS FOR TOLERANCE AND THE ETIOLOGY OF HUMAN PBC
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RISK OF CARDIOVASCULAR AND CEREBROVASCULAR EVENTS IN PRIMARY BILIARY CIRRHOSIS: A POPULATION BASED COHORT STUDY
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#691
GENETIC POLYMORPHISM OF HLA-DR AND CYTOKINE GENES IN JAPANESE PATIENTS WITH PRIMARY BILIARY CIRRHOSIS (PBC) – HLA-DRB1*0405 CONFRS SUSCEPTIBILITY TO GP210-TYPE PROGRESSION OF PBC
Minoru Nakamura1, Hisayoshi Kondo2, Yoshihiro Aiba1, Atsumasa Komori1, Mutsumi Matsuyama2, Masahiro Ito1, Kiyoshi Migita1, Hiroshi Yatsuhashi1, Takeo Saoshiro2, Michiaki Koga3, Yukio Watanabe3, Hideki Hayashi3, Masaaki Shimada3, Naohiko Masaki1, Hideo Morimoto3, Masakazu Kobayashi1, Koichi Honda3, Hiroki Oishi4, Kazuhiro Tsukamoto4, Mihoko Kikuchi3, Michio Yasunami3, Hiromi Ishibashi1
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A CRITICAL ROLE OF INVARIANT NKT CELLS IN EXACERBATING THE BILIARY LESIONS IN A TGF-β RECEPTOR II DOMINANT-NEGATIVE MOUSE MODEL OF PRIMARY BILIARY CIRRHOSIS
Ya-Hui Chuang1, Zhe-Xiong A. Lian1, Guo-Xiang Yang1, Shang-An Shu1, Yuki Moritoki1, William M. Ridgway2, Aftab A. Ansari3, Mitchell Kronenberg4, Richard A. Flavell5, Bin Gao6, M. Eric Gershwin1
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PATIENTS WITH PRIMARY BILIARY CIRRHOSIS AT HIGH RISK FOR LOW BONE MASS CAN ACCURATELY BE IDENTIFIED BY SIMPLE RISK SCORE INDICES
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**#695**

**CENTRAL CEREBRAL ACTIVATION IS REDUCED IN PRIMARY BILIARY CIRRHOSIS AND ASSOCIATED WITH EXCESSIVE DAYTIME SLEEPINESS**

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**AUTONOMIC DYSFUNCTION IS ASSOCIATED WITH STRUCTURAL BRAIN ABNORMALITIES, PARTICULARLY IN THE GLOBUS PALLIDUS**

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**RESULTS OF THE FRENCH STUDY OF RISK FACTORS FOR PRIMARY BILIARY CIRRHOSIS**

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**#698**

**PRIMARY BILIARY CIRRHOSIS (PBC) WITH INITIAL NORMAL BILIRUBIN CONCENTRATION: TREATMENT WITH UDCA DOES NOT AFFECT LIVER-RELATED SURVIVAL**

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**#699**

**COMBINATION ANTIRETROVIRAL THERAPY WITH COMBIVIR ATTENUATES AUTOIMMUNE BILIARY DISEASE IN THE NOD.C3C4 MOUSE MODEL OF PRIMARY BILIARY CIRRHOSIS**

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**HLA DRB1 ALLELES IN FRENCH PATIENTS ACCORDING TO THE CLINICAL PRIMARY BILIARY CIRRHOSIS PHENOTYPE**

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**CD8 T CELLS PLAY A CRITICAL ROLE IN PRIMARY BILIARY CIRRHOSIS OF DNTGFβRII MICE**

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**#702**

**ANTIBODIES TO SS-A/RO-52KD AND CENTROMERE IN PRIMARY BILIARY CIRRHOSIS**

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**#703**

**INTERCELLULAR TRANSPORT AND TRANSLLOCATION OF IGA AMA IN PRIMARY BILIARY CIRRHOSIS**

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**PROGRESSION IN PBC: NEW INSIGHTS FROM FOLLOW-UP LIVER BIOPSIES 10 YEARS AFTER DIAGNOSIS**

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RITUXIMAB FOR PRIMARY BILIARY CIRRHOSIS (PBC) REFRACTORY TO URSEDOXOXYCHOLIC ACID (UDCA)

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#706
BILIARY INFECTION WITH MOUSE MAMMARY TUMOR VIRUS IN THE NOD.C3C4 MOUSE AND OTHER MOUSE MODELS OF PRIMARY BILIARY CIRRHOSIS

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#707
INCIDENCE AND MORTALITY OF PRIMARY SCLEROSING CHOLANGITIS IN THE UK: A POPULATION-BASED COHORT STUDY

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#708
STIMULATION OF THE INNATE IMMUNE SYSTEM IS NECESSARY FOR LIVER-SPECIFIC CD8+ T LYMPHOCYTES TO INDUCE AN AUTOIMMUNE HEPATITIS

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#709
CLINICAL RELEVANCE OF GENETIC AND SEROLOGIC MARKERS IN AUTOIMMUNE HEPATITIS: A SINGLE CENTER EXPERIENCE WITH 296 PATIENTS

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#710
PERIPHERAL, BUT NOT CENTRAL, TOLERANCE NOR LEVEL OF AUTOANTIGEN EXPRESSION IS RESPONSIBLE FOR THE GENDER BIAS IN SUSCEPTIBILITY TO EXPERIMENTAL TYPE 2 AUTOIMMUNE HEPATITIS

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#711
PREDICTIVE FACTORS FOR HEPATOCELLULAR CARCINOMA IN TYPE 1 AUTOIMMUNE HEPATITIS: IMPACT OF GENDER, STAGE, HLA PHENOTYPE AND TREATMENT

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#712
GLUCOCORTICOID RESISTANCE IN AUTOIMMUNE HEPATITIS ASSOCIATES TO INCREASED EXPRESSION AND ACTIVITY OF P-GLYCOPROTEIN IN T CELL

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#713
POTENTIATION OF THE INHIBITORY EFFECT OF INTRAHEPATIC NKT LYMPHOCYTES BY A NOVEL NON-DEGRADABLE BETA-GLUCOSYLCERAMIDE ANALOG IS ASSOCIATED WITH DECREASED STAT 1 PHOSPHORYLATION

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#714
ASSOCIATION OF CYTOTOXIC T-LYMPHOCYTE ANTIGEN 4 GENE POLYMORPHISMS WITH TYPE 1 AUTOIMMUNE HEPATITIS IN JAPANESE

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#715
OUTCOME AND SURVIVAL IN CHILDHOOD ONSET AUTOIMMUNE SCHELOSING CHOLANGITIS AND AUTOIMMUNE HEPATITIS; A 13 YEARS FOLLOW-UP STUDY
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GENETICALLY DETERMINED ABNORMALITY OF THE PD-1/PD-LS PATHWAY MAY PREDISPOSE TO AUTOIMMUNE LIVER DISEASE
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#717
THE USE OF MYCOPHENOLATE MOFETIL IN THE TREATMENT OF AUTOIMMUNE HEPATITIS IN PATIENTS REFRACTORY TO OR INTOLERANT OF CONVENTIONAL TREATMENT
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#718
LYMPHOTOXIN ALPHA GENE POLYMORPHISMS IN PEDIATRIC AUTOIMMUNE HEPATITIS TYPE 1
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#719
BETA-GLUCOSYLCERAMIDE ATTENUATES THE IMMUNE-MEDIATED DAMAGE OF ALPHA-GALACTOSYLCERAMIDE IN A CD1D RECEPTOR DEPENDENT MANNER: A LIGAND DEPENDENT EFFECT ON NKT REGULATORY LYMPHOCYTES
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#720
ANTIBODIES TO DOUBLE-STRANDED DNA (ANTI-DSDNA) IN LIVER DISEASE: HIGH SPECIFICITY FOR TYPE 1 AUTOIMMUNE HEPATITIS (AIH-1) AND USEFUL MARKER FOR THE DIAGNOSIS OF PRIMARY BILIARY CIRRHOSIS (PBC) - AIH OVERLAP SYNDROME
Georgios Pappas, Alessandro Granito, Paolo Muratori, Silvana Maccariello, Luigi Muratori, Rodolfo Ferrari, Fabio Cassani, Silvia Ferri, Valentina Cipriani, Chiara Quarneti, Francesco B. Bianchi
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#721
THE IMPACT OF ETHNICITY ON THE NATURAL HISTORY OF AUTOIMMUNE HEPATITIS
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#722
EFFECT OF CD4+CD25+ REGULATORY T-CELL ON MONOCYTE TOLL-LIKE-RECEPTOR-4 EXPRESSION IN AUTOIMMUNE HEPATITIS
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SEROLOGICAL MARKERS DO NOT PREDICT LIVER HISTOLOGY IN AUTOIMMUNE HEPATITIS
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POPULATION-BASED STUDY OF ALASKA NATIVE PERSONS WITH AUTOIMMUNE HEPATITIS: CLINICAL PRESENTATION, RESPONSE TO TREATMENT AND RELAPSES
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**#726**
IDENTIFYING LEARNING NEEDS OF ADULT LIVER TRANSPLANT RECIPIENTS
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RESULTS OF A UNIQUE HEPATITIS C OUTREACH PROGRAM IN PROVIDING SCREENING, EDUCATION, AND REFERRAL IN A MIDWEST STATE
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**#728**
HEALTH-RELATED QUALITY OF LIFE IN PATIENTS WITH CHRONIC LIVER DISEASE WITH OR WITHOUT HEPATOCELLULAR CARCINOMA (HCC) AWAITING ORTHOPIC LIVER TRANSPLANTATION (OLT)
Teresa Casanovas1, Laia Jané1, Michael Herdman2, Alfonso Casado3, Joan Fabregat1
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**#729**
POST TRANSPLANT TREATMENT OF HEPATITIS C: IS THERE AN IDEAL MONITORING PARAMETER?
Victoria Zacharias, Nyingi Kemmer, Kamran Safdar, Ryan Neff, Grace Bell, Nicole Majoras, Tiffany E. Kaiser, Guy W. Neff
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**#730**
TITLE: MULTIDISCIPLINARY COLLABORATION IMPROVES HEPATITIS C MANAGEMENT AND CARE FOR HIGH-RISK PATIENTS IN A METHADONE MAINTENANCE CLINIC
Marian Kerbleski, Alexander Monto, Sue Currie, Patricia Lane, David Kan
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**#731**
IMPROVEMENT OF QUALITY AND EFFICIENCY IN HEPATOLOGY CLINIC AT A VA MEDICAL CENTER
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**#732**
SURVIVAL OUTCOMES IN LIVER TRANSPLANT RECIPIENTS SUFFERING FROM NON-ALCOHOLIC STEATOHEPATITIS
Grace Bell, George Tannous, Victoria Zacharias, Nyingi Kemmer, Muslim Atiq, Kamran Safdar, Nadia Malik, Beverley Borjas, Tiffany E. Kaiser, Guy W. Neff
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Portal Hypertension and Other Complications of Cirrhosis: Clinical

**#733**
OCTREOTIDE BOLUS INJECTION AND AZYGOS BLOOD FLOW (AZBF) IN PATIENTS WITH CIRRHOSIS: SUSTAINED DECLINE AND VISIBLE EFFECT AT READMINISTRATION
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**#734**
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Virendra Singh1, Prashant C. Dheerendra2, Baljinder Singh3, Chander K. Nain4, Divya Chawla5, Navneet Sharma2, Ashish Bhatta2, Sushil K. Mahi2
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#737
PROGNOSTIC FACTORS FOR HEPATORENAL SYNDROME (HRS) REVERSAL IN PATIENTS WITH TYPE 1 HRS ENROLLED IN A RANDOMIZED, DOUBLE-BLIND, PLACEBO-CONTROLLED TRIAL

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Jildou Hoekstra1, Sarwa Darwish Murad1, Aurelie Plessier2, Manuel Hernandez-Guerra3, Dominique Valla2, Juan Carlos Garcia-Pagan4, Elwyn Elias4, Massimo Primignani5, Frank W. Leebeek5, Harry L. Janssen1
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#739
UROTENSIN II INCREASES PORTAL PRESSURE AND INDUCES HEPATIC FIBROSIS

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#740
PROTON PUMP INHIBITOR USE IS ASSOCIATED WITH A HIGH RISK OF SPONTANEOUS BACTERIAL PERITONITIS

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#741
PREDICTORS OF INTERMEDIATE-TERM RISK IN CIRRHOSIS: IMPLICATIONS FOR MINIMAL LISTING CRITERIA IN LIVER TRANSPLANTATION (LT)

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#742
TERLIPRESSIN IMPROVES RENAL FUNCTION AND INDUCES NATRIURESIS IN PATIENTS WITH CIRRHOSIS AND ASCITES WITHOUT HEPATORENAL SYNDROME

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#743
THE DEGREE OF DISTURBANCE OF RENAL BLOOD FLOW AUTOREGULATION IN CIRRHOSIS DETERMINES THE SEVERITY OF RENAL DYSFUNCTION

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#744
HYPONATREMIA: A MAJOR DETERMINANT OF IMPAIRED HEALTH-RELATED QUALITY OF LIFE IN CIRRHOSIS WITH ASCITES

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#745
NOD2 GENE VariANTS CONFER INCREASED RISK FOR SPONTANEOUS BACTERIAL PERITONITIS IN DECOMPENSATED LIVER CIRRHOSIS

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#746
PLATELET COUNT/SPLEEN DIAMETER RATIO AND AASLD CRITERION FOR SCREENING ESOPHAGEAL VARICES IN PATIENTS WITH HEPATITIS C VIRUS-RELATED COMPENSATED CIRRHOSIS

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**#747**

**INTRANASAL DESMOPRESSIN IS EFFECTIVE IN PREVENTING BLEEDING AFTER DENTAL EXTRACTION IN CIRRHOTIC PATIENTS HAVING MODERATE DEGREES OF COAGULOPATHY**

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**#748**

**EVALUATION OF REGIONAL HEPATIC PERFUSION (RHP) BY CONTRAST-ENHANCED ULTRASOUND: AN OBJECTIVE, NON-INVASIVE METHOD TO STUDY LIVER PERFUSION IN PATIENTS WITH CIRRHOSIS**

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**#749**

**SILDENAFIL HAS NO EFFECT ON PORTAL PRESSURE BUT LOWERS ARTERIAL PRESSURE**

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**#750**

**OXYGEN DESATURATION DURING SLEEP IN HEPATOPULMONARY SYNDROME**

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**#751**

**A MULTI-CENTER CASE-CONTROL STUDY OF CLINICAL PREDICTORS AND FUNCTIONAL STATUS IN HEPATOMALIGNANT SYNDROME (HPS)**

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**#752**

**ASCITIC FLUID LACTOFERRIN: A NOVEL MARKER FOR DIAGNOSIS OF SPONTANEOUS BACTERIAL PERITONITIS (SBP)**


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**#753**

**AQUAPORIN-1 DEMONSTRATES ARTERIAL CAPILLARY PROLIFERATION AND HEPATIC SINUSOIDAL TRANSFORMATION IN HUMAN CIRRHOTIC LIVER-RELEVANCE TO PORTAL HYPERTENSION**

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**#754**

**LONG-TERM SURVIVAL IN PATIENTS WITH REGRESSION OF VIRAL-RELATED CIRRHOSIS**

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**#755**

**PROGNOSTIC VALUE OF LIVER STIFFNESS MEASUREMENT AND HEPATIC VENOUS PRESSURE GRADIENT IN PATIENTS WITH CHRONIC LIVER DISEASE: A PROSPECTIVE STUDY**

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**#756**

**PROGNOSTIC INDICATORS OF REBLEEDING IN POOR HEMODYNAMIC RESPONDERS TO TREATMENT OF PORTAL HYPERTENSION**

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ADRENAL IMPAIRMENT IS FREQUENT FINDING IN STABLE CIRRHOSIS AND IS RELATED TO DISEASE SEVERITY

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#758
HEPATIC VENOUS PRESSURE GRADIENT PREDICTS THE FIRST CLINICAL DECOMPENSATION IN PATIENTS WITH CHRONIC HEPATITIS C-RELATED CIRRHOSIS

Diego Rincón, Ana Hernando, Oreste Lo Iacono, Alain Huerta, Judith Gómez, Magdalena Salcedo, Maria Vega Catalina, Cristina A. Ripoll, Cecilia Sanz, Oscar Nuñez, Ana Matilla, Gerardo Clemente, Rafael Banares
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#759
COMPARISON BETWEEN ENDOGENOUS THROMBIN POTENTIAL (ETP) AND INR TO ASSESS LIVER FUNCTION DECREASE IN PATIENTS WITH LIVER CIRRHOSIS

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#760
EARLY PRIMARY PROPHYLAXIS WITH BETA-BLOCKERS AND ROLE OF HEPATIC VENOUS PRESSURE GRADIENT ASSESSMENT IN PREVENTION OF GROWTH OF SMALL ESOPHAGEAL VARICES IN CIRRHOSIS

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#761
SPLICE STIFFNESS MEASUREMENT BY MAGNETIC RESONANCE ELASTOGRAPHY IS AN INDEPENDENT PREDICTOR OF ESOPHAGEAL VARICES IN PATIENTS WITH COMPENSATED CIRRHOSIS

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LEUKOPENIA AND THROMBOCYTOPENIA PREDICT A POOR PROGNOSIS IN COMPENSATED CIRRHOSIS

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#763
STEM CELLS MOBILIZATION INDUCES HEPATOCYTE LINEAGE PROLIFERATION: A PROOF OF CONCEPT IN MAN

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#764
HUMAN IMMUNODEFICIENCY VIRUS-ASSOCIATED NODULAR REGENERATIVE HYPERPLASIA IS LINKED TO PORTAL OBSTRUCTIVE VENOPATHY AND AUTO-IMMUNE PROTEIN S DEFICIENCY

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#765
HEPATOPULMONARY SYNDROME (HPS): A PROSPECTIVE STUDY ON THE PROGRESSION OF HIPOXEMIA

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SURVIVAL BENEFIT IN PATIENTS WITH ACUTE VARICEAL BLEEDING AND HEMODYNAMIC REPERCUSSIONS FOLLOWING EARLY ADMINISTRATION OF VAPREOTIDE
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#767
NODULAR REGENERATIVE HYPERPLASIA: A CLINICOPATHOLOGICAL EVALUATION
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#769
GROWTH HORMONE-STIMULATED IGF-1 GENERATION IN CIRRHOSIS REFLECTS HEPATOCELLULAR DYSFUNCTION
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SECONDARY PARENCHYMAL DAMAGES IN CIRRHOTIC LIVERS ASSOCIATED WITH ACTIVATED PLATELET AGGREGATION IN SINUSOIDUS
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HISTOLOGICAL-CLINICAL CORRELATION IN CIRRHOSIS - VALIDATION OF A HISTOLOGICAL CLASSIFICATION OF THE SEVERITY OF CIRRHOSIS
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INCIDENCE OF CIRRHOSIS IN THE UK: A POPULATION-BASED STUDY
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#773
CLOSTRIDIUM DIFFICILE INFECTION IS ASSOCIATED WITH PROTON PUMP INHIBITORS IN PATIENTS WITH CIRRHOSIS
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WHAT IS THE BEST NON INVASIVE METHOD FOR EARLY PREDICTION OF CIRRHOSIS IN CHRONIC HEPATITIS C? PROSPECTIVE COMPARISON BETWEEN FIBROSCAN AND SERUM MARKERS (LOK INDEX, APRI, AST/ALT RATIO, PLATELET COUNT AND FIBROTEST)
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#775
SIX YEARS OF “ON DEMAND” LAMADV COMBINATION THERAPY SIGNIFICANTLY REDUCES THE DEVELOPMENT AND PROGRESSION OF ESOPHAEGAL VARICES IN PATIENTS WITH HBEAG-NEGATIVE CIRRHOSIS
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Paolo Angeli, Elena Mazza, Silvano Fasolato, Erika Zola, Alessandra Galioto, Alessia Callegaro, Antonietta Sticca, Freddy Salinas, Stefano Fagiuli, Emanuela Miola, Umberto Cillo, Angelo Gatta, Lajos Okolicsanyi

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PORTAL, SYSTEMIC, AND PULMONARY HEMODYNAMICS IN PORTAL HYPERTENSIVE GASTROPATHY (PHG) - EVIDENCE OF ADDITIONAL VASODILATION: A STUDY OF 254 CIRRHOTICS

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LIMITED EFFICACY OF PROPRANOLOL ALONE OR IN COMBINATION WITH ISOSORBIDE-5-MONONITRATE FOR PRIMARY PROPHYLAXIS OF VARICEAL BLEEDING: A STEP-WISE HEMODYNAMIC EVALUATION

Praveen Sharma, Ashish Kumar, Smruti R. Mishra, Sanjeev K. Jha, Barjesh C. Sharma, Shiv Kumar Sarin

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**URINARY LIPOCALIN2 REFLECTS HEPATIC DYSFUNCTION IN PATIENTS WITH CHRONIC LIVER DISEASE**

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**THE IMPACT OF SERUM POTASSIUM CONCENTRATION ON MORTALITY FOLLOWING LIVER TRANSPLANTATION: A COHORT MULTI-CENTRE STUDY**

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**THE RELATIONSHIP BETWEEN DECREASED HEART RATE VARIABILITY AND HEPATIC ENCEPHALOPATHY IN PATIENTS WITH CIRRHOSIS**

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Maria Teresa Melgosa2, Juan Carlos Garcia Pagan3, Giovanni L. Ricci1, Juan Turnes1, Isabel Blanco Vich2, Sandra Pizarro2, Lucia Miglioresi1, Jose Luis Valera2, Jaime Bosch1, Josep Roca2, Roberto Rodriguez Raisin2, Joan Albert Barbera2

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IS VON WILLEBRAND FACTOR A PREDICTIVE PARAMETER FOR CLINICALLY SIGNIFICANT PORTAL HYPERTENSION IN PATIENTS WITH LIVER CIRRHOSIS?

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MOLECULAR MECHANISMS UNDERLYING MUSCLE LOSS IN CIRRHOTIC PATIENTS

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**PROGNOSTIC VALUE OF ALGORITHMS COMBINING FIBROTEST®FIBROSURE® (FT) AND ASHTEST® (HT) IN COMPARISON WITH MELD AND PUGH PROGNOSTIC INDEXES IN PATIENTS WITH SEVERE CIRRHOSIS**

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**MONITORING OF LOW MOLECULAR HEPARINS IN PATIENTS WITH LIVER CIRRHOSIS**

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**THE ROLE OF B TYPE NATRIURETIC PEPTIDE (BNP) AS A MARKER OF STRUCTURAL CARDIAC DISEASE IN PATIENTS WITH CIRRHOSIS**

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**PATIENTS WITH HEPATITIS C VIRUS CIRRHOSIS HAVE A WORSE OUTCOME WHEN ADMITTED TO THE INTENSIVE CARE UNIT COMPARED TO OTHER CAUSES OF CIRRHOSIS**

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**DISCREPANCIES BETWEEN CLINICAL AND AUTOPSY DIAGNOSES AND THE SIGNIFICANCE OF THE DIFFERENCE TO RESIDENT EDUCATION**

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**VWF FUNCTIONS, ADAMTS-13 ACTIVITY AND ANTIGEN LEVELS IN PATIENTS WITH LIVER CIRRHOSIS**

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**RELATIONSHIP BETWEEN SEVERITY OF LIVER DISEASE AND RISK OF GALLSTONES IN MEN WITH CHRONIC HEPATITIS C: A CLINICOPATHOLOGICAL STUDY**

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**SURVIVALS OF HEPATIC ENCEPHALOPATHY: RELEVANCE TO ETIOLOGY AND INITIAL COMPLICATION OF CIRRHOSIS**

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**HEPATIC VENOUS PRESSURE GRADIENT IN CIRRHOSIS: RELATIONSHIP WITH THE GRADE OF VARIX, CHILD-PUGH SCORE AND MELD SCORE AND ASCITES**

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Ana Moreno1, Luis A. Gil-Grande2, Fernando García-Hoz2, Javier Blázquez3, José R. Foruny2, Carmen Quereda1, Antonio López-San Román2, Miguel García-González2, Javier Moreno2, Maria J. Pérez-Elias1, Juan Sánchez-Corral2, Catalina Rodríguez-Martín2, Santiago Moreno1, Rafael Bárceña2
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Emilia Prakoso1,2, Jade D. Jamias1, Martin W. James1, David J. Koorey1, Simone I. Strasser1, Geoffrey W. McCaughan1,2, Nicholas A. Shackell1,2
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Jose Castellote, Anna Girbau, Sandra Maisterra, Carles Pons, Ana Berrozo, Carme Baliellas, Xavier Xiol
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Marie-Luise Berres1, Eray Yagmur2, Barbara Schnyder1, Alexander Koch1, Ron Winograd1, Axel M. Gressner1, Christian Trautwein1, Hermann E. Wasmuth1
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SERUM-ASCITES ALBUMIN CONCENTRATION GRADIENT (SAAG) MORE THAN 1.5 G/DL PREDICTS ESOPHAGEAL VARICEAL HEMORRHAGE
Pises Pisesponsa, Suthinee Sripotong, Taned Chitapanarux, Apinya Leerapun, Sutawat Thongsawat, Ong-ard Prasontarangkul
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MEDICATION ADHERENCE TO BETA-BLOCKER THERAPY FOR PRIMARY PROPHYLAXIS OF ESOPHAGEAL VARICEAL HEMORRHAGE IN A VETERAN POPULATION
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SELECTIVE IMPAIRMENTS OF COVERT VISUAL ATTENTION AND MOVEMENT PREPARATION IN MINIMAL HEPATIC ENCEPHALOPATHY
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Portal Hypertension and Other Complications of Cirrhosis: Experimental

LASTING AMELIORATION IN THE CLINICAL COURSE OF DECOMPENSAED ALCOHOLIC CIRRHOSIS WITH BOOST INFUSIONS OF MOBILIZED PERIPHERAL BLOOD STEM CELLS
Dimitrios Kapetanos¹, Evangelia Yannaki², Achilles Anagnostopoulos², Angeliki Xagorari², Fatis Iordanidis³, Ioannis Batis³, Panayotis Kalyanidis³, Evangelia Athanasiou², Georgios Douvas², Athanasios Fassas², George E. Kitsi¹
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PROTECTIVE EFFECTS OF ERYTHROPOIETIN IN RATS WITH CIRRHOTIC CARDIOMYOPATHY
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Gavin A. Wright¹, Nathan A. Davies ¹, Lars M. Ytreba², Vanessa Stadlbauer¹, Ole-Martin Fuskevág³, Claudia Zwingerman⁴, Stephen Hodges¹, Rajiv Jalan¹
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EVALUATION OF PULMONARY HYDROGEN SULFIDE GENERATION IN EXPERIMENTAL HEPATOPULMONARY SYNDROME (HPS)
Junlan Zhang¹, Jo Morrison², Jeanette Doeller², David Kraus²,³, Bao Luo¹, Leping Tang¹, Joseph Barney¹, Yongming Wang¹, Michael B. Fallon¹
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Tom S. Chan¹, Claudia Zwingmann¹, Sven Gottschalk¹, Valerie-Ann Raymond¹, Peter Darby², David Mazier², Marc Bilodeau¹
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Jonel Trebicka, Andreas Eckhardt, Martin Hennenberg, Erwin Biecker, Frank Lammert, Tilman Sauerbruch, Jörg Heller
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Internal Medicine, University of Bonn, Bonn, Germany

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Srinivasan Dasarathy¹, Sean Muc¹, Prabhu S. Parimi², Milan Dodig¹, Satish C. Kalhan¹, Arthur J. McCullough¹
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Lien Van Landeghem, Wim Lalame, Marcel Zeegers, Ingrid Vander Elst, Jos van Pelt, David Cassiman, Frederik Nevens
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Debbie L. Shawcross, Gavin A. Wright, Vanessa Stadlbauer, Stephen Hodges, Rajiv Jalan
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Christian J. Steib, Markus Bystron, Christine Opelz, Josef M. Härtl, Ingrid Liss, Burkhard Göke, Manfred Bilzer, Alexander L. Gerbes
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**UPREGULATION OF RHOA- AND RHO-KINASE-EXPRESSION AND RHO-KINASE ACTIVITY IN LIVERS FROM RATS WITH CCL4-INDUCED MICRONODULAR CIRRHOSIS**

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**#844**

**EFFECTS OF BILE ACIDS ON PULMONARY MICROVASCULAR ENDOTHELIAL CELL PROLIFERATION: IMPLICATIONS FOR EXPERIMENTAL HEPATOPULMONARY SYNDROME (HPS)**

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Acute Liver Failure and Artificial Liver Support

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PROTEIN MICROARRAY STUDY REVEALS TOXIN-SELECTIVE CYTOKINE PROFILES IN EXPERIMENTAL ACUTE LIVER FAILURE: BENEFICIAL EFFECTS OF MILD HYPOTHERMIA AND N-ACETYLCYSTEINE

Chantal Bémeur, Javier Vaquero, Paul Desjardins, Roger F. Butterworth
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**#848**
CEREBRAL HYPEREMIA AND INTRACRANIAL HYPERTENSION INDUCED BY CO-ADMINISTRATION OF ENDOTOXIN AND AMMONIA IN THE RAT ARE MEDIATED BY CYCLOOXYGENASE

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ACETAMINOPHEN THERAPEUTIC MISADVENTURE: A PROSPECTIVE STUDY

Alexandre Louvet, Benoît Quesnel, Jeanne Boitard, Sébastien Dharmaraju, Faustine Wartel, Valérie Canva, Pierre Deltenre, Philippe Mathurin
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Wenlei Jiang, Paul Desjardins, Roger F. Butterworth
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EVIDENCE OF ENDOTOXIN TOLERANCE AND SEVERELY IMPAIRED T-HELPER 1 RESPONSE IN ACUTE HEPATIC FAILURE

Charalampos G. Antoniades, Philip A. Berry, Ivana Carey, Astrid Scali, Julia Wendon, Diego Vergani
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Caroline M. Bates, Narendra Kocher, Janice S. Davidson, Peter C. Hayes, Kenneth Simpson
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David Rudnick1, Dennis Dietzen1, Ross Shepherd1, Yumirle P. Tumelle1, Song Zhang2, Robert Squires2, Pediatric Acute Liver Failure Study Group The2
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Institute of Liver Studies, Kings College Hospital, London, United Kingdom

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Gadi Lalazar, Tomer Adar, Yaron Ilan
Liver Unit, Hadassah Hebrew University Medical Center, Jerusalem, Israel

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William Bernal, Georg Auzinger, Elizabeth Sizer, Julia Wendon
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Deanna Oliver1, Fatma Barakat1, Meghan Carlson1, William Perry1, Jan Stange2,1, Tarek Hassanein1
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Andrew J. Portal, Matthew Bruce, Mark Austin, Constantine J. Karvellas, Mark McPhail, Elizabeth Sizer, Georg Auzinger, Julia Wendon, William Bernal
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Athina Zira1,2, Stamatos E. Theocharis1, Soren Engelsen3, Ioannis Stamos1, Ioanna Andreadou2, Emmanuel Mikros2
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Eleanor S. Gilchrist1, Catherine Payne1, Kay Samuel2, Patricia Lee1, Philip N. Newsome1, John Plevris1
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Andrew J. Portal1, Mark Austin1, Matthew Bruce1, Constantine J. Karvellas1, Funmi Awopetu2, Roy Sherwood2, Elizabeth Sizer1, William Bernal1, Georg Auzinger1, Michael Heneghan1, Julia Wendon1
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Yoshiyuki Yamagishi, Hidetsugu Saito, Shinichiro Tada, Hirotsoshi Ebinuma, Masahiro Kikuchi, Yoshinori Horie, Shinzo Kato, Toshifumi Hibi
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Chia C. Wang1, Linda Cook2, Sarah Holte1, Meighan Krows1, Arthur Bagabag2, Kaying Ng2, Keith Jerome1,2, Lawrence Corey1,2
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Christine Meffre1, Elisabeth Delarocque-Astagneau1, Corinne Pioche1, Frédéric Dubois1, Françoise Roudot-Thoraval1, Dominique Guyader1, Christine Silvain1, Yann Le Strat1, Jean Claude Desenclos1
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Sigal Fishman¹, Guy Rosner¹, Philippe Halfon², Hava Peretz³, Guillaume Penaranda², Moshe Leshno¹, Oren R. Mohamed, Amanda DeVoss, Katherine Wherity, Donald M. Jensen Medicine, Center for Liver Diseases, University of Chicago, Chicago, IL, USA

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Ikuo Nakamura¹,², Kaori Ochiai³, Yasuhiro Tanaka², Fuminori Moriyasu¹, Masashi Mizokami³, Michio Imawari⁴
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Michael J. Williams, Adam Lawson, Keith R. Neal, Stephen Ryder, William L. Irving
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Leila B. Pereira¹, Regina Moreira¹, Cynthia Braga², Ulisses Montarroyos¹, Celina Martelli¹, Marilia Turchi¹, Luiza Lima², Luiz C. Arraes², Marcelo A. Costa³, Ricardo Ximenes¹,⁵
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LOW-DENSITY LIPOPROTEIN RECEPTOR (LDLR) POLYMORPHISMS ARE ASSOCIATED WITH SPONTANEOUS AND TREATMENT-INDUCED RECOVERY FROM HEPATITIS C VIRUS (HCV) INFECTION

Tobias Mueller1, Andreas Mas Marques2, Christoph Sarrazin3, Manfred Wiese4, Juliane Halangk1, Heiko Witt1, Hartmut Wasmuth5, Golo Ahlenstiel6, Ulrich Spengler7, Frank Lammer7, Eckart Schott1, Viola Weich1, Eckart Schreier2, Betram Wiedenmann1, Thomas Berg1
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CORRELATES OF INCIDENT HCV INFECTION AND SERONEGATIVE-IMMUNE STATUS IN HIGH RISK, IDU PRISONERS

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ANALYSIS OF HCV IN SOUTH EAST ASIA

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THE PRACTICE OF HEPATITIS VACCINATION IN PATIENTS WITH HEPATITIS C AND THE VALIDITY OF PROCEDURE AND DRUG CODES FOR HEPATITIS A AND B VACCINATIONS IN VA ADMINISTRATIVE DATABASES

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COMMUNITY TRENDS IN DIAGNOSIS AND TREATMENT OF HEPATITIS C

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DOES GENDER MATTER? A PROSPECTIVE EVALUATION OF RISK FACTORS FOR HCV INFECTION AND TREATMENT CANDIDACY IN WOMEN

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PREVALENCE AND PREDICTORS OF OBESITY AMONG INDIVIDUALS TESTING POSITIVE FOR HEPATITIS C ANTIBODY IN A MULTICULTURAL, URBAN, TERTIARY CARE REFERRAL CLINIC

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**#892**

COMPARTMENTALIZATION OF HEPATITIS C VIRUS QUASISPECIES FOLLOWING LIVER TRANSPLANTATION

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**#893**

STEATOHEPATITIS: RISK FACTORS AND IMPACT ON DISEASE SEVERITY IN HIV-HCV COINFECTION

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**#894**

ACUTE HEPATITIS C IN HIV-INFECTED MEN WHO HAVE SEX WITH MEN IN FRANCE IN 2006 AND 2007

Christine Larsen 1, Laurent Alric 2, Isabelle Aupérin 3, Marie-Laure Chaix 4, Elisabeth Delarocque-Astagneau 1, Stéphanie Dominguez 2, Xavier Duval 6, Anne Gervais 6, Jade Ghosn 7, François Linard 8, Yann Le Strat 1, Jean-Yves Le Talec 9, Lionel Piroth 10, Stanislas Pol 11, Annie Velter 1

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**#895**

FACTORS ASSOCIATED WITH SEVERE LIVER DISEASE IN NEWLY REFERRED HEPATITIS C VIRUS-INFECTED FRENCH DRUG USERS: A MULTICENTER STUDY OF 4 373 PATIENTS, 2001-2004

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**#896**

APOLIPOPROTEIN E4 (APOE4) ALLELE PROTECTS FROM CHRONIC HEPATITIS C VIRUS (HCV) INFECTION

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**#897**

IN VITRO INFECTION OF IMMORTALIZED HUMAN HEPATOCYTES BY HEPATITIS B VIRUS AND ITS APPLICATION TO ANTI-VIRAL TREATMENT

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**#898**

THE ROLE OF SERIAL MEASUREMENT OF SERUM HBV-DNA LEVELS IN PATIENTS WITH CHRONIC HBEAG(-) HEPATITIS B INFECTION; ASSOCIATION WITH LIVER DISEASE PROGRESSION. A PROSPECTIVE COHORT STUDY

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**#899**

ACTIVE REPLICATION OF HEPATITIS B VIRUS (HBV) STARTS AFTER BIRTH

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#900
**LARGE SCALE LONGITUDINAL STUDY OF CHRONIC HEPATITIS B PATIENTS WITH HEPATITIS B SURFACE ANTIGEN SEROCLEARANCE**

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#901
**FIBROTEST® (FT) AND ACTITEST® (AT) ACCURATELY PREDICT RISK OF LIVER DECOMPENSATION AND DEATH IN PATIENTS WITH CHRONIC HEPATITIS B (CHB)**

Yen Ngo1, Yves Benhamou1, Mona Munteanu2, Vincent Thibault1, Pascal Lebray1, Dominique Thabut1, Rachel Morra1, Djamila Messo1, Francoise Imbert-Bismut1, Vlad Ratzia1, Thierry Poynard1

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#902
**REDUCED HBV REPLICATION IN HBEAG-NEGATIVE PATIENTS IS DUE TO LOWER COVALENTLY CLOSED CIRCULAR DNA CONTENT AND IMPAIRED VIRION PRODUCTIVITY**

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#903
**HIGH VIRAL LOAD AND HEPATITIS B VIRUS (HBV) SUBGENOTYPE CE HAVE INCREASED RISK OF HEPATOCELLULAR CARCINOMA (HCC) – A 7-YEAR PROSPECTIVE FOLLOW-UP STUDY**

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#904
**ANALYSIS OF HBV MUTATIONS IN PATIENTS WITH EPIDEMIC FULMINANT HEPATITIS**

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#905
**HBV SUPPRESSES TLR-MEDIATED INNATE IMMUNE RESPONSES IN PARENCHYMAL AND NON-PARENCHYMAL LIVER CELLS**

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#906
**CHANGES IN SERUM HBV DNA LEVEL USING A TRAJECTORY MODEL PREDICT THE RISK OF HCC IN CHRONIC HEPATITIS B PATIENTS: THE R.E.V.E.A.L.-HBV STUDY**

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#907
**HBV VIRAL LOAD LESS THAN 10^4 COPIES/ML IS ASSOCIATED WITH SIGNIFICANT RISK OF HEPATOCELLULAR CARCINOMA IN CHRONIC HEPATITIS B PATIENTS: AN UPDATE FROM THE R.E.V.E.A.L.-HBV STUDY**

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#908
**HEPATITIS B VIRUS VACCINE ESCAPE MUTANTS HAVE IMPAIRED VIRION SECRETION EFFICIENCY IN CELL CULTURE**

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#909
**REAL-TIME PCR QUANTIFICATION OF TOTAL INTRACELLULAR HBV DNA AND CCCDNA IN HBSAG-POSITIVE PATIENTS WITH AND WITHOUT HDV CO-INFECTION AND IN PATIENTS WITH OCCULT HBV INFECTION**

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#910
**HEPATITIS B VIRUS PROTEINS INDUCE ACTIVATION OF HFGL2 TRANSCRIPTION THROUGH C-ETS-2 TRANSCRIPTION FACTOR AND MAPK SIGNAL PATHWAY**

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EFFICIENT CROSSTALK BETWEEN HBV-SPECIFIC T HELPER AND CYTOTOXIC T CELLS CHARACTERIZES SUCCESSFUL CONTROL OF HEPATITIS B VIRUS INFECTION

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EVOLUTION OF VIRAL QUASISPECIES IN THE POLYMERASE GENE OF HEPATITIS B VIRUS DURING ANTIVIRAL TREATMENT: FROM NAIVE TO VIRAL BREAKTHROUGH

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FACTORS ASSOCIATED WITH HBSAG SEROCLEARANCE IN ASYMPTOMATIC CARRIERS OF ENDEMIC AREAS DURING A LONG FOLLOW-UP PERIOD OF UP TO 17 YEARS

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HEPATITIS B VIRUS QUASISPECIES EVOLUTION IN HBEAG SEROCONVERTERS

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TLR9 EXPRESSION OF PLASMACYTOID DENDRITIC CELLS IN PATIENTS WITH CHRONIC HEPATITIS B INFECTION

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HEPATITIS B VIRUS X PROTEIN (HBX) TRIGGERS HEPATIC STELLATE CELLS (HSCS) ACTIVATION THROUGH TRANSFORMING GROWTH FACTOR BETA (TGF-β) SIGNALLING PATHWAY

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INFLUENCE OF SERUM HBV DNA LEVEL ON A RECURRENT OF HEPATOCELLULAR CARCINOMA AFTER CURATIVE TREATMENT WITH RADIOFREQUENCY ABLATION

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HEPATITIS B VIRUS (HBV) X PROTEIN (HBX) PROMOTES HUMAN HEPATIC STELLATE CELLS ACTIVATION AND PROLIFERATION MODULATED BY MATRIX METALLOPROTEINASE 2 (MMP-2)

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EXPRESSION LEVELS OF HNF4A LINK EFFICIENT HBV REPLICATION TO HEPATOCYTE DIFFERENTIATION STATE

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IMPACT OF CHRONICALLY ELEVATED HBV DNA VIRAL LOAD ON RISK OF HCC OCCURRENCE: AN UPDATE FROM THE R.E.V.E.A.L.-HBV STUDY

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#921
SPECTRUM OF STEATOSIS IN CHRONIC HEPATITIS B (CHB) AND ITS RELATION TO BIOCHEMICAL, METABOLIC, VIROLOGICAL AND HISTOLOGICAL PARAMETERS
Deepak K. Singh1, Archana Rastogi1, Puja Sahuja1, Ranjana Gonal1, Akshat Jain1, Sayed Hisser2, Ashish Kumar2, Shiv K. Sarin2
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#922
PREVALENCE OF FIBROSIS AND CIRRHOSIS IN CHRONIC HEPATITIS B: IMPLICATIONS FOR TREATMENT AND MANAGEMENT
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#923
ANALYSIS OF FULL HEPATITIS B VIRUS GENOME IN PATIENTS WITH AND WITHOUT HEPATOCARCINOMA
kaiyu zhang, Fumio Imazeki, Kenichi Fukai, Keiichi Fujiwara, Makoto Arai, Yukata Yonemitsu, Osamu Yokosuka
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#924
HEPATITIS B VIRUS INHIBITS TLR9-MEDIATED INTERFERON ALPHA SECRETION IN PLASMACYTOID DENDRITIC CELLS
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#925
TRACING HEPATITIS B VIRUS DNA BACK TO THE 16TH CENTURY IN A KOREAN MUMMY
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#926
EFFECT OF ANTIVIRAL THERAPY ON THE IMMUNOHISTOCHEMICAL EXPRESSION OF BCL-XL AND BAX PROTEIN IN PATIENTS WITH HBEAG-NEGATIVE CHRONIC HEPATITIS B
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#927
DIFFERENCES OF EARLY DYNAMICS AND LIVER DAMAGE AMONG HEPATITIS B VIRUS GENOTYPES IN UPA/SCID MICE WITH HUMAN HEPATOCYTES
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#928
GENDER DIFFERENCE IN THE NATURAL COURSE OF HBEAG-POSITIVE CHRONIC HEPATITIS B
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#929
BOW BODY MASS INDEX (BMI) PREDICTS DISCORDANCE BETWEEN TRANSIENT ELASTOGRAPHY (TE) AND LIVER BIOPSY (LB) ASSESSMENT OF LIVER FIBROSIS
Mirella Fraquelli1, Cristina Rigamonti2, Giovanni Casazza3, Maria Francesca Donato2, Dario Conti1, Maria Grazia Rumi2, Pietro Lampertico2, Guido Ronchi3, Massimo Colombo2
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#930
RAPID IMPROVEMENT OF INNATE IMMUNE RESPONSES AND MONOCYTE TOLL-LIKE RECEPTOR-2 (TLR2) EXPRESSION DURING LAMIVUDINE AND PEGYLATED INTERFERON THERAPY FOR CHRONIC HEPATITIS B (CHB)
Kumar Visvanathan1, Narelle Skinner1, Alex J. Thompson3,2, Paul V. Desmond3, William Abbott4, Ed Gane4, Stephen Locarnini2
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#931
DIFFERENTIAL EXPRESSION OF HEPATIC APURINIC/apyrimidinic ENDONUCLEASE 1, A DNA REPAIR ENZYME, IN CHRONIC HEPATITIS B VIRUS AND HEPATITIS C VIRUS INFECTION
Shinichi Sumiyoshi, Yoshimasa Kubayashi, Kenichi Souda, Kinya Kawamura, Kazuhito Kawata, Yurimi Takahashi, Satsuki Makino, Hidenoa Nortake, Hirotoshi Nakamura
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#932
GENETIC POLYMORPHISMS AT THE APOLIPOPROTEIN E LOCUS AFFECT THE OUTCOME OF CHRONIC HEPATITIS B
Davide Bitetto 1, Rosalba Minisini 2, Carlo Fabbris 1, Michela Burlone 2, Stefano Fangazio 2, Edmondo Falleti 1, Carlo Smirne 2, Pierluigi Tonitelli 1, Giovanna Fattovich 3, Mario Pirisi 2
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#933
PREDICTIVE MODEL FOR FIBROSIS AND CIRRHOSIS IN CHRONIC HEPATITIS B USING LIVER STIFFNESS MEASUREMENT
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#934
INHIBITION OF VIRAL REPLICATION WITH PEGYLATED INTERFERON ALFA-2A INCREASES IL-17 IN CHRONIC HEPATITIS B
Hai-ying Zhang, Jian Li, Chee-Kin Hui, Kwok-fan Cheung, Yui-Hung Yueng, Kan Leung, Lei Lu, George K. Lau
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#935
INTRACELLULAR LEVELS OF HEPATITIS B VIRUS DNA AND PREGENOMIC RNA IN PERIPHERAL BLOOD MONONUCLEAR CELLS OF CHRONICALLY INFECTED PATIENTS
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Hepatitis B: Treatment

#936
PREDICTORS OF SIGNIFICANT HISTOLOGICAL FINDINGS IN CHRONIC HEPATITIS B PATIENTS WITH PERSISTENTLY NORMAL ALT LEVELS
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TREATMENT-INDUCED HBEAG SEROCONVERSION IS A POOR THERAPEUTIC ENDPOINT
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Denotes AASLD Presidential Poster of Distinction

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#939
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Francisco Rodriguez-Frias 1,3, Rosendo Jardi 1,3, Maria Buti 2,3, Melanie Schaper 1,3, David Tabernero 1, Rafael Esteban 2,3
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ONCOLOGISTS AND HEPATITIS B: RESULTS OF A QUESTIONNAIRE SURVEY TO DETERMINE THEIR CURRENT LEVEL OF AWARENESS AND CLINICAL PRACTICE OF ANTIVIRAL PROPHYLAXIS TO PREVENT REACTIVATION

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CHARACTERIZATION OF HEPATITIS B VIRUS POLYMERASE MUTATIONS DETECTED DURING ANTIVIRAL TREATMENT WITH ADEFOVIR DIPIVOXIL (ADV)

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#952
**COMBINATION ANTIVIRAL EFFECTS OF 2’, 3’-DIDEOXY-3’FLUOROGUANOSINE WITH NUCLEOS(T)IDE ANALOGS AGAINST HEPATITIS B VIRUS IN VITRO**

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#954
**INCREASING ADEFOVIR AND ENTECAVIR UTILIZATION DRIVE HEPATITIS B RESISTANCE PATTERNS IN A US NATIONAL REFERENCE LABORATORY DATABASE**

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#956
**ADEFOVIR DIPIVOXIL PLUS LAMIVUDINE COMBINATION TREATMENT IS SUPERIOR TO ADEFOVIR DIPIVOXIL MONOTHERAPY IN LAMIVUDINE-RESISTANT HEPATITIS B E ANTIGEN-NEGATIVE CHRONIC HEPATITIS B PATIENTS**

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#957
**PRETREATMENT ALANINE TRANSAMINASE LEVEL MIGHT NOT BE THE MOST IMPORTANT FACTOR IN PREDICTING HBEAG LOSS IN OLDER PATIENTS WITH MORE PROLONGED HBV INFECTION**

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**IN VITRO ANTI-HBV ACTIVITY OF TELBIVUDINE/TENOFOVIR AND TELBIVUDINE/ENTECAVIR COMBINATIONS**

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**DOES THE GENOTOXIC EFFECT OF LAMIVUDINE TREATMENT IN CHRONIC HEPATITIS B TO THE HOST DNA?**

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#960

**TENOFOVIR SHOWS LIMITED EFFICACY IN TREATMENT OF HBV INFECTIONS RESISTANT AGAINST ADEFOVIR**

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#961

**THE EFFECT OF LAMIVUDINE AND ADEFOVIR DIPIVOXIL ON PREVENTING HEPATOCELLULAR CARCINOMA IN HBV-RELATED LIVER CIRRHOSIS**

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#962

**THE INDICATION AND LIMITATION OF INTERFERON THERAPY AS THE FIRST LINE THERAPY OF CHRONIC HEPATITIS B - FROM THE HISTOLOGICAL ANALYSIS OF 800 CHRONIC HEPATITIS B PATIENTS**

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**EMERGENCE OF HBV VIRUS GENE MUTATION RELATED TO ENTECAVIR-RESISTANCE IN CHRONIC HBV PATIENTS PARTICIPATED IN THE PHASE 2 CLINICAL STUDIES OF ENTECAVIR IN JAPAN**

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#964

**MUTATIONS OF THE HBV-POLYMERASE GENE ASSOCIATED WITH ADV DRUG RESISTANCE IN PATIENTS UNDERGOING A FIRST ADV THERAPY**

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#965

**SUSTAINED HBEAG AND HBSAG LOSS AFTER LONG-TERM FOLLOW-UP OF HBEAG POSITIVE PATIENTS TREATED WITH PEGINTERFERON ALPHA-2B**

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#966

**PREDICTION OF LONG-TERM MAINTENANCE OF VIROLOGIC RESPONSE DURING LAMIVUDINE TREATMENT IN HBEAG NEGATIVE CHRONIC HEPATITIS B**

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#967

**AMINO ACID VARIABILITY WITHIN HEPATITIS B SURFACE ANTIGEN AND THE OVERLAPPING REVERSE TRANSCRIPTASE REGION IN HBSAG NEGATIVE/HBCAB POSITIVE PATIENTS PRESENTING HBV REACTIVATION WHILE UNDERGOING CHEMOTHERAPY AND/OR STEM CELL TRANSPLANTATION FOR CANCER**

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#968

**ESTIMATING THE IMPACT OF CHRONIC HEPATITIS B ON FUTURE LIVER-RELATED MORBIDITY, MORTALITY AND COST IN SPAIN**

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#969

**NEUTRALIZING ANTIBODIES TO INTERFERON ALPHA IN CHRONIC HEPATITIS C PATIENTS NON-RESPONDING TO PEGYLATED INTERFERON PLUS RIBAVIRIN RETREATED TO PEG-INTERFERON α-2A AND RIBAVIRIN (ANRS GAMMATTI)**

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#970

**LIVER BIOPSY: STILL ESSENTIAL IN THE MANAGEMENT OF CHRONIC HEPATITIS B**

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#971

**PREDICTORS OF LIVER DECOMPENSATION IN HBEAG– PATIENTS WITH CHRONIC HEPATITIS B**

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#972

**LONG TERM FOLLOW-UP OF CHINESE PATIENTS WITH HBEAG-NEGATIVE CHRONIC HEPATITIS WHO DISCONTINUED TREATMENT AFTER 2 YEARS OF LAMIVUDINE MONOTHERAPY**

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#973
ASSOCIATION OF PRETREATMENT SERUM INTERFERON-GAMMA INDUCIBLE PROTEIN 10 (IP-10) LEVELS WITH VIROLOGICAL RESPONSE TO LAMIVUDINE MONOTHERAPY AND LAMIVUDINE INTERFERON SEQUENTIAL TREATMENT IN CHRONIC HEPATITIS B
Shinya Nagaoka, Hiroshi Yatsuhashi, Rumiko Nakao, Mika Fukuda, Satoru Hashimoto, Akiko Nishikawa, Naruhiro Hai, Masakuni Tateyama, Eiisuke Ozawa, Naota Taura, Seigo Abiru, Koji Yano, Atsumasa Komori, Kiyoshi Migita, Hikaru Fujioka, Hiromi Ishibashi
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#974
NOT HBEAG-STATUS BUT HBV GENOTYPE PREDICTS RESPONSE TO IFN IN CHRONIC HEPATITIS B
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#975
INTRAHEPATIC AND SERUM MARKERS OF HBV REPLICATION AND THEIR RELATIONSHIP TO SERUM HBEAG TITRES: IMPLICATIONS FOR THE USE OF QUANTITATIVE HBEAG TESTING AS A PREDICTIVE TOOL FOR TREATMENT OUTCOME
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#976
DRUG RESISTANCE MUTATION ANALYSIS BY DIFFERENT METHODS IN A HIGHLY TREATMENT-EXPERIENCED CHRONIC HEPATITIS B PATIENT POPULATION
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#977
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Elisabetta Loggi1, Florian K. Bihi1,2, Cinzia Fortini1, Carmela Curso1, Elena Grandini1, Lorenzo Micco1, Annagliuma Garenzini1, Mauro Bernardi1, Christian Brander3, Pietro Andreone4
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#978
TWENTY-FOUR WEEKS THERAPY WITH PEGINTERFERON ALFA-2A IS SIMILAR TO 48 WEEKS THERAPY IN PATIENTS WITH HBEAG POSITIVE CHRONIC HEPATITIS B AND GOOD PREDICTORS OF RESPONSE
Lucretia Rezzonico1, Omar Galdame1, Bernardo Frider2, Joaquin Solari1, Alejandra Villamil1, Paola Cاسciato1, Maria Reig1, Juan Bandi1, Anaia Alessio2, Adrian Gadano3
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#979
LONG-TERM FOLLOW-UP OF HBSAG CLEARANCE IN PATIENTS WITH HBEAG-NEGATIVE CHB TREATED WITH PEGINTERFERON ALFA-2A: INCREASE IN HBSAG CLEARANCE RATE FROM 3% 6 MONTHS POST-TREATMENT TO 8% AFTER 3 YEARS
Patrick Marcellin1, Maurizia Brunetto2, Ferruccio Bonino3, George K. Lau4, Patrizia Farci5, Cihan Yurdaydin6, Teerha Piratvisuth7, K. Loo8, Y. Wang9, Stephanos J. Hadziyannis10, Eva Wolf11, Matei Popescu12
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#980
VERY HIGH PREVALENCE OF PRECORE (PREC) AND/OR BASAL CORE PROMOTER (BCP) MUTATIONS (MUT) IN HBEAG-POSITIVE (EAG+) AND NEGATIVE (EAG-) CHRONIC HEPATITIS B (CHB) ESPECIALLY AMONG OLDER PATIENTS
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#981
CLINICAL COVARIATES ASSOCIATED WITH VIROLOGICAL RESPONSE AND ADEFOVIR RESISTANCE IN CHRONIC HEPATITIS B HBEAG NEGATIVE LAMIVUDINE-RESISTANT PATIENTS TREATED WITH ADEFOVIR ALONE OR IN COMBINATION WITH LAMIVUDINE FOR 28 MONTHS

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#982
FACTORS AFFECTING RESPONSE TO ADEFOVIR TREATMENT IN PATIENTS WITH CHRONIC HEPATITIS B AND LAMIVUDINE RESISTANCE

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#983
PREDICTORS OF VIROLOGIC RESPONSE AND RESISTANCE TO ADEFOVIR IN PATIENTS WITH LAMIVUDINE-RESISTANT CHRONIC HEPATITIS B VIRUS INFECTION

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#984
MANAGEMENT OF CHRONIC HEPATITIS B VIRUS (HBV) INFECTION BY PRIMARY CARE PHYSICIANS IN URBAN HOSPITALS AND CLINICS IN NEW YORK CITY

Henry Pollow1, Kejia Wan1, Thomas Miyoshi1, Sonali Tawdekar1, Paige Baker1, Dean McEwen2, Cindy Weinbaum3, Stephanie R. Bioble3, George Fryer1, Ronald Low4
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#985
HEPATITIS B SURFACE ANTIGEN TITER WAS DECREASED DURING CLEVUDINE THERAPY

Kwan Soo Byun1, Byung Chul Yoo2, Kwan Sik Lee3, Young-Hwa Chung3, Soo Hyung Ryu3, Mong Cho6, Joong-Won Park7, Byung-Ik Kim2, Heon-Ju Lee8, Joong-Yoel Han9, Seong Gyu Hwang10, Haak Cheoul Kim11, Kwon Yoo12, Young-Suk Lee13, Youn-Jae Lee14, Chae Yoon Chan3, Se-Hyun Cho9, Jin-Mo Yang15, Young Soo Kim16, Sung-Kyu Choi17, Chul-Ju Han18, Myung-Seok Lee19, Jong-Young Choi9, Hye-Suk Lee20, Ju Hyun Kim21
1Korea University Hospital, Seoul, South Korea. 2Samsung Medical Center, Seoul, South Korea. 3Yonsei University Hospital, Seoul, South Korea. 4Asan Medical Center, Seoul, South Korea. 5Inje University Seoul Paik Hospital, Seoul, South Korea. 6Pusan National University Hospital, Busan, South Korea. 7National Cancer Center, Goyang-si, Gyeonggi-do, South Korea. 8Youngnam University Medical Center, Daegu, South Korea. 9The Catholic University of Korea, Seoul, South Korea. 10Pochon CHA University Bundang CHA Hospital, Seongnam-si, Gyeonggi-do, South Korea. 11Wonkwang University Hospital, Iksan-si, Jeollabuk-do, South Korea. 12Ewha Womans University Mokdong Hospital, Seoul, South Korea. 13The Catholic University of Korea, Bucheon-si, Gyeonggi-do, South Korea. 14Inje University Busan Paik Hospital, Busan, South Korea. 15The Catholic University of Korea, Suwon-si, Gyeonggi-do, South Korea. 16Inha University Hospital, Incheon, South Korea. 17Chonnam National University Hospital, Gwangju, South Korea. 18Korea Institute of Radiological and Medical Sciences, Seoul, South Korea. 19Hallym University Medical Center Kangnam Sacred Heart Hospital, Seoul, South Korea. 20Seoul National University Hospital, Seoul, South Korea. 21Gachon Medical School, Incheon, South Korea

#986
A HEPATITIS B VIRUS MUTANT IMPLICATED IN NON-RESPONSE TO SEQUENTIAL TREATMENT WITH ADEFOVIR, LAMIVUDINE AND ENTECAVIR SHOWS CROSS-RESISTANCE TO MULTIPLE ANTIVIRAL AGENTS

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#987
COST-EFFECTIVENESS OF NEW TREATMENT PARADIGMS FOR E-AG NEGATIVE CHRONIC HEPATITIS B

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#988
SCREENING FOR HEPATITIS B IN CHEMOTHERAPY PATIENTS: SURVEY OF CURRENT ONCOLOGY PRACTICES

Tram Tran1, Mina Oh1, Fred Poordad1, Paul Martin2
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#989
PROFILES OF HBV DNA, ALT AND HBEAG STATUS AFTER STOPPING LAMIVUDINE IN PATIENTS WITH HBV SEROCONVERSION

James Fung1, Ching Lung Lai1, Yasuhiro Tanaka2, Masashi Mizokami2, John Yuen1, Danny Wong1, Man-Fung Yuen1
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#990
REDUCTION IN SERUM HBSAG LEVEL IN PATIENTS WITH CHRONIC HEPATITIS B INFECTED WITH GENOTYPE D INDUCED BY (PEGYLATED)INTERFERON ALFA-2A ALONE OR IN COMBINATION WITH NUCLEOS(T)IDE ANALOGS: A LONG-TERM SINGLE CENTRE COHORT STUDY

Maurizia Brunetto1, F. Moriconi1, D. Cavallone1, F. Oliveri1, A. M. Maina1, P. Ciccorsi1, P. Columbato1, B. Coco1, G. Moscati3, Ferruccio Bonino3
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#991
HIGH RATES OF HBSAG SEROCONVERSION IN CHRONIC HEPATITIS B PATIENTS responding to INTERFERON THERAPY: A LONG TERM FOLLOW-UP STUDY

Anneke Korevaar1, Rami Moucari1,2, Tarik Asselah1,2, Olivier Lada2, Nathalie Boyer1, Michèle Martinot-Peignoux2, Pierre Bedossa3, Patrick Marcellin1,2
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#992
HBSAG SEROCONVERSION: DIFFERENT KINETICS OF SERUM HBSAG LEVEL DECREASE IN INACTIVE CARRIERS AND CHRONIC HEPATITIS B PATIENTS TREATED WITH INTERFERON

Rami Moucari1,2, Vincent Mackiewicz3, Olivier Lada2, Agnes Devergne3, Nathalie Boyer1, Tarik Asselah1,2, Michèle Martinot-Peignoux2, Michel Vidaud3, Marie-Hélène Nicolas-Chanoine3, Patrick Marcellin1,2
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#993
HIGH PREVALENCE OF SIGNIFICANT HISTOLOGIC DISEASE IN PATIENTS WITH CHRONIC HEPATITIS B (CHB) AND NORMAL ALT

Mindie H. Nguyen1,2, Huy N. Trinh2, Ruei T. Garcia2, Jeannine Phan2, Gloria H. Nguyen2, Gerald Weiss3, Huy Nguyen2, Khanh Nguyen2, Emmet B. Keeffe1
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#994
BASELINE PARAMETERS PREDICT BOTH EARLY VIROLOGIC RESPONSE AND LONGER TERM OUTCOMES FOR TELBIVUDINE-TREATED PATIENTS WITH CHRONIC HEPATITIS B (THE GLOBE STUDY)

Stefan Zeuzem1, Maria Buti2, Edward J. Gane3, Yun-Fan Liaw4, Adrian M. Di Bisceglie5, E. Jenny Heathcote6, Nikolai V. Naoumov7, Jens Rasenack8, Seng Gee Lim9, Jin Lin Hou10, Xin-Jian Qiao11, Karin Gall11
1Johann Wolfgang Goethe University, Frankfurt, Germany. 2 Vall d’Hebron University Hospital, Barcelona, Spain. 3University of Auckland, Auckland, New Zealand. 4Chang Gung Memorial Hospital and University, Taipei, Taiwan. 5Saint Louis University School of Medicine, St. Louis, MO, USA. 6Toronto Western Hospital, Toronto, ON, Canada. 7Novartis Pharmaceuticals, East Hanover, NJ, USA. 8University of Virginia Health Science Center, Charlottesville, VA, USA. 9National University Hospital, Singapore, Singapore. 10Nanfang University of Medical Science, Guangzhou, China. 11Idenix Pharmaceuticals, Cambridge, MA, USA

#995
ON-TREATMENT VIROLOGIC SUPPRESSION AT WEEK 24 DECREASES THE RISK OF HISTOLOGIC PROGRESSION AT 1 YEAR; DATA FROM THE GLOBE TRIAL

Yves Benhamou1, Adrian M. Di Bisceglie2, Zachary D. Goodman3, Pierre Lamportico4, Michael P. Manns5, Pamela Vig6, Xin-Jian Qiao6, Karin Gall6
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#996
IMPACT OF ADEFOVIR DIPIVOXIL ON LIVER FIBROSIS AND ACTIVITY ASSESSED WITH FIBROTEST-ACTITEST IN PATIENTS INFECTED BY HEPATITIS B VIRUS

Thierry Poynard1, Yen Ngo1, Patrick Marcellin3, Stephanos J. Hadziyannis4, Vlad Ratziu5, Yves Benhamou1, Carol Brosgraff2
1APHP GHPS, Paris, France. 2Gilead, Foster City, CA, USA. 3APHP Beaujon, Paris, France. 4Henri Dunant Hospital, Athens, Greece

#997
LONG-TERM FOLLOW-UP OF ENTECAVIR TREATED PROTOCOL-DEFINED NON-RESPONDERS IN ROLLOVER STUDY ETV-901

Morris Sherman1, Mario Rizzetto2, Ching Lung Lai1, Yun-Fan Liaw4, Adrian Gadano5, Ira M. Jacobson9, Eugene R. Schiff6, Joanna Yang7, Richard Colombo7, Bruce Kreter8, Robert Hindes7
1Toronto General Hospital, Toronto, ON, Canada. 2Ospedale S. Giovanni Battista, Turin, Italy. 3Queen Mary Hospital, University of Hong Kong, Hong Kong, China. 4Linkou Chang Gung Memorial Hospital, Taoyuan, Taiwan. 5Hospital Italiano Regional del Sur, Buenos Aires, Argentina. 6University of Miami, Miami, FL, USA. 7Bristol-Myers Squibb Pharmaceutical Research Institute, Wallingford, CT, USA. 8Bristol-Myers Squibb Pharmaceutical Research Institute, Princeton, NJ, USA. 9Department of Medicine, Weill Medical College of Cornell University, New York, NY, USA
#998
FACTORS AFFECTING INITIAL VIROLOGIC RESPONSE AND EMERGENCE OF RESISTANT MUTANTS AFTER ADEFOVIR TREATMENT IN LAMIVUDINE RESISTANT CHRONIC HEPATITIS B PATIENTS
Jaeyoun Cheong1,2, Jinhee Cho1, Sungwon Cho1,2
1Gastroenterology, Ajou University School of Medicine, Suwon, South Korea. 2Liver Cirrhosis Clinical Research Center, Seoul, South Korea

#999
PROGNOSTIC FACTORS THAT ASSOCIATED WITH HEPATITIS B VIRUS DNA BREAKTHROUGH IN CHRONIC HEPATITIS B PATIENTS WITH LAMIVUDINE RESISTANT HEPATITIS B: MULTI-CENTER LONG-TERM RESULTS
Zheng Zeng, Guobao Tian, Di Tian, Jianjun Cui, Haiying Lu Peking University First Hospital, Beijing, China

#1000
ADEFOVIR DEPIVOXIL FOR DECOMPENSATED LIVER CIRRHOSIS PATIENTS WITH LAMIVUDINE-RESISTANT HEPATITIS B: MULTI-CENTER LONG-TERM RESULTS
Hyun Young Woo1, Jong Young Choi1, Seung Kew Yoon1, Dong Jin Suh2, Seung Woon Paik3, Kwang Hyub Han4, Soon Ho Um5, Byung Ik Kim6, Heon Ju Lee7, Mong Cho8, Chun Kyon Lee9, Dong Jun Kim10, Jae Seok Hwang11
1The Catholic University of Korea, Seoul, South Korea. 2University of Ulsan College of Medicine, Seoul, South Korea. 3Samsung Medical Center, Seoul, South Korea. 4Yonsei University College of Medicine, Seoul, South Korea. 5Korea University College of Medicine, Seoul, South Korea. 6Kangbuk Samsung Hospital, Seoul, South Korea. 7Yeungnam University College of Medicine, Daegu, South Korea. 8Pusan National University College of Medicine, Pusan, South Korea. 9National Health Insurance Corporation Ilsan Hospital, Koyang, South Korea. 10Hallym University College of Medicine, Chuncheon, South Korea. 11Keimyung University College of Medicine, Daegu, South Korea

#1001
CLINICAL FEATURES OF LYMPHOMA CHEMOTHERAPY ASSOCIATED HEPATITIS VIRUS B REACTIVATION
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#1002
PREDICTING SUSTAINED HBsAG LOSS AFTER TREATMENT WITH PegIFN ALPHAS-2B: DEVELOPMENT AND VALIDATION OF A PRACTICAL MODEL
Erik H. Buster1, Bettina E. Hansen1,2, Stefan Zeuzem3, Solko W. Schalm1, Ewout W. Steyerberg2, Harry L. Janssen3
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#1003
THREE-YEAR ASSESSMENT OF ENTECAVIR RESISTANCE IN GENOTYPE C CHRONIC HEPATITIS B PATIENTS IN JAPAN REVEALS DIFFERENT CLINICAL OUTCOMES REGARDING BREAKTHROUGH HEPATITIS PREDICTED BY THE RESISTANCE SUBSTITUTIONS USING RECENTLY DEVELOPED INNO-LIPA HBV ASSAY
Motokazu Mukaido1,8, Yasuhiro Tanaka1, Etsuro Orito2, Fuat Kurbanoğlu1, Kenichi Fukai2, Osamu Yokosuka3, Michio Sata4, Tatsuya Ide5, Karin Yoshiyasu5, Yamada Goutaro6, Kohsaku Sakaguchi7, Masashi Mizokami1
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#1004
THE EFFECTS OF α-GALACTOSYLCERAMIDE ON CHRONIC HEPATITIS B INFECTION IN A RANDOMIZED PLACEBO CONTROLLED PHASE I/II TRIAL
Martijn J. ter Borg1, Dave Sprengers1, Andrea M. Woltman1, B. Mary E. von Blomberg2, Karin C. Van Nieuwkerk3, Wanda C. Tielmans1, Rekha Binda1, Renate van der Molen4, Harry L. Janssen1
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#1005
CROSS-RESISTANCE CHARACTERIZATION OF THE MAIN HBV DRUG RESISTANT MUTANTS
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#1006
DETECTION OF HEPATITIS B VIRUS YMDD MUTANTS BY MULTIPLEX-PCR USING DUAL PRIMING OLGONUCLEOTIDE (DPO)
Park Ji Young1, Lee Tae Hoon1, Park Jeong Hoon1, Kim Sang Gyun1, Park Do Hyun2, Lee Suk He2, Jang Jae Young1, Kim Young Seok1, Chung Il Kwon1, Kim Hong Soo1, Park Sang Heum2, Kim Sun Joo1, Kim Seok Hyun1, Lee Heon Young2, Kim Chang Jin2, Jeong Dong Jun1
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Denotes AASLD Presidential Poster of Distinction
#1007
FIVE-YEAR RETROSPECTIVE SURVEY OF HBV GENOTYPIC DRUG RESISTANCE PATTERNS IN A DATABASE OF 381 HBV REVERSE TRANSCRIPTASE SEQUENCES IN SOUTHERN FRANCE
Philippe Colson1,2, Rene Gerolami3, Christian Tourres1, Anne Motte1,2, Isabelle Ravaux4, Isabelle Poizot-Martin5, Jacques Moreau6, Patrick Borentain3, Mireille Henry1,2, Catherine Tamalle1,2
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#1008
INFLAMMATION AND FIBROSIS IN PATIENTS WITH CHRONIC HEPATITIS B DESPITE LOW VIRAL LOAD AT THE TIME OF LIVER BIOPSY
Evan B. Grossman1, Maya Gambarin-Gelwan2,3, Thomas A. Hahambis2, Rhonda K. Yantiss4, Brian R. Edlin2,3, Ira M. Jacobson2,3
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Inflammation and Immunobiology

#1009
ECTOPTIC EXPRESSION OF NEURAL AUTOANTIGEN IN LIVER SUPPRESSES AUTOIMMUNE NEUROINFLAMMATION BY INDUCING ANTIGEN-SPECIFIC CD4+ CD25+ FOXP3+ REGULATORY T CELLS
Johannes Herkel1, Stefan Lüth1, Samuel Huber1, Christoph Schramm1, Thorsten Buch2, Stefan Zander1, David C. Wraith3, Ansgar W. Lohse1
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#1010
CRITICAL ROLE FOR CD1D-RESTRICTED Vα14 INKT CELLS IN STIMULATING INTRAHEPATIC CD8 T CELL RESPONSES TO LIVER-EXPRESSED ANTIGEN
Dave Sprengers1,2, Fenna C. Sillé2, Gurdyal S. Besra3, Harry L. Janssen1, Eckart Schott4, Marianne Boes2,5
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Inflammation and Immunobiology

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#1011
CTS-1027, A POTENT MMP INHIBITOR, PROTECTS AGAINST TNFα- AND α-FAS-INDUCED LIVER INJURY
Patricia C. Contreras, Karen Valentino
Conatus Pharmaceutical, San Diego, CA, USA

#1012
INVESTIGATING THE ROLE OF AUTOACTIVE CD8+ AND CD4+ T CELLS IN THE PATHOGENESIS OF AUTOIMMUNE HEPATITIS IN A TRANSGENIC MOUSE MODEL
M. Zierden, E. Kühnen, M. Odenthal, H. P. Dienes
Institute for Pathology, University Hospital of Cologne, Cologne, Germany

#1013
DELETION OF SOCS3 GENE IN THE LIVER PARENCHYMAL CELLS PROTECTS FROM T CELL-MEDIATED LIVER INJURY
Hisanobu Ogata1,2, Koichi Azuma1, Jacquelyn J. Maher2
1Department of Medicine and Clinical Science, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan. 2Liver Center and Department of Medicine, University of California, San Francisco, San Francisco, CA, USA

#1014
DELETION OF CD39 INHIBITS DEVELOPMENT OF METASTATIC LIVER CANCER
Xiaofeng Sun, Yan Wu, Mika Ogawa, Keichi Enjojiyi, Eva Csizmadia, Guido Beldi, Simon C. Robson
Liver Center, Department of Medicine, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA, USA

#1015
EVIDENCE FOR ALTERNATIVE- AND CLASSICAL-PATHWAY INDEPENDENT ACTION OF COMPLEMENT FACTOR C3 DURING LIVER REGENERATION
Amelia Clark, Alexander Weymann, Eric Hartman, Yumirle P. Turnelle, Dennis Hourcade, David Rudnick
Washington University School of Medicine, St. Louis, MO, USA
#1016

**HEPATOCYTE SURVIVAL IN ACUTE HEPATITIS IS DUE TO C-JUN/AP-1-DEPENDENT EXPRESSION OF INDUCIBLE NITRIC OXIDE SYNTHASE**

Peter Hasselblatt¹, Martina Rath¹, Vukoslav Komnenovic¹, Kurt Zatloukal², Erwin Wagner¹  
¹Research Institute of Molecular Pathology, Vienna, Austria. ²Institute of Pathology, Medical University of Graz, Graz, Austria

#1018

**INHIBITION OF NKT CELL ACTIVATION AND THE DEVELOPMENT OF HEPATITIS BY A GLYCOSAMINOGLYCAN-BINDING DEFICIENT RANTES MUTANT**

Maureen N. Ajuebor¹, Amanda Proudfoot², Cory Hogaboam³, Tai Le⁴, Mitch Kronenberg⁵, Mark Swain⁶  
¹Dept of Molecular and Cellular Physiology, Louisiana State University, Shreveport, LA, USA. ²Merck Serono Research Center, Geneva, Switzerland. ³Dept of Pathology, University of Michigan Medical School, Ann Arbor, MI, USA. ⁴Developmental Immunology, La Jolla Institute for Allergy and Immunology, San Diego, CA, USA. ⁵Dept of Medicine, University of Calgary, Calgary, AB, Canada

#1019

**CONTRIBUTION OF TRIF TO SECRETION OF MATURE IL-1β AND IL-18 VIA THE TLR-MEDIATED CASPASE-1 ACTIVATION DURING EXTRACELLULAR BACTERIAL INFECTION IN MICE**

Michiko Imamura¹, Koubou Yasuda², Jiro Fujimoto¹, Shizuo Akira³, Hiroko Tsutsui¹, Kenji Nakanishi²  
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#1020

**ALCOHOL CONSUMPTION INDUCES EXPRESSHION OF SOCS3 AND SOCS1 AND INHIBITS SIGNALING VIA STAT3 AND STAT1 PATHWAYS IN HUMAN MONOCYTES**

Oxana Norkina, Angela Dolganiuc, Karen Kodys, Pranoti Mandrekar, Gyongyi Szabo  
Umass Med School, Worcester, MA, USA

#1021

**ACTIVATION OF CYCLIC-AMP RESPONSE ELEMENT BINDING PROTEIN CONTRIBUTES TO ADIPONECTIN-STIMULATED INTERLEUKIN-10 EXPRESSION IN RAW 264.7 MACROPHAGES**

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#1022

**TRIMMING OF BRANCHED OLIGOSACCHARIDE STRUCTURES ON HBV INHIBITS DC-SIGN BINDING**

Marjoleine L. Op den Brou¹, Marein A. de Jong², Renate G. van der Molen¹, Theo B. Geijtenbeek², Harry L. Janssen¹, Andrea M. Woltman¹  
¹Gastroenterology & Hepatology, Erasmus MC, University Medical Center Rotterdam, Rotterdam, Netherlands. ²Molecular Cell Biology & Immunology, VU University Medical Center, Amsterdam, Netherlands

#1023

**CONCANAVALIN A-INDUCED HEPATITIS IS DEPENDENT ON TOLL-LIKE RECEPTOR 4 (TLR4) SIGNALING PATHWAYS**

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¹Department of Physiology, University of Otago, Dunedin, New Zealand. ²Malaghan Institute of Medical Research, Wellington, New Zealand

#1024

**INHIBITION OF RIG-I/CARDIF-INDUCED INTERFERON RESPONSES BY N TERMINUS OF HCV NS4B PROTEIN**

Megumi Tasaka¹, Naoya Sakamoto²,¹, Yoshie Itakura¹, Mina Nakagawa²,¹, Yasushiro Itsui¹, Yuku Sekine-Osajima¹, Yuki Nishimura-Sakurai¹, Cheng-Hsin Chen¹, Mamoru Watanabe¹  
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#1025

**EXPRESSION OF HEPATITIS C VIRUS PROTEINS IS SUFFICIENT TO INDUCE RECOGNITION BY RESIDENT HEPATIC AND INVARIANT CD1D-RESTRICTED T CELLS**

Kazuhiro Yanagisawa¹, Roujie Wang¹, Wenyu Lin², Rachel Baden¹, Khadija Iken¹, Stanley M. Lemon², Raymond T. Chung², Lucy Golden-Mason³, Hugo R. Rosen³, Margaret J. Koziel¹, Mark A. Eley¹  
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#1026
PROSPECTIVE ANALYSIS OF HEPATIC CD1D-RESTRICTED T CELLS IN ADVANCED HCV LIVER DISEASE
Roujie Wang1, Lyly Tran1, Kristin K. Snow2, Karen L. Lindsay3, Margaret J. Koziel1, Mark A. Exley1
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#1027
BLYS/BAFF RECEPTOR-LIGAND SYSTEM IN HCV INDUCED B-CELL CLONAL DISORDERS
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#1028
HCV-SPECIFIC CELLULAR RESPONSES IN ANTIBODY NEGATIVE, HCV RNA NEGATIVE INJECTION DRUG USERS
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#1029
DETECTION OF CD4+ T CELL RESPONSES IN PATIENTS WITH ACUTE HCV INFECTION IRRESPECTIVE OF CLINICAL OUTCOME
Julian C. Schulze zur Wiesch1,2, Victoria Kasprowicz2, Arthur Y. Kim2, Steve Longworth2, Brian Nolan2, Thomas Kuntzen2, Barbara McGovern2, Lia Lewis-Ximenas6,2, Raymond T. Chung3, William Kwock4, Bruce D. Walker2,7, Georg M. Lauer2
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#1030
CHRONIC EVOLUTION OF ACUTE HEPATITIS C IS ASSOCIATED WITH LOWER FREQUENCIES OF HCV-SPECIFIC CD4+ T-CELLS BUT UNRELATED TO HCV-SPECIFIC FOXP3+ REGULATORY CD4+ T-CELLS
Malte H. Heeg1,2, Axel Ulsenheimer1,2, Norbert H. Gruener1,2, Maria C. Jung1,2, Reinhard Zachoval1,2, Winfried Schraut2, Martin Waechtler2, Gerold Pape1,2, Herlmut M. Diepolder1,2
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#1031
HIGHER PERCENTAGES OF INTRA-HEPATIC REGULATORY T CELLS ARE PRESENT IN CHRONIC HEPATITIS B PATIENTS WITH A HIGH VIRAL LOAD
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#1032
ANTIGEN-PULSED DENDRITIC CELLS ARE CAPABLE OF INDUCING BOTH INNATE AND ADAPTIVE IMMUNITY: A LESION LEARNT FROM CROSS TALK BETWEEN NATURAL KILLER CELLS AND DENDRITIC CELLS AND THEIR APPLICATION FOR IMMUNE THERAPY OF CHRONIC HEPATITIS B VIRUS INFECTION
Osamu Yoshida, Sh. Md. Fazle Akbar, Teruki Miyake, Mao Hamada, Yoichi Hiasa, Kojiro Michitaka, Morikazu Onji
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#1033
HLA CLASS II AND RACE-DEPENDENT DIFFERENCES IN IMMUNE RESPONSE TO HCV, SUSCEPTIBILITY TO CHRONIC INFECTION, AND RESPONSE TO THERAPY
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#1034
ADHESION OF IN VITRO GENERATE DENDRITIC CELLS TO HUMAN HEPATIC ENDOTHELIUM UNDER CONDITIONS OF SHEAR STRESS
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#1035
INTRAHEPATIC REGULATORY T CELLS DIFFER PHENOTYPICALLY FROM THEIR CIRCULATING COUNTERPARTS IN CHRONIC THERAPY NAIVE HCV PATIENTS
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#1036
THE ROLE OF NANOBACTERIA IN CHOLECYSTITIS AND CHOLELITHIASIS
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#1037
CRITICAL ROLE OF CD44 IN HEPATOTOXIN-MEDIATED LIVER INJURY
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#1038
INCREASED ACETYLATION OF ALPHA-TUBULIN AND DECREASED EXPRESSION OF FIBROCYSTIN ARE SENSITIVE MARKERS OF CELLULAR DAMAGE AND ACTIVATION IN INFLAMMATORY LIVER DISEASE
Sarah Blair-Reid1, Gary Reynolds1, Jane Hartley3, Deirdre A. Kelly2, Stefan Hubscher2, Peter C. Harris5, Colin A. Johnson4, Christopher J. Ward5, Simon Afford1
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#1039
CRITICAL ROLE OF EARLY GROWTH RESPONSE-1 TO GALACTOSAMINE / LIPOPOLYSACCHARIDE-INDUCED ACUTE LIVER INJURY IN MICE
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#1040
INHIBITION OF THE KUPFFER CELL INCREASED SERUM HMGB1 LEVELS AND THE MORTALITY IN A RAT SEPTIC PERITONITIS MODEL
Hiroshi Kono, Kenichi Ishii, Naohiro Hosomura, Nobuyuki Tanaka, Masanori Matsuda, Hideki Fuji
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#1041
MRNA EXPRESSION ANALYSIS OF IMMUNOREGULATION, APOPTOSIS AND FIBROSIS IN LIVER DISEASES
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#1042
EXPRESSION OF NOTCH SIGNALING AND ANTIGEN PROCESSING MOLECULES IN PROGRESSIVE PATHOGENENIC BIOPSIES OF CHRONIC HEPATITIS B INFECTION
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#1043
INTRAHEPATIC STATUS OF REGULATORY T CELLS IN AUTOIMMUNE HEPATITIS, PRIMARY BILIARY CIRRHOSIS, CHRONIC HEPATITIS C, AND CHRONIC HEPATITIS B
Masashi Sakaki1, Kazumasa Hiroishi1, Toshiyuki Baba1, Miki Kushima2, Michio Imawari1
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#1044
SPECIFIC CONTRIBUTION OF NADPH-OXIDASE IN DISTINCT CELL TYPES DURING HEPATIC FIBROSIS.
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#1045
TLR4/MYD88/NF-KB-MEDIATES HEPATIC STELLATE CELLS ACTIVATION BY THE REGULATION OF TGF-B PSEUDORECEPTOR BAMBI IN HEPATIC FIBROSIS
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#1046
GLYCATED FIBRONECTIN STIMULATES HEPATIC STELLATE CELL (HSC) ACTIVATION AND TYPE I COLLAGEN PRODUCTION IN PRIMARY CULTURES
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#1047
BENEFICIAL EFFECT OF ANGIOTENSIN-BLOCKING AGENTS ON LIVER FIBROSIS IN PATIENTS WITH HEPATITIS C
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#1048
ROLE OF P75 SIGNALLING IN THE PATHOGENESIS OF LIVER FIBROSIS IS CONTEXT AND LIGAND STRUCTURE DEPENDENT

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#1049
LIVER FIBROSIS PERSISTS FOLLOWING NEUTROPHIL DEPLETION DURING LIVER REPAIR

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#1050
EVIDENCE OF SNAIL1 TRANSCRIPTION FACTOR INVOLVEMENT IN HEPATIC STELLATE CELLS ACTIVATION PROCESS

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#1051
INCREASED MORTALITY, ENHANCED LIVER FIBROSIS AND DIMINISHED ACUTE-PHASE RESPONSE AFTER BILE-DUCT LIGATION IN HEPATOCYTE SPECIFIC C-MET/GP130 DEFICIENT MICE

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#1052
AMELIORATION OF BILIARY FIBROSIS BY AN ALPHAVBETA 6 INTEGRIN ANTAGONIST IS MEDIATED BY INHIBITION OF CHOLANGIOCYTE ADHESION TO FIBRONECtin AND CHOLANGIOCYTE TGFbeta1 ACTIVATION

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#1053
TOLL-LIKE RECEPTOR-9 ACTIVATION AMELIORATES HEPATIC FIBROSIS ASSOCIATED WITH LYMPHOCYTE INTERACTIONS

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#1054
ANGIOTENSIN 1–7 REDUCES BILE DUCT PROLIFERATION AND HEPATIC FIBROSIS IN THEBILE DUCT LIGATED RAT

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#1055
ROLE OF C-C CHEMOKINE RECEPTOR 2 (CCR2) DURING CONSTITUTION AND RESOLUTION OF FIBROSIS INDUCED BY CARBONE TETRACHLORIDE IN MICE

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#1056
INCREASED SUSCEPTIBILITY TO HEPATIC STEATOSIS, INFLAMMATION AND ADVANCED FIBROSIS IN APOLIPOPROTEIN-E DEFICIENT MICE

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#1057
DELETION OF THE THROMBIN RECEPTOR GENE, PAR 1, AMELIORATES LIVER FIBROSIS AND DECREASES TGFβ EXPRESSION BY HEPATIC STELLATE CELLS IN A MURINE MODEL OF CIRRHOSIS

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#1058
HUMAN DISCOIDIN DOMAIN RECEPTOR 1: INTRAHEPATIC FORMS AND IN VITRO FUNCTIONS
Sunmi Song, Nicholas A. Shackel, Xin M. Wang, Katerina Ajami, Geoffrey W. McCaughan, Mark D. Gorrell
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#1059
BXD RECOMBINANT INBRED MOUSE LINES - A GENETIC REFERENCE POPULATION FOR DISSECTION OF THE COMPLEX GENETICS OF LIVER FIBROSIS
Rabea Hall, Sonja Hillebrandt, Katrin Hochrath, Frank Grunhage, Yildiz Yildiz, Susanne Weber, Stephanie Schwartz, Tilman Sauverbruch, Frank Lammert
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#1060
GENETIC ASSOCIATION STUDY OF PROFIBROGENIC GENE VARIANTS USING TRANSIENT ELASTOGRAPHY FOR PHENOTYPIC CHARACTERIZATION OF LIVER FIBROSIS
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#1061
SYNERGISTIC ANTI-FIBROTIC EFFICACY OF STATIN AND PROTEIN KINASE C INHIBITOR IN HEPATIC FIBROSIS
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#1062
THE IMPACT OF BONE MARROW STEM CELLS ON LIVER FIBROSIS IS CRITICALLY DETERMINED BY THEIR ROUTE OF CELL TRAFFICKING
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#1063
TGFβ1 STIMULATES THE HUMAN α1(I) COLLAGEN PROMOTER BY COOPERATION BETWEEN SMAD2/4 AND SP1 TRANSMITTING FACTORS
Polina Sysa, James J. Potter, Xiaopu Liu, Esteban Mezey Medicine, Johns Hopkins University School of Medicine, Baltimore, MD, USA

#1064
A HUMAN RECOMBINANT ANTIBODY FOR TARGETING ANTI-FIBROGENIC THERAPEUTICS TO MYOFIBROBLASTS IN VIVO
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#1065
ENHANCED IL-6 PRODUCTION BY HEPATIC STELLATE CELLS IN RESPONSE TO IMATINIB MESYLATED STI571 - (GLEEVEC ®), A NOVEL LINK BETWEEN RECEPTOR TYROSINE KINASE INHIBITION AND HEPATIC REGENERATION
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#1066
CD147 REGULATION OF HEPATOCYTE DERIVED MATRIX METALLOPROTEINASES: A NOVEL PATHWAY INVOLVED IN LIVER FIBROGENESIS
Sarah R. Richardson1, Fiona J. Warner1, Geoff W. McCaughan1,2, Mark D. Gorrell1,2, Rosa Lam1, Nicholas A. Shackel1,2
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#1067
DETECTION AND PREVENTION OF HEPATIC FIBROSIS TARGETING TGF-β ACTIVATION REACTION
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#1068
C-REACTIVE PROTEIN AMELIORATES HEPATIC FIBROGENESIS THROUGH INHIBITION OF HEPATIC STELLATE CELL TRANSACTIVATION
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#1069
THE FUNCTIONAL CONTRIBUTION OF BONE MARROW-DERIVED CELLS TO LIVER FIBROSIS IN A MOUSE MODEL OF CHRONIC LIVER INJURY
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ACCELERATED ORTHOTOPIC HCC GROWTH IS LINKED WITH INCREASED PRO-ANGIOGENIC AND PRO-METASTATIC FACTORS IN MURINE LIVER FIBROSIS

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CONCORDANCE BETWEEN FIBROTEST® (FT) AND FIBROSCAN® (FS): A NEW NON-INVASIVE METHODOLOGY FOR IMPROVING ACCURACY IN A WORLD WITHOUT A GOLD STANDARD

Thierry Poynard¹, Patrick Ingliz¹, Mona Munteanu², Pascal Lebray¹, Rachel Morra¹, Djamilia Messous¹, Françoise Imbert-Bismut¹, Yves Benhamou¹, Vlad Ratziu¹
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LACK OF INDUCIBLE NITRIC OXIDE SYNTHASE IN A MOUSE MODEL WITH CARBON TETRACHLORIDE INJURY LEADS TO INCREASED APOPTOSIS AND DECREASED FIBROSIS

Ghazaleh Aram, James J. Potter, Xiaopu Liu, Esteban Mezey Medicine, Johns Hopkins University, Baltimore, MD, USA

EXTRA-CELLULAR FIBRONECTIN INDUCED HEPATIC OVAL CELL PROLIFERATION THROUGH CYCLOOXYGENASE-2-INDUCED PROSTAGLANDIN SIGNALING

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TREATMENT OF ESTABLISHED CIRRHOSIS WITH SV40 VECTORS Encoding INSULIN-LIKE GROWTH FACTOR I

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MULTIPLE GENETIC MARKERS IN TLR4 REGION ARE ASSOCIATED WITH CIRRHOSIS RISK IN CHC PATIENTS

Hongjin Huang¹, Olivia Abar¹, Charley Rowland¹, Ramasubbu Venkatesh¹, Joseph Catanesi¹, Mitchell L. Shiftman², Ramsey Chiman Cheung³, Teresa L. Wright⁴, Thomas J. Layden⁵, Nathalie H. Bzowej⁶, Thomas White¹, Scott L. Friedman⁷
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BMP-7: THE KEY ANTAGONIST OF TGF-ß IN LIVER FIBROSIS?

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KUPFFER CELL-DERIVED CONNECTIVE TISSUE GROWTH FACTOR (CTGF) AND TRANSFORMING GROWTH FACTOR BETA (TGFß) MEDIATE THE PROFIBROGENIC EFFECTS OF LEPTIN IN VITRO

Jianhua Wang, Isabelle Leclercq, Joanne Brymora, Mehdi Ramezani-Moghadam, Roslyn M. London, Kumar Subramaniam, Jacob George
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LOSARTAN REDUCES THE EXPRESSION OF PROFIBROGENIC GENES AND INFLAMMATION IN PATIENTS WITH CHRONIC HEPATITIS C

Jordi Colmenero¹, Ramón Bataller¹, Pau Sancho-Bru¹, Xavier Forns¹, Miquel Bruguerà¹, Marlene Dominguez¹, Montserrat Moreno¹, Vicente Arroyo¹, David Brenner², Pere Gines¹
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ATORVASTATIN ATTENUATES ANGIOTENSIN-II INDUCED INFLAMMATORY AND FIBROGENIC ACTIONS IN THE LIVER

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#1080
**T CELL DERIVED MEMBRANE MICROPARTICLES CONTAINING EMMPRIN INDUCE PROFIBROLYTIC ACTIVATION IN HEPATIC STELLATE CELLS**

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#1081
**CELECOXIB SHOWS ANTI-FIBROTIC EFFECT IN HEPATIC FIBROSIS MODELS IN RAT AND INDUCES APOPTOSIS OF HEPATIC STELLATE CELLS THROUGH INHIBITION OF AKT ACTIVATION**

Yong-Han Paik¹, KwanSik Lee¹, SoHee Kang¹, MoonYoung Kim¹, JungIl Lee², DoYoung Kim¹, Sang Hoon Ahn¹, Kwang-Hyub Han¹, ChaeYoon Chon¹, YoungMyoung Moon², David Brenner³

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#1082
**TELMISARTAN PREVENTS HEPATIC FIBROSIS AND ENZYME-ALTED PRENEOPLASTIC LESIONS IN RAT LIVER CIRRHOSIS INDUCED BY A CHOLINE-DEFICIENT L-AMINO ACID-DEFINED DIET**

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#1083
**NATRIURETIC PEPTIDE IMPROVED LIVER FIBROSIS IN RAT MODEL USING NEW TREATMENT SYSTEM**

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#1084
**TRANSIENT ELASTOGRAPHY (FIBROSCAN®) IN PATIENTS WITH CHRONIC HEPATITIS C VIRUS INFECTION AND HAEMOPHILIA A**

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#1085
**INHIBITION OF ANGIOGENESIS BY A VITRONECTIN RECEPTOR ANTAGONIST WORSENS LIVER FIBROSIS**

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Pediatric Hepatology

#1086
**HYPERDYNAMIC CIRCULATION IN INFANTS WITH BILIARY ATRESIA AND PORTAL HYPERTENSION**

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#1087
**ADAPTIVE CHANGES OF HEPATOCYTE TRANSPORTERS AND NUCLEAR RECEPTORS IN PEDIATRIC PATIENTS WITH EARLY AND LATE-STAGE CHOLESTASIS**

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#1088
**THE ROLE OF ENDOSCOPIC RETROGRADE CHOLANGIOPANCREATOGRAPHY IN DIAGNOSIS OF BILIARY ATRESIA IN INFANTS**

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#1089
**PREDOMINANCE OF T HELPER 1 VIRUS SPECIFIC IMMUNE RESPONSE FAVOURS VIRUS CONTROL IN CHILDHOOD HEPATITIS B VIRUS INFECTION**

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#1090
UNIQUE FULL LENGTH HEPATITIS B VIRUS GENOMES ISOLATED FROM CHILDREN WITH HEPATOCELLULAR CARCINOMA

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#1091
INTRAFAMILIAL TRANSMISSION OF HEPATITIS C VIRUS IN CHILDREN: CORRELATION WITH VIROLOGICAL AND IMMUNOLOGICAL PARAMETERS

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#1092
HCV AUTOIMMUNITY IN A U.S. MULTI-CENTER COHORT OF TREATMENT NAÏVE CHILDREN

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#1093
TREATMENT OF HEPATITIS B USING INTRON A VS PEGININTERFERON IN COMBINATION WITH LAMIVUDINE IN CHILDREN

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#1094
HBEAG SEROCONVERSION CORRELATES WITH A HIGH NUMBER OF CORE GENE MUTATIONS AND WITH GENOTYPES B AND D IN PAEDIATRIC PATIENTS

Ivana Carey, Maria Mytilinaiou, Antonietta Giannattasio, Sanjay Bansal, Paul Cheeseman, Dita Cebecauerova, Giorgina Mieli-Vergani, Diego Vergani
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#1095
GRAFT HISTOLOGY UP TO 10 YEARS AFTER PEDIATRIC LIVER TRANSPLANTATION: HIGH INCIDENCE OF SEVERE FIBROSIS, CORRELATED WITH TRANSPLANT RELATED FACTORS

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#1096
IMPROVED PREDICTIVE VALUE OF PELD WITH THE ADDITION OF SERUM SODIUM AND PLATELET COUNT

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#1097
PEDIATRIC LIVER RETRANSPANTATION: OUTCOMES AND A PROGNOSTIC SCORING TOOL

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#1098
LIPID-ENRICHED DIET INDUCES GROWTH RETARDATION AND LIVER STEATOSIS IN CYSTIC FIBROSIS MICE

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#1099
CAN INSULIN-LIKE GROWTH FACTOR BINDING PROTEIN-1 (IGFBP-1) IDENTIFY OVERWEIGHT CHILDREN AT RISK OF DEVELOPING STEATOHepATITIS?
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#1100
PREDICTORS FOR SEVERE COURSE OF CYSTIC FIBROSIS-RELATED LIVER DISEASE IN CHILDREN
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#1101
PATTERN OF DIAGNOSTIC EVALUATION FOR THE CAUSES OF PEDIATRIC ACUTE LIVER FAILURE (PALF): OPPORTUNITIES FOR QUALITY IMPROVEMENT
Michael Narkewicz1, Dominic Dell Olio6, Saul J. Karpen5, Susan Krug7, Karen F. Murray4, Kathleen B. Schwarz8, Nada Yazigi7, Song Zhang3, Steven H. Belle2, For the Pediatric Acute Liver Failure Study Group3
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#1102
CLINICAL SIGNIFICANCE OF THE ABERNETHY MALFORMATIONS (CONGENITAL ABSENCE OR HYPOPLASIA OF THE PORTAL VEIN)
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#1103
SCLEROSING CHOLANGITIS IN CHILDREN—RETROSPECTIVE SINGLE CENTER REVIEW
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#1104
ROLE OF LIVER HISTOLOGY IN THE MANAGEMENT OF ACUTE LIVER FAILURE IN CHILDREN
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#1105
BUDD CHIARI SYNDROME IN CHILDREN: A SINGLE CENTRE EXPERIENCE IN THE UNITED KINGDOM
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Steatosis and Steatohepatitis: Clinical

#1106
CLINICAL CORRELATES OF HISTOPATHOLOGY IN PEDIATRIC NONALCOHOLIC FATTY LIVER DISEASE
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#1107
NON-ALCOHOLIC FATTY LIVER DISEASE AS AN IMPORTANT RISK FACTOR OF CORONARY HEART DISEASE
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#1108
INHERITED VARIANTS OF GENES INVOLVED IN INSULIN SIGNALING AFFECT THE RISK OF HEPATIC FIBROSIS IN NAFLD
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#1109
DAILY CANNABIS USE, A NOVEL RISK FACTOR OF STEATOSIS SEVERITY IN PATIENTS WITH CHRONIC HEPATITIS C

Christophe Hezode1,2, Elie-Serge Zafrani3,2, Françoise Roudot-Thoraval3,4, Charlotte Costentin1, Ali Hessami3, Fatima Medkour1, Magali Bouvier-Alias4,2, Jean-Michel Pawlotsky4,2, Sophie Lotersztajn2, Ariane Mallat1,2
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#1110
PIGMENT EPITHELIUM-DERIVED FACTOR (PEDF) REGULATES HEPATOCYTE LIPID CONTENT THROUGH ITS INTERACTION WITH ADIPOSE TRIGLYCERIDE LIPASE (ATGL)

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#1111
ALCOHOL CONSUMPTION IN SEVERELY OBESE PATIENTS WITH NONALCOHOLIC FATTY LIVER DISEASE: RELATIONSHIP WITH HEPATIC FIBROSIS

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#1112
CHOLESTEROL ACCUMULATION IN PATIENTS WITH FATTY LIVER DISEASE CORRELATES WITH INDUCTION OF CHOLESTEROL-REGULATING ENZYMES AND SIGNS OF STEATOHEPATITIS

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#1113
ARTERIALIZATION OF CENTRAL ZONES IN NONALCOHOLIC STEATOHEPATITIS (NASH)

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#1114
MODEST WINE DRINKING IS ASSOCIATED WITH A DECREASED PREVALENCE OF SUSPECTED NONALCOHOLIC FATTY LIVER DISEASE

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#1115
ANALYSIS OF HEPATIC EXPRESSION OF GENES INVOLVED IN LIPID AND IRON METABOLISM IN NONALCOHOLIC FATTY LIVER DISEASE

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#1116
NON-INVASIVE DIAGNOSTIC BIOMARKERS FOR NON-ALCOHOLIC STEATOHEPATITIS (NASH)

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#1117
EFFECT OF PENTOXIFYLLINE ON CLINICAL, BIOCHEMICAL AND METABOLIC PARAMETERS, HEPATIC NECROINFLAMMATION, FIBROSIS AND STELLATE CELL ACTIVATION IN PATIENTS WITH NONALCOHOLIC STEATOHEPATITIS

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ADIPOSE TISSUE OF INSULIN RESISTANCE IN NASH PATIENTS

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#1119
HIGH SENSITIVITY C-REACTIVE PROTEIN IS AN INDEPENDENT CLINICAL FEATURE OF NONALCOHOLIC STEATOHEPATITIS (NASH) AND ALSO OF THE SEVERITY OF FIBROSIS IN NASH
Yuichi Nozaki, Masato Yoneda, Takuma Higurashi, Hiroshi lida, Hironori Mawatari, Hiroki Endo, Ayako Tamimoto, Kyoko Yonemitsu, Tomoyuki Akiyama, Koji Fujita, Hirokazu Takahashi, Masahiko Inamori, Noritoshi Kobayashi, Yasunobu Abe, Kensuke Kubota, Hiroyuki Kinkoshi, Satoru Saito, Atsushi Nakajima Division of Gastroenterology, Yokohama City University, Yokohama, Japan

#1120
PRESENCE OF OBSTRUCTIVE SLEEP APNEA IS ASSOCIATED WITH FIBROSIS IN PATIENTS WITH NONALCOHOLIC FATTY LIVER DISEASE
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#1121
PROTEOMIC ANALYSIS OF SERUM BIOMARKERS IN PATIENTS WITH NONALCOHOLIC STEATOHEPATITIS USING SELDI-TOF/MS OR MALDI-TOF/MS
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#1122
PIOGLITAZONE ENHANCES HEPATIC, MUSCLE AND ADIPOSE TISSUE INSULIN SENSITIVITY AND AMELIORATES SYSTEMIC INFLAMMATION IN PATIENTS WITH IGT OR T2DM AND NASH
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#1123
OXIDATIVE STRESS PROFILES IN PEDIATRIC NONALCOHOLIC FATTY LIVER DISEASE
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#1124
SHORT-TERM VARIABILITY IN COMMONLY USED LIVER TESTS: IMPLICATIONS FOR CLINICAL PRACTICE
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#1125
ADIPOPHILIN EXPRESSION AND OXIDIZED PHOSPHATIDYLCHOLINE LOCALIZATION IN BALLOONED HEPATOCYTES IN NONALCOHOLIC STEATOHEPATITIS
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#1126
ASSOCIATION OF AST AND ALT WITH LIVER HISTOLOGY IN ADULTS WITH NAFLD
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#1127
METABOLIC SYNDROME AMONG CHILDREN WITH VARIABLE FEATURES OF NONALCOHOLIC FATTY LIVER DISEASE
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#1128
ADVANCED FIBROSIS IN NAFLD IS ASSOCIATED WITH LIFETIME ALCOHOL USE, DIABETES, AND AGE BUT NOT WITH LIFETIME CIGARETTE SMOKING
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#1129
NON-ALCOHOLIC FATTY LIVER DISEASE CAN INDUCE PORTAL HYPERTENSION, DEMONSTRATED BY HEPATIC VENOUS PRESSURE GRADIENT MEASUREMENT, RELATED TO THE SEVERITY OF STEATOSIS BUT NOT FIBROSIS IN OVERWEIGHT AND OBESE PATIENTS
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#1130
STUDY OF A POLYMORPHISM IN THE GLUTAMATE-CYSTEINE LIGASE (GCLC) GENE IN NONALCOHOLIC FATTY LIVER DISEASE
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#1131
INFLUENCE OF IMPAIRED GLUCOSE TOLERANCE, GLUCOSE CONTROL STATUS, LIGHT TO MODERATE ALCOHOL CONSUMPTION, AND REDUCED CARBOHYDRATE DIETS ON SERUM ALT CHANGE DURING RAPID WEIGHT LOSS IN THE RESIDENTIAL WEIGHT LOSS PROGRAM
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#1132
NON-ALCOHOLIC FATTY LIVER DISEASE IS ASSOCIATED WITH TRANSCRIPTIONAL DYSREGULATION OF THE CORTICOSTEROID METABOLISING ENZYME 11βHSD-1 LEADING TO EXCESS INTRAHEPATIC STEROID EXPOSURE
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#1133
NORMAL ALT IN NAFLD SHOULD NOT PRECLUDE LIVER BIOPSY
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#1134
ULTRASTRUCTURAL EVALUATION OF HEPATOCYTES WITH TYPE 2 MALLORY-DENK BODIES: CLUES TO BALLOONING IN NONALCOHOLIC STEATOHEPATITIS (NASH)
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RELATIONSHIP OF BMI AND THE METABOLIC SYNDROME IN NON-DIABETIC PATIENTS WITH NAFLD
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#1136
FATIGUE IN NAFLD IS SEVERE AND ASSOCIATES WITH EXCESSIVE DAYTIME SOMNOLENCE BUT NOT WITH LIVER DISEASE SEVERITY OR INSULIN RESISTANCE
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#1137
INFLUENCE OF ADIPONECTIN GENE POLYMORPHISMS IN PATIENTS WITH NONALCOHOLIC FATTY LIVER DISEASE
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#1138
HISTOLOGICAL CHARACTERISTICS OF PATIENTS WITH 'CRYPTOGENIC' CIRRHOSIS AND PRIOR BIOPSY SHOWING NASH
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#1140
ETHNIC AND GENDER DIFFERENCES IN LIVER HISTOLOGY IN OBESITY SURGERY PATIENTS
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#1141
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John Kaskinas1, Maria J. Schina1,3, Emanuel K. Manesis1, Dina G. Tiniakos2, Emilia Hadziyannis1, Savvas Savvas1, Basilios Karanam1, Athanasios Archimandritis1
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#1142
IMPACT OF NEW UPPER LIMITS OF NORMAL FOR ALANINE AMINOTRANSFERASE IN THE US POPULATION
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#1143
NON-ALCOHOLIC FATTY LIVER DISEASE IN CHINESE DOES LEAD TO PROGRESSIVE DISEASE
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NON-ALCOHOLIC FATTY LIVER DISEASE IN NON-OBESE AMERICAN CHEMICAL WORKERS
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#1145
A NEW APPROACH IN THE TREATMENT OF PATIENTS WITH NASH—RESULTS OF A PILOT STUDY
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#1146
A NASH PREDICTIVE INDEX (NPI) FOR USE IN PATIENTS WITH NONALCOHOLIC FATTY LIVER DISEASE
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#1147
PHOSPHOPROTEOMIC BIOMARKERS PREDICTING WEIGHT LOSS AFTER BARIATRIC SURGERY IN PATIENTS WITH NON-ALCOHOLIC FATTY LIVER DISEASE (NAFLD)
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#1148
NUTRITIONAL ASSESSMENT OF PATIENTS WITH NON-ALCOHOLIC FATTY LIVER DISEASE (NAFLD) AND CHRONIC VIRAL HEPATITIS
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#1149
DIRECT COMPARISON OF TWO NON INVASIVE BIOMARKERS FOR THE DIAGNOSIS OF ADVANCED FIBROSIS IN PATIENTS WITH NON ALCOHOLIC FATTY LIVER DISEASE (NAFLD) : NAFLD SCORE AND FIBROTEST
Mona Munteanu1, Frederic Charlotte1, Vlad Ratziu1, Sophie Jacqueminet1, Djamilia Messous1, Philippe Podevin3, Laurence Sarfay1, Eric Bruckert1, Andre Grimal1, Thierry Poyard1
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#1151
SEVERITY OF NON-ALCOHOLIC FATTY LIVER DISEASE IS RELATED TO MTHFR 677C→T HOMOZYGOSITY, LOWER GLUTATHIONE AND INSULIN RESISTANCE
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#1152
UPPER LIMIT OF NORMAL FOR ALANINE AMINOTRANSFERASE LEVEL IN KOREAN POPULATION: EFFECT OF BMI
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#1153
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#1154
ANGIOTENSIN II CAUSES PROGRESSIVE NONALCOHOLIC FATTY LIVER DISEASE MEDIATED BY OXIDATIVE STRESS IN THE REN-2 RAT MODEL
Yongzhong Wei1, Suzanne Clark1, E. M. Morris1, John Thyfault1, Grace Uptegrove1, Adam Whaley-Connel1, Carlos M. Ferriano2, James R. Sowers1, Jamil A. Ibdah1
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#1155
MONOCYTE CHEMOTACTIC PROTEIN-1 SECRETED BY ADIPOSE TISSUE INDUCES LIPID ACCUMULATION IN HEPATOCYTES
Sophie Clément, Cristiana Juge-Aubry, Stéphanie Conzelmann, Valeria Pazienza, Brigitte Pittet-Cuenod, Christoph Meier, Francesco Negro
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#1156
THE ZEBRAFISH FOIE GRAS MUTANT IS A MODEL OF FATTY LIVER DISEASE WITH ACTIVATED UNFOLDED PROTEIN RESPONSE
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#1157
C-REACTIVE PROTEIN INDUCES INSULIN RESISTANCE IN BOTH HUMAN HEPATOCYTES AND ADIPOCYTES
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#1158
MB07811, A HEPDIRECT PRODRUG OF A LIVER-TARGETED THYROID HORMONE RECEPTOR (TR) AGONIST, AND OTHER SYNTHETIC NON-LIVER-TARGETED TR AGONISTS, BUT NOT T3, REDUCE HEPATIC STEATOSIS
Edward E. Cable, Xiaohong Yang, Patricia D. Finn, Jian Li, Jeffery W. Stebbins, Michael P. Haughey, Jinzhao Hou, Bruce R. Ito, Paul D. van Poelje, David L. Linemeyer, Mark D. Erion
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#1159
HOMOCYSTEINE SUPPLEMENTATION ATTENUATES, RATHER THAN INCREASES, THE UNFOLDED PROTEIN RESPONSE IN A MURINE NUTRITIONAL MODEL OF STEATOHEPATITIS
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#1160
GENETIC EVIDENCE THAT LIPID TRAFFICKING PLAYS A KEY ROLE IN FIBROSIS IN NON ALCOHOLIC FATTY LIVER DISEASE
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#1161
ALTERATIONS IN LYMPHOCYTE LIPID RAFT COMPOSITION AND STRUCTURE VIA BETAGLYCOLIPIDS IS ASSOCIATED WITH AMELIORATION OF NON ALCOHOLIC STEATOHEPATITIS: A NOVEL THERAPEUTIC TARGET FOR NASH
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#1162
HIGH FAT DIET CHANGES EXPRESSION OF IRON RELATED MOLECULES IN MICE LIVER AND DUODENUM
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#1163
THE CAR AGONIST, TCPOBOP, ATTENUATES STEATOHEPATITIS IN THE MCD-FED MOUSE
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#1164
ROLE OF VISCERAL FAT REMOVAL IN THE PREVENTION OF HEPATIC INSULIN RESISTANCE AND TRIGLYCERIDE ACCUMULATION IN RESPONSE TO HIGH FAT DIET IN VIVO
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#1165
RESOLUTION OF HEPATIC STEATOSIS DESPITE PERSISTENT OBESITY IN MICE FED STANDARD RODENT CHOW AFTER INDUCTION OF TYPE 2 NAFLD USING THE AMERICAN DIET INDUCED OBESITY SYNDROME MODEL
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#1166
THE DEVELOPMENT OF NEW DRUG SCREENING SYSTEM USING STEATOHEPATITIS MEDAKA FISH MODEL INDUCED BY HIGH-FAT DIET
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#1167
HYPOXIA WORSENS NON-ALCOHOLIC STEATOHEPATITIS
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#1168
DISTINCT REQUIREMENT OF APOB-100 FOR INCREASED SECRETION OF VLDL-TRIGLYCERIDE IN RESPONSE TO HEPATIC OVEREXPRESSION OF MTP IN OB/OB MICE
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#1169
5-LIPOXYGENASE INHIBITION ABROGATES HEPATIC STEATOSIS BY REDUCING LIVER FATTY ACID-BINDING PROTEIN EXPRESSION AND INCREASING HEPATOCYTE MICROSONAL TRIGLYCERIDE TRANSFER PROTEIN ACTIVITY
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#1170

DAILY EXERCISE INCREASES HEPATIC FATTY ACID OXIDATION AND PREVENTS STEATOSIS IN OTSUCA LONG-EVANS TOKUSHIMA FATTY (OLETF) RATS

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#1176

STUDY OF THE MITOCHONDRIAL COMPLEX I BY BLUE NATIVE ELECTROPHORESIS IN LEAN AND OB/OB MICE

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#1171

PHYLLANTHUS URINARIA AMELIORATES THE SEVERITY OF NUTRITIONAL STEATOHEPATITIS

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OXIDATIVE STRESS-RELATED DAMAGE IS IMPLICATED IN LPS-INDUCED ACTIVATION OF HEPATIC STELLATE CELLS

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#1173

PIOGLITAZONE RESTORES REGENERATION FAILURE FOLLOWING PARTIAL HEPATECTOMY IN OBSE & DIABETIC KK-AY MICE

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#1174

MECHANISM OF SERUM ALT INCREASE IN EXPERIMENTAL NASH

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#1175

THE MITOCHONDRIAL SUPEROXIDE DISMUTASE 2 (SOD2) TARGETING SEQUENCE POLYMORPHISM IS ASSOCIATED WITH FIBROTIC NAFLD: CONSISTENT EVIDENCE FROM CASE-CONTROL AND INTRA-FAMILIAL ALLELIC ASSOCIATION STUDIES

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TRB3 IS ASSOCIATED WITH HEPATIC LIPGENESIS AND DYSLIPIDEMIA IN NAFLD

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#1178

HEPATIC ADIPOR2 SIGNALING REGULATES THE DEVELOPMENT OF NONALCOHOLIC STEATOHEPATITIS IN MICE

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PREVENTION OF HEPATIC FIBROSIS IN A MURINE MODEL OF METABOLIC SYNDROME WITH NON-ALCOHOLIC STEATOHEPATITIS (NASH)

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#1180

HEPATOCELLULAR SPECIFIC GP130 PROTECTS AGAINST THE DEVELOPMENT AND PROGRESSION OF STEATOHEPATITIS

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#1181
NOVEL THERAPEUTIC APPROACH FOR NAFLD USING ANTIPLATELET AGENTS IN AN ANIMAL MODEL
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#1182
ENDOCANNABINOID PRODUCTION BY MONOCYTES IS INCREASED IN NONALCOHOLIC STEATOHEPATITIS
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#1183
THE TRADITIONAL JAPANESE KAMPO FORMULA KEISHIBUKURYOGAN AMELIORATES STEATOSIS, REDUCES OXIDATIVE STRESS AND INFLAMMATION, AND ULTIMATELY PREVENTS LIVER FIBROSIS IN A RABBIT MODEL OF NON ALCOHOLIC STEATOHEPATITIS
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#1184
PALMITATE-INDUCED LIPID ACCUMULATION IN HEPATOCYTES LEADS TO ACTIVATION AND PRO-FIBROTIC GENE EXPRESSION IN HEPATIC STELLATE CELLS – A NEW IN VITRO MODEL TO STUDY FIBROGENESIS IN NASH
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#1185
DIETARY CONSTITUENTS IN MICE ARE REFLECTED IN THE HEPATIC LIPID PROFILE AS DETECTED BY CARBON-13 MAGIC ANGLE SPINNING MAGNETIC RESONANCE SPECTROSCOPY
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#1186
ADENOVIRUS-MEDIATED TRANSFER AND INDUCTION OF HEPATIC HEME OXYGENASE-1 IN THE LIVER PROTECTS AGAINST STEATOHEPATITIS IN VITRO AND IN VIVO
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#1187
DELAYED LIVER REGENERATION AND IMPAIRED FATTY ACID METABOLISM IN ADIPOPOETIN KNOCKOUT MICE AFTER PARTIAL HEPATECTOMY
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#1188
GREEN TEA POLYPHENOLS IMPROVE LIVER INJURY OF NONALCOHOLIC STEATOHEPATITIS (NASH) MODEL MICE EXPRESSING NUCLEAR STEROL REGULATORY ELEMENT-BINDING PROTEIN 1C (NSREBP-1C) IN ADIPOSE TISSUE
Takato Ueno1,2, Toru Nakamura2,1, Ryuichiro Sakata2,1, Osamu Hashimoto2,1, Kinya Inoue2,1, Hiromi Koga2,1, Takui Torigura2,1, Masako Shinkawa1, Hitomi Nakayama2, Syuichi Otabe2, Naotoshi Hitori3, Kentaro Yamada2, Michio Sata2,1
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#1189
TRANS-FATTY ACID POTENTIATES ACCUMULATION OF TRIGLYCERIDE AND SUSCEPTIBILITY TO OXIDATIVE STRESS IN HEPATOCYTES
Kazuyoshi Kon, Kenichi Ikejima, Tomonori Aoyama, Kyoko Okumura, Kumiko Arai, Sumio Watanabe Gastroenterology, Juntendo University, Tokyo, Japan
#1190
LOSS OF β-CATENIN EXACERBATES MEHIONINE-CHOLINE-DEFICIENT DIET INDUCED STEATOHEPATITIS IN MICE
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#1191
DIETARY ZINC SUPPLEMENTATION REVERSES ALCOHOLIC FATTY LIVER IN THE PRESENCE OF CHRONIC ALCOHOLIC ADMINISTRATION
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Cell and Molecular Biology

#1192
CAVEOLIN-1 UPREGULATES ENDOTHELIAL CAPILLARY-LIKE TUBULAR FORMATION AND FENESTRAL CONTRACTION IN ASSOCIATION WITH RHO GTPASES IN SINUSOIDAL ENDOTHELIAL CELLS

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#1193
ROLE OF THE N-TERMINAL SEQUENCE OF BSEP (ABCB11) IN PROTEIN TRAFFICKING AND STABILITY

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#1194
LIVER CELL VOLUME REGULATION AND ATP RELEASE REQUIRE INTACT VESICULAR TRAFFICKING PATHWAYS

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#1195
THE SORTING OF ENDOCYTIC PROTEIN ALONG ACTIN AND MICROTUBULES IN THE HEPATOCYTE

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#1196
PROTEOMIC AND IMMUNOFLOUORESCENCE ANALYSIS OF EARLY AND LATE ENDOCYTIC VESICLES ISOLATED FROM RAT HEPATOCYTES

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#1197
THE ADRENOLEUKODYSTROPHY PROTEIN (ALDP) AND THE 70-KDA PEROXISOMAL MEMBRANE PROTEIN (PMP70) ARE DIFFERENTIALLY ASSOCIATED WITH LIPID MICRODOMAINS.

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#1198
EMODIN IMPAIRS THE REARRANGEMENT OF ADHESION MOLECULES INDUCED BY HCV

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#1199
EFFECTS OF HINT2, A PUTATIVE TUMOR SUPPRESSOR, ON MITOCHONDRIAL FUNCTIONS

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#1200
HEPATITIS C VIRUS INDUCES IFN-A IN PLASMACYTOID DENDRITIC CELLS AND AFFECTS DIFFERENTIALLY STIMULATION VIA TOLL-LIKE RECEPTORS 7 AND 9

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#1201
HEPATIC STEATOSIS AND GLOMERULAR DISEASE IN MICE WITH C-TERMINALLY TRUNCATED GP73 (GOLPH2)
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#1202
PRIMARY HUMAN HEPATOCYTES UNDERGO DNA SYNTHESIS IN RESPONSE TO ENGINEERED FORMS OF HEPATOCYTE GROWTH FACTOR (HGF/SF): A PRE-REQUISITE TO USING HGF ANALOGUES CLINICALLY
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#1203
THE ROLES OF LYSOSOMAL PROTEINASES IN PRIMARY HEPATOCYTE DEATH INDUCED BY TNF-α AND ACTINOMYCIN D
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#1204
NATURAL POLYMORPHISM OF NS3 PROTEASE DOMAIN STRAINS HCV-1 IN HCV AND HIV-HCV COINFECTED PATIENTS: VIROLOGICAL AND CLINICAL IMPLICATION FOR DRUG RESISTANT VIRUSES
Philippe Halffon1, Hawwa Dia1, Philippe Benech2, Patrick Philipbert3, Agnès Martineau1, Guillaume Pénaranda4, Jérôme Courcambeck2, Mourad Bouzidi2, Hacène Khiri1
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#1205
MICROENVIRONMENTAL REGULATION OF SINUSOIDAL ENDOTHELIAL CELL PHENOTYPE
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#1206
DEVELOPMENT AND CHARACTERIZATION OF MICROSCALE MODELS OF RAT AND HUMAN LIVERS
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#1207
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#1208
OVEREXPRESSION OF CYP2E1 IN LIVER INCREASES INSULIN RESISTANCE AND INHIBITS INSULIN SIGNALING IN AN ANIMAL MODEL OF NASH
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#1209
POST-TRANSLATIONAL MODIFICATION OF HEPATIC SCAVENGER RECEPTOR CLASS B, TYPE I AND CHYLOMICRON METABOLISM IN A MOUSE MODEL
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#1210
WNT11 INHIBITS THE CANONICAL WNT PATHWAY THROUGH THE PKC-MEDIATED β-CATENIN PHOSPHORYLATION IN HUMAN HEPATOMA CELLS
Takashi Toyama, Han Chu Lee, Jack R. Wands, Miran Kim
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#1211
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Orlando Musso1, Ismail Hendauoui1, Elise Lavergne1, Harri Elamaa2, Nathalie Theret1, Delphine Quelard1, Bruno Turlin1, Taina Pihlajaaniemi2, Bruno Clement1
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#1212
INACTIVATION OF CASPASE-8 AFFECTS HEPATOCYTE PROLIFERATION AFTER PARTIAL HEPATECTOMY IN MICE
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#1213
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#1214
PTEN ASSOCIATION WITH P13Kα SUBUNIT IS A NEGATIVE REGULATORY MECHANISM FOR INSULIN SIGNALING IN THE LIVER
Jiman He, Suzanne de la Monte, Jack R. Wands
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#1215
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#1216
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#1217
CONDITIONAL ABLATION OF C-FLIP RENDERS HEPATOCYTE MORE SENSITIVE TOWARDS CD95 MEDIATED APOPTOSIS IN VIVO
Marcus Schuchmann1, Daniel Sammet1, Jörn M. Schattenberg1, Jürgen Siebler1, You-Wen He2, Peter R. Galle1
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#1218
MICRONRNA-370 REGULATES INTERLEUKIN-6 DEPENDENT P38 MAP KINASE SIGNALING IN HUMAN CHOLANGIOCARCINOMA
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#1219
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#1220
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Hironori Koga1, Michiko Maeyama1, Sivakumar Ramadoss1, Selvendran Karuppiah2, Takaumi Yoshida1, Osamu Hashimoto1, Mitsuhiko Abe1, Chikatomi Yanagimoto1, Hiroti Kummur1, Shinichiro Hanada1, Eitaro Taniguchi1, Takumi Kawaguchi1, Masaru Harada1, Takuji Torimura1, Hirohisa Yano3, Takato Ueno4, Michio Sata1
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#1221
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#1222
HEPATIC STAT3 ATTENUATES SYSTEMIC INFLAMMATORY RESPONSE AND LETHALITY IN SEPTIC MICE
Ryoitaro Sakamori, Tetsuo Takehara, Hayato Hikita, Akira Sasakawa, Keisuke Kohga, Akio Uemura, Shinjiro Yamaguchi, Tomohide Tatsuki, Kazuyoshi Ohkawa, Norio Hayashi
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#1223
**DISRUPTED CELL ADHESION BUT NOT PROLIFERATION OR ANTI-APOPTOSIS MEDIATES POLYCYSTIC LIVER DISEASE**

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#1224
**GP130-STAT3-DEPENDENT SOCS3 INDUCTION CONTROLS TIMING OF HEPATOCYTE PROLIFERATION DURING LIVER REGENERATION**

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#1225
**LIVER TETRAPLOIDIZATION IS TRIGGERED BY WEANING UNDER AN INSULIN SIGNAL**

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#1226
**MOLECULAR MECHANISMS OF ANTI-APOPTOTIC AND ANTI-OXIDANT EFFECTS OF GRAPE AND ITS COMPOUNDS**

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#1227
**EXPRESSION AND LOCALIZATION OF ATYPICAL PKC ISOFORMS IN LIVER PARENCHYMAL CELLS**

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#1228
**ESTROGEN RECEPTORS ACTIVATE THE EXPRESSION OF THE HUMAN ENTEROHEPATIC TRANSPORTER GENES SLC10A2 AND SLC01B1 BY ENHANCING THE DNA-BINDING OF HNF-1α**

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#1229
**S6K1 INHIBITION BY A NOVEL CLASS OF DITHIOLETHIONES ABROGATES INSULIN RESISTANCE INDUCED BY HYPEROSMOTIC STRESS**

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#1230
**ADAM17 PROMOTES ERK-MEDIATED PHOSPHORYLATION OF SER112 IN BAD**

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#1231
**OVEREXPRESSION OF THE LIVER TRANSCRIPTION FACTOR HNF6 ACCELERATES HEPATOCYTE PROLIFERATION DURING MOUSE LIVER REGENERATION**

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#1232
**THE HUMAN SMALL HETERODIMER PARTNER (SHP) PROMOTER CONTAINS INDEPENDENT AND UNCONVENTIONAL 9-CIS RETINOIC ACID- AND BILE SALT-RESPONSIVE ELEMENTS**

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#1233
**ROLE OF FARNESOID X RECEPTOR IN REGULATING HEPATIC LIPID HOMEOSTASIS**

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#1234
**HOMOCYSTEINE-INDUCED INHIBITORY EFFECTS ON ADIPONECTIN PRODUCTION IN ALCOHOLIC LIVER DISEASE**

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#1235
PROTEIN KINASE C REGULATES H2O2-INDUCED HEPATOCYTES NECROSIS BY SUPPRESSION OF PROTECTIVE SIGNALING VIA AMP ACTIVATED KINASE AND AKT
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#1236
ALTERING TRANSPLANTED CELL ENGRAFTMENT AND PROLIFERATION IN THE LIVER THROUGH PARACRINE SIGNALING WITH COTRANSPLANTATION OF LIVER SINUSOIDAL ENDOTHELIAL CELLS (LSEC) AND HEPATOCYTES IN MICE
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#1237
USEFULNESS OF ONCOSTATIN M GENE THERAPY ON LIVER DAMAGE INDUCED BY DIMETHYLNITROSAMINE IN RATS
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#1238
SEROTONIN 2B RECEPTOR SIGNALING: A NOVEL NEGATIVE REGULATOR OF LIVER GROWTH
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#1239
THE SODIUM-COUPLED NEUTRAL AMINO ACID TRANSPORTER (SNAT) 4 IS EXPRESSED IN DEVELOPING MOUSE LIVER AND REGULATED BY HEPATOCYTE NUCLEAR FACTOR (HNF) 4ɑ
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#1240
MONOCYTE CHEMOATTRACTANT PROTEIN-1 ENHANCED ANTITUMOR EFFECTS OF SUICIDE GENE THERAPY AGAINST HEPATOCELLULAR CARCINOMA VIA ACTIVATING TH1-POLARIZED IMMUNE RESPONSES
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#1241
DIFFERENTIAL FUNCTIONS OF CYCLIN E1 AND E2 FOR CELL CYCLE CONTROL AND ENDOREPLICATION DURING LIVER REGENERATION IN MICE
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#1242
S-ADENOSYLMETHIONINE AND METHYLTHIOADENOSINE INHIBIT LIPOPOLYSACCHARIDE-INDUCED TUMOR NECROSIS FACTOR α EXPRESSION VIA MODULATION OF HISTONE METHYLATION
Ainhoa Iglesias Ara, Meng Xia, Komal Ramani, Shelly C. Lu Medicine, Keck School of Medicine USC, Los Angeles, CA, USA

#1243
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Christina Baehr1, Jörg Haupenthal2, Otto Kollmar3, Bernd Kronenberg1, Simone Kiernymayer2, Thomas Pieli2, Oliver Waidmänn3, Stefan Zeuzem1, Albrecht Piiper2
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#1244
GENE EXPRESSION BASED RECURRENT PREDICTION OF HBV-RELATED HUMAN HEPATOCELULAR CARCINOMA
Yoon Jun Kim1, Hyun Goo Woo2, Yong Jin Jung1, Dong Hee Kim1, Bum Joon Park1, Nam-Joon Yi2, Kyung-Suk Suh1, Kuhn Ulke2, Jung-Hwan Yoon1, Snorri S. Thorgeirsson4, Hyo-Suk Lee1
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#1245
MITOCHONDRIAL CHOLESTEROL IN HEPATOMA CELLS MODULATES APOPTOSIS SUSCEPTIBILITY AND CANCER THERAPY IN TUMOR XENOGRAFTS
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#1246
HEPATOCYTE GROWTH FACTOR ATTENUATED MOUSE LIVER FIBROSIS BY ADENO-ASSOCIATED VIRUS VECTOR
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#1247
SUBCUTANEOUS VACCINATION WITH DENDRITIC CELLS ENGINEEREED TO EXPRESS MAFP AND IL-12 INDUCES INHIBITION OF AFP-EXPRESSING SUBCUTANEOUS HEPATOCELLULAR TUMOR GROWTH IN MICE
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#1248
TRANSFORMING GROWTH FACTOR-β GENE EXPRESSION SIGNATURE PREDICTS CLINICAL OUTCOME IN CANCER
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#1249
A UNIQUE METASTASIS-RELATED MICRORNA EXPRESSION SIGNATURE IS A PROGNOSTIC INDICATOR OF SURVIVAL AND RECURRENT IN HEPATOCELLULAR CARCINOMA
Anuradha Budhu1, Hu-Liang Jia1,2, Marshonna Forguers1, Chang-Gong Liu1, David Goldstein3, Amy Lam4, Krista Zanetti1, Qing-Hai Ye2, Lun-Xiu Qin2, Carlo Croce1, Zhao-You Tang2, Xin Wang1
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#1250
HUGL-2, THE HUMAN HOMOLOGUE OF THE DROSOPHILA TUMOR SUPPRESSOR LGL, IS REGULATED BY EGF AND SNAIL, A PRIME REGULATOR OF EPITHELIAL-MESENCHYMAL TRANSITION (EMT)
Tim Zimmerman1, Anubha Kashyap1, Urs Hartmann1, Gerd Otto2, Peter R. Galle1, Susanne Strand1, Dennis Strand1
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#1251
ENHANCED RETINOIC ACID-INDUCED APOPTOSIS IN HYPOXIC HEPATOCELLULAR CARCINOMA CELLS VIA ER STRESS-RELATED JNK ACTIVATION IS ATTENUATED BY HYPOXIA INDUCTION OF CRABP-II
Jung-Hwan Yoon1, Jeong-Hoon Lee1, Goh Eun Chung1, Bo Hyun Kim1, Sun Jung Myung1, Jong In Yang1, Won Kim1, Yoon Jun Kim1, Kyung-Suk Suh2, Hyo-Suk Lee3
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#1252
INVESTIGATION OF THE ROLE OF GLYPCAN-3 IN RAT HEPATOCYTE GROWTH AND LIVER REGENERATION
Bowen Liu, William C. Bowen, Aaron W. Bell, Shirish Paranjpe, Wendy M. Mars, Kari N. Nejak-Bowen, Jianhua Luo, George Michalopoulos
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#1253
THE ROLE OF NF-κB ACTIVITY IN THE ANTIVIRAL ACTION OF INTERFERON
Lawrence Pfeffer
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#1254
GANGLIOSIDE GD3 SYNTHASE OVEREXPRESSION SENSITIZES HUMAN HEPATOMA CELLS TO HYPOXIA BY SUPPRESSING THE NUCLEAR FACTOR-κB-DEPENDENT SURVIVAL PATHWAY
Josep M. Lluis1, Albert Morales1, Scott Welford2, Amato Giaccia2, Jose C. Fernandez-Checa1
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#1255
TRANSLATIONAL CONTROL OF GENE EXPRESSION DURING LIVER DIFFERENTIATION
Desiree M. Espiet, Richard Stockert
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#1256
**IN VIVO SUPPRESSION OF MAFA MRNA WITH SIRNA AND ALTERATION OF THE GENE EXPRESSION PROFILE, ESPECIALLY OF ADIPOCYTOKINE GENES, IN MOUSE LIVER ANALYZED BY THE MICROARRAY METHOD**

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#1257
**AUGMENTER OF LIVER REGENERATION (ALR): THE FACTOR THAT REGULATES HEPATOCYTE APOPTOSIS IN LIVER REGENERATION AFTER PARTIAL HEPATECTOMY**

Lorenzo Polimeno, Barbara Pesetti, Emanuele Annoscia, Marcella Margiotta, Floriana Giorgio, Thomas Lisowsky, Annacinzia Amoruso, Antonio Francavilla  
DETO, Università degli Studi di Bari, Bari, Italy

#1258
**COBALT PROTOPORPHYRIN BINDS TO BACH1, A TRANSCRIPTIONAL REPRESSOR OF HEME OXYGENASE 1 GENE**

Weihong Hou¹, Ying Shan¹, Richard W. Lambrecht², Jianyu Zheng¹, Susan E. Donohue¹, Herbert L. Bonkovsky²  
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#1259
**POSSIBLE ROLE OF TYROSINE KINASE INHIBITOR AND PROTEASOME INHIBITOR FOR HCC TREATMENT**

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#1260
**GENE EXPRESSION PROFILE OF PERIPHERAL BLOOD MONONUCLEAR CELLS MAY REFLECT THOSE OF TUMOR-INFILTRATING LYMPHOCYTES IN HEPATOCELLULAR CARCINOMA**

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#1261
**HUMAN GANKYRIN OVEREXPRESSION DOWNREGULATES P53 EXPRESSION AND PROMOTES CELL PROLIFERATION IN ZEBRAFISH**

Seung Kew Yoon, So Yeon Kim, Wonhee Hur, Jung-Eun Choi, Daniel Kim, Lian-Shu Piao, Chan Ran You, Hyun Young Woo, Soung Won Jeong, Si Hyun Bae, Jong Young Choi  
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#1262
**IN VITRO VALIDATION THAT PHARMACOLOGIC INHIBITION OF FRIZZLED RECEPTORS CAN EXERT ANTI-ONCOGENIC PROPERTIES IN HUMAN CANCEROUS HEPATOCYTES**

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#1263
**HYPOXIA INDUCED GENE EXPRESSION SIGNATURE IN NORMAL HEPATOCYTES AND HUMAN HEPATOCELLULAR CARCINOMA**

Salvador Naranjo-Suarez, Valentina M. Factor, Cedric Coulouarn, Snorri Thorgrimsson, NCI, NIH, Bethesda, MD, USA

#1264
**CHROMATIN-BASED ANALYSIS OF THE IFNα TRANSCRIPTOME REVEALS FUNCTIONALLY DISTINCT SUBSETS OF STAT2 DIRECT TARGET GENES**

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#1265
**COMPREHENSIVE GENE EXPRESSION ANALYSIS IN HEPATOCELLULAR CARCINOMA (HCC) BY NEWLY DEVELOPED 5'-END SAGE METHOD AND IDENTIFICATION OF NOVEL UP-REGULATED INTRONIC TRANSCRIPT**

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#1266
**HYDRODYNAMICS-BASED INJECTION OF A SHORT HAIRPIN RNA-PRODUCING VECTOR TARGETING C-JUN IN RAT LIVERS**

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#1267
CLINICOPATHOLOGICAL SIGNIFICANCE OF EZH2 AND BMI1 EXPRESSION IN PATIENTS WITH HEPATOCELLULAR CARCINOMA

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#1268
RNA INTERFERENCE MEDIATED SILENCING OF EGFR IN REGENERATING RAT LIVER FOLLOWING A PARTIAL HEPATECTOMY

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#1269
RNAI THERAPEUTICS FOR THE TREATMENT OF LIVER DISEASES

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#1270
INHIBITION OF TNF ALPHA STIMULATION BY PRETREATING HUMAN HEPATOCYTES WITH STAUROSPORINE AND LY294002 ON INTRINSIC APOPTOSIS PATHWAYS

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#1271
MODULATION OF STEMNESS IN DYSFUNCTIONAL PROGENITOR/STEM CELLS ALTERS CANCER FORMATION IN THE LIVER

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#1272
BETA-CATENIN IS INDISPENSABLE FOR LIVER DEVELOPMENT AND SURVIVAL

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#1273
HUMAN TGF-β-REGULATED LIVER STEM/PROGENITOR CELLS IN NORMAL POST TRANSPLANT LIVING DONOR RECIPIENTS ARE ALTERED IN HEPATOCELLULAR CANCERS

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#1274
EXPRESSION OF NEIGHBOR OF PUNC E11 (NOPE) DURING MURINE LIVER DEVELOPMENT

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#1275
SELECTIVE PROLIFERATION OF HUMAN HEPATOCYTE PROGENITOR CELLS IN COMBINATION WITH HYALURONIC ACID AND SERUM-FREE MEDIUM

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#1276
REGULATION OF HEPATIC LINEAGE ADVANCEMENT IN HESC THROUGH A MESODERMAL INTERMEDIATE RECAPITULATES FETAL STAGE-SPECIFIC PROCESSES

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#1277
ANALYSIS OF DIFFERENTIATION OF HEPATIC PROGENITOR CELLS DERIVED FROM SEVERELY INJURED RAT LIVERS

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#1278
INDUCTION OF PANCREATIC BETA-CELL PHENOTYPE IN HEPATIC PROGENITOR CELLS (HPC) ISOLATED FROM ADULT DIABETIC MICE
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#1279
DIFFERENTIATION OF RAT MULTIPOTENT ADULT PROGENITOR CELLS TOWARDS THE HEPATIC LINEAGE BY RECAPITULATING LIVER EMBRYOLOGY
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Drug Metabolism and Toxicity

#1280
DIFFERENTIAL ACETAMINOPHEN-INDUCED HEPATOTOXICITY BETWEEN TWO TYPES OF DIETARY STEATOSIS OF THE LIVER IN MICE
Yuqing Wang1, Makota Oketani1, Masahisa Horiiuchi2, Yasushi Imamura3, Akihiro Morichi1, Susumu Hasegawa1, Hirofumi Uto1, Akio Ido1, Hirohito Tsubouchi1
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#1281
REPEATED EXPOSURE TO ACETAMINOPHEN PROTECTS AGAINST A SUBSEQUENT LETHAL DOSE THROUGH SELECTIVE DEPLETION OF CYTOSOLIC GLUTATHIONE
Marjorie Bon Homme, Sreelatha Channareddy, William W. Tucker, Roland Valdes, Mark W. Linder
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#1282
MALLORY BODY FORMATION IS AN EPGENETIC PHENOMENON WHICH IS PREVENTED BY TREATMENT WITH SAME
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#1283
THE SPECTRUM OF LIVER DISEASE IN HIV-INFECTED INDIVIDUALS IN EAST AFRICA
Ponsiano Ocama, Jordan J. Feld, Michael Katwater, Theresa Piloya, Kenneth Opio, Andrew Kambugu, Elly Katabira, David L. Thomas, Robert Colebunders, Allan Ronald
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#1284
QUANTITATIVE PROTEOMIC ANALYSIS OF THE ETHANOL RESPONSE IN HEPATOCYTES USING THE ISOTOPE CODED AFFINITY TAG METHOD AND A SYSTEMS BIOLOGY APPROACH
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#1285
B-LYMPHOCYTES AS AN IN-VITRO MODEL TO STUDY INTERINDIVIDUAL VARIATION IN SENSITIVITY TO ACETAMINOPHEN TOXICITY
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#1286
ALCOHOL INDUCES GLOBAL HEPATIC PROTEIN HYPERACETYLACTION
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#1287
USEFULNESS OF FIBROSCAN FOR THE FOLLOW UP OF PATIENTS TREATED WITH METHOTREXATE
David Laharie1, Edouard Chabrun1, Thierry Schoeverbeke2, Thomas Hubiche3, Marie-Sylvie Doutre4, Maiet Longy5, Jean-Luc Pellegrin6, Juliette Fouche1, Laurent Castella1, Florence Salaun1, Sandrine Villars1, Frank Zerbib7, Victor de Ledinghen1,8
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#1288
RELATIONSHIP BETWEEN MEDICATION DOSE AND IDIOSYNCRATIC DRUG INDUCED LIVER INJURY (DILI)
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THE CHOICE OF CLINICAL LABORATORY MAY CHANGE STUDY OUTCOME: AN INTER-LABORATORY EVALUATION OF AMINOTRANSFERASE MEASURES
Jody L. Green, Elizabeth Campagna, Gregory M. Bogdan, Kennon Heard, Richard C. Dart
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INHIBITION OF ATM ACTIVITY AMELIORATES THE ETHANOL METABOLISM-MEDIATED CELL CYCLE ARREST
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GENETIC POLYMORPHISMS OF IL-10, IL-4 AND TNF α. AND SUSCEPTIBILITY TO DEVELOP DRUG-INDUCED IDIOSYNCRATIC LIVER INJURY (DILI)
Ketevan Pachkoria2, Esperanza Crespo5,6, Francisco Ruiz-Cabello6,5, Yolanda Borraz2, Alfonso Serrano4, Gloria Pelaez3, M. C. Fernández3, Manuel Romero-Gomez7, Ana Madrazo7, Jose Maria Navarro9, Joan Costa10, Nuria Lopez10, Maribel Lucena9, Raúl J. Andrade2
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GILBERT’S SYNDROME ASSOCIATED VARIABILITY: COMBINATION OF UDP GLUCURONOSYLTRANSFerase (UGT) 1A1 AND UGT1A7 GENE POLYMORPHISMS INCREASE THE RISK OF IRINOTECAN TOXICITY
Tim O. Lankisch1, Christoph Schulz2, Thomas Zwingers2, Thomas J. Erichsen1, Michael P. Manns1, Volker Heinemann3, Christian P. Strassburg1
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ACETAMINOPHEN BIOTRANSFORMATION AT SINGLE AND REPEAT MAXIMUM DOSES IS SIMILAR BETWEEN ADULTS WITH AND WITHOUT CHRONIC LIVER DISEASE
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METABOLOMIC ANALYSIS OF URINE AND LIVER AFTER CHRONIC ALCOHOL TREATMENT IN C57BL/6J MICE
Blair U. Bradford1, Jun Han1, Oksana Kosyk1, Svitlana Shymonyak1, Jason Winnike2, Hiroshi Kono3, Thomas M. O’Connell2, Ivan Rusyn1
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A PROSPECTIVE STUDY OF THE RELIABILITY OF THE RUCAM FOR ASSESSING CAUSALITY IN DRUG-INDUCED LIVER INJURY
James Rochon1, Leonard B. Seeff2, Robert J. Fontana4, Suthat Liangpunsakul5, Paul H. Hayashi3, Timothy J. Davern5, John G. McHutchison1
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DETECTION OF ACETAMINOPHEN PROTEIN ADDUCTS IN SERUM DURING THERAPEUTIC DOSING OF ACETAMINOPHEN IN HEALTHY VOLUNTEERS
Laura James1,5, Pippa Simpson1,5, Susan Rahman2, Mark Russo4, Paul B. Watkins3
1University of Arkansas for Medical Sciences, Little Rock, AR, USA. 2Children’s Mercy Hospital, Kansas City, MO, USA. 3University of North Carolina, Chapel Hill, NC, USA. 4University of North Carolina, Greensboro, NC, USA. 5Arkansas Children’s Hospital Research Institute, Little Rock, AR, USA

ACETAMINOPHEN-PROTEIN ADDUCTS IN ALCOHOLIC PATIENTS RECEIVING ACETAMINOPHEN 4 G/DAY FOR 5 DAYS
Sean H. Rhyee1, Jody L. Green1, Laura James2, Kennon Heard1, Richard C. Dart1
1Denver Health Rocky Mountain Poison and Drug Center, Denver, CO, USA. 2Arkansas Children’s Hospital Research Institute, Little Rock, AR, USA
Response-Guided Therapy in a Prospective Trial of Peginterferon Alfa-2a (40kd)/Ribavirin Treatment in Patients with Genotypes 1 or 4

Peter Ferenci1, Hermann Lauer2, Thomas-Matthias Scherzer1, Harald Brunner3, Andreas Maierson4, Michael Gschwander5, Rudolf E. Stauber6, Rainer Hubmann7, Katharina Staufner1, Christian Datz8, Martin Bischof9, Harald Hofer1, Karin Löschenberger10, Petra E. Steindl-Mundla1, the Austrian Hepatitis Study Group On behalf of 1
1Department of Internal Medicine IV, Medical University of Vienna, Vienna, Austria. 2Department of Medicine, General Hospital, Graz, Graz, Austria. 3General Hospital, Salzburg, Austria. 4Krankenhaus der Elisabethinen, Linz, Austria. 5Wilhelminenhospital, Vienna, Austria. 6Medical University Graz, Graz, Austria. 7General Hospital, Linz, Austria. 8Krankenhaus Oberndorf, Salzburg, Austria. 9Krankenhaus Rudolfstiftung, Vienna, Austria. 10Roche Austria, Vienna, Austria

Patients Coinfected with HCV and HIV Who Achieve an RVR (HCV RNA <50 IU/ML at Week 4) or CEVR (HCV RNA <50 IU/ML at Week 12) Have Similar Rates of SVR to Monoinfected Patients Treated with Peginterferon Alfa-2a (40kd) (Pegasys®) and Ribavirin (Copegus®)

Maribel Rodriguez-Torres1, Francesca Torriani2, Juergen Rockstroh3, Jean Depamphilis4, Giampiero Carosi5, Douglas T. Dieterich6 1Fundación de Investigación de Diego, Sanurce, PR, USA. 2University of California, San Diego, CA, USA. 3University of Bonn, Bonn, Germany. 4Roche, Nutley, NJ, USA. 5Università Degli Studi di Brescia, Brescia, Italy. 6Mount Sinai School of Medicine, New York, NY, USA
#1304

**HCV-SPECIFIC CELLULAR IMMUNITY, RNA REDUCTIONS, AND NORMALIZATION OF ALT IN CHRONIC HCV SUBJECTS AFTER TREATMENT WITH GI-5005, A YEAST-BASED IMMUNOTHERAPY TARGETING NS3 AND CORE: A RANDOMIZED, DOuble-blind, PLACEBO CONTROLLED PHASE 1B STUDY**

Eugene R. Schiff2, Gregory T. Everson4, Naoky Tsai8, Nathalie H. Bzowej7, Robert G. Gish7, John G. Mchutchison9, Ira M. Jacobson5, Myron J. Tong4, Donald M. Jensen6, Geor M. Lauer10, Scott Cruickshank11, John Ferraro1, Aurelia Haller1, Richard Duke1, Timothy Rodell1, David Apelian1

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#1305

**ASSOCIATION OF PRE-TREATMENT AND ON-TREATMENT FACTORS WITH RAPID VIROLOGIC RESPONSE IN HCV GENOTYPE 1 INFECTED PATIENTS TREATED WITH PEGIFNα-2A/RBV**

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#1306

**TREATMENT OF PEGINTERFERON/RIBAVIRIN NONRESPONDERS WITH DAILY DOSING OF CONSENSUS INTERFERON AND RIBAVIRIN - PRELIMINARY RESULTS OF THE GERMAN CONSENSUS INTERFERON MULTICENTER STUDY**

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#1307

**PF-03491390 INHIBITS LIVER FIBROSIS IN PATIENTS WITH CHRONIC HEPATITIS C VIRUS INFECTION VIA SUPPRESSION OF PRO-APOPTOTIC CASPASE-ACTIVATION.**

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#1308

**DIFFERENTIATION OF EARLY VIROLOGIC RESPONSE (EVR) INTO RVR, COMPLETE EVR (CEVR) AND PARTIAL EVR (PEVR) ALLOWS FOR A MORE PRECISE PREDICTION OF SVR IN HCV GENOTYPE 1 PATIENTS TREATED WITH PEGINTERFERON ALFA-2A (40KD) (PEGASYS®) AND RIBAVIRIN (COPEGUS®)**

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#1309

**FINAL RESULTS OF PATIENTS RECEIVING PEGINTERFERON-ALFA-2A (PEG-IFN) AND RIBAVIRIN (RBV) AFTER A 14-DAY STUDY OF THE HEPATITIS C PROTEASE INHIBITOR TELAPREVIR (VX-950), WITH PEG-IFN**

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#1310

**RETREATMENT OF HCV GENOTYPE 1 RELAPSE PATIENTS TO PEGINTERFERON/RIBAVIRIN THERAPY WITH AN EXTENDED TREATMENT REGIMEN OF 72 WEEKS WITH CONSENSUS INTERFERON/RIBAVIRIN VERSUS PEGINTERFERON ALPHA/RIBAVIRIN**

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#1311

**LONG-TERM LOW DOSE TREATMENT WITH PEGYLATED INTERFERON ALPHA 2B LEADS TO A SIGNIFICANT REDUCTION IN FIBROSIS AND INFLAMMATORY SCORE IN CHRONIC HEPATITIS C NONRESPONDER PATIENTS WITH FIBROSIS OR CIRRHOSIS**

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**#1312**

**CORRELATION BETWEEN POLYMORPHISMS OF NS5B AND EARLY CLEARANCE OF HEPATITIS C VIRUS BY PEG-INTERFERON PLUS RIBAVIRIN TREATMENT**

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**#1313**

**EFFECT OF INFlixIMAB ON THE EFFICACY OF PEGINTERFERON ALFA-2B (PEG-2B) PLUS RIBAVIRIN (RBV) THERAPY IN TREATMENT-NAIVE PATIENTS WITH HEPATITIS C GENOTYPE 1 AND HIGH TUMOR NECROSIS FACTOR α (TNF-α) LEVELS**

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**#1314**

**TREATMENT OF ALCOHOLIC HCV PATIENTS WITH ACETAMINOPHEN 4 G/DAY FOR 5 DAYS DOES NOT AFFECT HEPATIC TESTS COMPARED TO PLACEBO**

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**#1315**

**FAVORABLE QUALITY OF LIFE (QOL) WITH ALBINTERFERON ALFA-2B PLUS RIBAVIRIN IN GENOTYPE 1, IFN-NAIVE, CHRONIC HEPATITIS C (CHC) PATIENTS**

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1Monash University, Melbourne, VIC, Australia. 2University of British Columbia, Vancouver, BC, Canada. 3J.W. Goethe-University Hospital, Frankfurt, Germany. 4Hospital Pitie-Salpetriere, Paris, France. 5University of Alberta, Edmonton, AB, Canada. 6Duke Clinical Research Institute, Durham, NC, USA. 7Human Genome Sciences, Rockville, MD, USA

**#1316**

**HIGH CHANCE OF CURE IN HCV GENOTYPE 1 PATIENTS WITH A LOW VIRAL LOAD ACHIEVING AN RVR TREATED FOR 24 WKS WITH PEGINTERFERON ALFA-2A (PEGASYS®) PLUS RIBAVIRIN (COPEGUS®): PROSPECTIVE, RANDOMIZED, CONTROLLED STUDY COMPARING 24 AND 48 WEEKS OF TREATMENT**

Ming-Lung Yu1,2, Chia-Yen Dai1,3, Jee-Fu Huang4, Li-Po Lee1, Ming-Yen Hsieh1, Chang-Fu Chiu5, Nai-Jen Hou4, Zu-Yau Lin1,2, Shin-Cherng Chen1,2, Ming-Yuh Hsieh1,2, Liang-Yen Wang1,2, Wen-Yu Chang1,2, Wan-Long Chuang1,2

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**#1317**

**TREATMENT OF HEPATITIS C PATIENTS WITH CHILD A AND B CIRRHOSIS WITH CONSENSUS INTERFERON AND RIBAVIRIN IN A LOW ASCENDING DOSING REGIMEN LEADS TO SIGNIFICANT VIRAL ELIMINATION RATES**

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**#1318**

**RESULTS OF A PHASE I PLACEBO-CONTROLLED TRIAL IN HEALTHY VOLUNTEERS TO EXAMINE THE SAFETY, TOLERABILITY AND PHARMACOKINETICS OF THE HCV PROTEASE INHIBITOR TMC435350 AFTER SINGLE AND REPEATED DOSING**

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**#1319**

**ANALYSIS OF RELAPSE RATES IN PEGYLATED INTERFERON AND RIBAVIRIN NON-RESPONDERS TREATED WITH DAILY CONSENSUS INTERFERON AND RIBAVIRIN**

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#1320
THE EFFECT OF COMPLETE AND PARTIAL RESPONSE AT WEEK 12 ON SUSTAINED VIROLOGIC RESPONSE: RESULTS FROM CONTROLLED TRIALS IN NAIVE HCV GENOTYPE 1 PATIENTS TREATED WITH PEGYLATED INTERFERON AND RIBAVIRIN
Mitchell L. Shiffman1, Hank Mansbach2, Janet Hammond2, Mark O’Neill2
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#1321
COMPARISON OF STANDARD VS EXTENDED PEGYLATED INTERFERON PLUS RIBAVIRIN TREATMENT ACCORDING TO THE TIME OF HCV RNA NEGATIVE IN PATIENTS WITH GENOTYPE 1 AND HIGH VIRAL LOAD
Tatsuya Ide, Teruko Arinaga, Ichiro Miyajima, Kei Ogata, Reichiuro Kuwahara, Yuriko Koga, Kouichiro Kuhara, Ryukichi Kumashiro, Michio Sata
Division of Gastroenterology, Kurume University School of Medicine, Kurume, Japan

#1322
TOLERABILITY OF LOW-DOSE PEGINTERFERON ALFA-2B IN HEPATITIS C PATIENTS WITH CIRRHOSIS: RESULTS FROM THE COPILOT TRIAL
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#1323
DAILY CONSENSUS INTERFERON VERSUS PEG-INTERFERON ALFA2B WITH WEIGHT BASED OR 800 MG RIBAVIRIN IN TREATMENT-NAIVE PATIENTS WITH CHRONIC HEPATITIS C GENOTYPES 2 OR 3
Fareed Rahman1, Marcus Schuchmann1, Hanns F. Lühr2, Ralf Link3, Peter Buggisch4, Michael Fuchs5, Stefan Kaiser6, Christoph Antoni7, Thomas Withöff8, Jörg Schlaak9, Peter R. Galle1, Wulf Bocher1
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#1324
DAILY CONSENSUS INTERFERON VERSUS PEG-INTERFERON ALFA2B WITH WEIGHT BASED RIBAVIRIN IN TREATMENT-NAIVE PATIENTS WITH CHRONIC HEPATITIS C GENOTYPE 1
Fareed Rahman1, Marcus Schuchmann1, Hanns F. Lühr2, Ralf Link3, Peter Buggisch4, Michael Fuchs5, Stefan Kaiser6, Christoph Antoni7, Thomas Withöff8, Jörg Schlaak9, Peter R. Galle1, Wulf Bocher1
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#1325
CORRELATION BETWEEN LIVER BIOPSY AND FIBROSURE™ DURING SCREENING FOR A PHASE II STUDY TO ASSESS THE ANTIFIBROTIC ACTIVITY OF FARGLITIZAR IN CHRONIC HEPATITIS C INFECTION
Keyur Patel1, John G. McHutchison1, Zachary D. Goodman2, Dickens Theodore3, Alison Webster3, Margaret Schultz3, Brit Stancil3, Martin Garland3, Stephen Gardner3
1Gastroenterology, Duke Clinical Research Institute and Duke University Medical Center, Durham, NC, USA. 2Armed Forces Institute of Pathology, Washington, DC, USA. 3GlaxoSmithKline, Research Triangle Park, NC, USA

#1326
A 52 WEEK MULTI-CENTRE, RANDOMIZED, DOUBLE-BLIND PLACEBO-CONTROLLED TRIAL EVALUATING THE EFFICACY AND SAFETY OF GLYCRRHIZIN IN PATIENTS WITH CHRONIC HEPATITIS C NOT RESPONDING TO IFNα OR PEG-IFN PLUS RIBAVIRIN THERAPY
1CEE, PharmaPart AG, Thalwil, Switzerland. 2Gastroenterology and Hepatology, Hannover Medical School, Hanover, Germany. 3Military Therapy, Moscow City Hospital No. 29, Moscow, Russian Federation. 4Moscow Hepatology Center, Moscow Clinical Hospital for Infectious Diseases No. 1, Moscow, Russian Federation. 5Infectious Diseases, Lugansk Medical University, Lugansk, Ukraine. 6Department of Morphology and Molecular Pathology, University Hospitals Leuven, Leuven, Belgium. 7Department of Pathology, University Hospital of Cologne, Cologne, Germany. 8Development & Planning Department, Minophagen Pharmaceutical Co., Ltd, Tokyo, Japan. 9Clinical Operations, PharmaPart AG, Thalwil, Switzerland
#1327

**COMPARISON OF PEGINTERFERON ALFA-2A AND RIBAVIRIN FOR 12 OR 24 WEEKS IN PATIENTS WITH HCV GENOTYPE 2 OR 3: THE CLEO TRIAL**

Fabrizio Mecenate1, Giuseppe Barbaro2, Adriano Pellicelli3, Angelo Barbattani4, Ettore Mazzoni5, Maria Elena Bonaventura6, Mario Romano7, Lorenzo Nosotti7, Pasquale Arcuri8, Antonio Picardi9, Giorgio Barbarini10, Fabrizio Soccorsi11

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7Medicine, IRCCS S. Gallicano Hospital, Rome, Italy.
8Hepatology, University of Rome, Rome, Italy.
9Malattie Infettive, IRCCS San Matteo Hospital, Pavia, Italy.
10Hepatology, S Camillo Forlanini Hospital, Rome, Italy.
11Hepatology, Pertini Hospital, Rome, Italy.
12Hepatology, Policlinico Casilino, Rome, Italy.
13Hepatology, S. Giacomo Hospital, Rome, Italy.
14Medical Biostatistics, Sumitomo Riko, PR, USA.
15Department of Medicine, SA Matheo Hospital, Pavia, Italy.
16Hepatology, S. Camillo Forlanini Hospital, Rome, Italy.
17Hepatology, S. Giovanni Hospital, Rome, Italy.
18Hepatology, Campus Biomedico University, Rome, Italy.
19Medical Biostatistics, Sumitomo, Puerto Rico, PR, USA.
20Dept of Medicine, Georgetown Univ., Washington, DC, DC, USA.
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#1328

**INTERFERON THERAPY PREEMPTIVE PARTIAL SPLENIC EMBOLIZATION FOR HEPATITIS C PATIENTS WITH THROMBOCYTOPENIA**

Hiroki Tahara1,2, Yasushi Shimada1,2, Hiroki Tjojima1,2, Tomoyuki Hirokawa2, Tatsuya Oyama2, Katsuhiko Horiuchi2, Atsushi Naganuma2, Hirota Kari2, Hitoshi Takagi2

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#1329

**VIRAL KINETICS OF HCV GENOTYPE 4 DURING PEGYLATED INTERFERON ALPHA 2A –RIBAVIRIN THERAPY**

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#1330

**EFFECTS OF CHRONIC HCV INFECTION ON HEALTH-RELATED QUALITY OF LIFE AND FATIGUE: THE ROLE OF LIVER FIBROSIS PROGRESSION**

Gerlinde Teuber1, Arne Schaefer2, Jasmin Rimpel1, Kathrin Paul1, Christian Keicher2, Michael Scheuven2, Stefan Zeuzem1, Michael R. Kraus2

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#1331

**RETREATMENT OF TREATMENT EXPERIENCED HCV PATIENTS WITH PEGYLATED INTERFERON ALFA-2A AND THYMOSIN ALPHA-1: POOLED ANALYSIS OF TWO RANDOMIZED CONTROLLED TRIALS**

Kenneth E. Sherman1, Stuart C. Gordon2, Rehan Ifikar3, Maribel Rodriguez-Torres4, Vinod K. Rustgi5, Adrian M. Di Bisceglie3

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#1332

**U.S. MULTICENTER PILOT STUDY OF DAILY CONSENSUS INTERFERON (INFERGEN) PLUS RIBAVIRIN FOR “DIFFICULT-TO-TREAT” HCV GENOTYPE 1 PATIENTS: TOLERANCE AND ON-THERAPY VIROLOGIC RESPONSE**

Samuel B. He1,2,13, Bashar Ageel2,14, Eric Dieperink3,14, Lori Tetrick2, Yngve Falck-Ytter4, Charles A. de Comarmond5, Coleman I. Smith11,14, Daniel McKee2,14, Anastasios A. Mihas6, William Boyd7, Clark C. Kulig8, Marcos Pedrosa9, Edmund J. Bini10

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#1333

**OUTCOMES IN HIV-HCV CO-INFECTED GENOTYPE 1 PATIENTS TREATED WITH PEGYINTERFERON ALFA-2A (40KD) PLUS RIBAVIRIN (RBV) 1000/1200 MG/D: PREDICTIONS BASED ON A GENERALIZED ADDITIVE MODEL (GAM)**

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HCV: Diagnosis and Natural History

**#1334**

**HEPATIC SOCS3 EXPRESSION IS STRONGLY ASSOCIATED WITH NON-RESPONSE TO IFN TREATMENT IN CHRONIC HCV**

Kyung Ah Kim, Wenyu Lin, Ethan Weinberg, Carolina B. Borges, Andrew W. Tai, Yoshitaka Kamegaya, Raymond T. Chung. Gastrointestinal Unit, Department of Medicine, Massachusetts General Hospital, Harvard Medical School, Boston, MA, USA

**#1335**

**EUROPEAN LIVER FIBROSIS (ELF) PANEL OF SERUM MARKERS CAN PREDICT CLINICAL OUTCOME IN A COHORT OF PATIENTS FROM ENGLAND WITH MIXED AETIOLOGY CHRONIC LIVER DISEASE**

Julie Parkes1,2, Paul Roderick1,2, Scott Harris1, Carol Gough3, Mark Wheatley2, Graeme J. Alexander4, Jane D. Collier5, Christopher P. Day5, David J. Mutimer7, Day6, David J. Mutimer7, John Ramage8, Alistair Burt9, William M. Rosenberg2

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**#1336**

**TRACKING THE COURSE OF HEPATITIS C WITH PAIRED BIOPSIES - A CRITICAL ANALYSIS**

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**#1337**

**LONG-TERM MORBIDITY AND MORTALITY OF HCV INFECTION: A 25 YEARS FOLLOW-UP**

Sanaa Kamal1,2, Imad Nasser1, Samer El Kamary3, Michelle Shardell1, Mohamed Hashem1, Amany Ibrahim2, Ashraf Moustafa2, Iman Fathy4,2, Maha Sobhi4,2, Amal Abdel Baky4, Hoda Mansour2,4

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**#1338**

**DIAGNOSTIC ACCURACY OF THE APRI FOR THE PREDICTION OF HEPATITIS C-RELATED FIBROSIS: A SYSTEMATIC REVIEW**

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**#1339**

**COMPARISON OF REPRODUCIBILITY OF HISTOLOGY, BLOOD TESTS AND FIBROSCAN FOR LIVER FIBROSIS**

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**#1340**

**HISTOLOGICAL OUTCOMES AFTER 30 YEARS IN UNTREATED IRISH WOMEN WITH CHRONIC HCV GENOTYPE 1B, DO GENETIC FACTORS INFLUENCE?**

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**#1341**

**HIGHER CAFFEINE CONSUMPTION IS ASSOCIATED WITH Milder FIBROSIS IN PATIENTS WITH CHRONIC LIVER DISEASES**

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**#1342**

**LARGE SCALE VALIDATION OF THE SAFE (SEQUENTIAL ALGORITHMS FOR FIBROSIS EVALUATION) BIOPSY IN CHRONIC HEPATITIS C**

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#1343
ANTI-LIVER KIDNEY MICROSOME TYPE ONE (\(\alpha\)LKM1) POSITIVE HCV-RELATED CHRONIC LIVER DISEASE: PRESENTATION AND FOLLOW UP
Silvia Ferri, Giorgio Ballardini, Valentina Cipriano, Alessandro Granito, Alberto Grassi, Luigi Muratori, Paolo Muratori, Georgios Pappas, Chiara Quarneri, Francesco B. Bianchi, Marco Lenzi
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#1344
INTEROBSERVER REPRODUCIBILITY OF LIVER STIFFNESS MEASUREMENT BY TRANSIENT ELASTOGRAPHY (FIBROSCAN®)
Damien Lucidarme\(^1\), Gerard Forzy\(^2\), Veronique Gremaux\(^1\), Bernard Filoche\(^1\)
\(^1\)Pathologie Digestive, groupe Hospitalier de l’Institut Catholique de Lille, Lomme, France. \(^2\)Laboratoire de Biologie, Groupe Hospitalier de l’Institut Catholique de Lille, Lomme, France

#1345
THE USE OF NEW NORMAL ALT VALUES DIFFERENTIATE BETTER PATIENTS WITH HBEAG NEGATIVE CHRONIC HEPATITIS B FROM INACTIVE CHRONIC CARRIERS
Nimer Assy\(^1,3\), Zaza Beniashvili\(^1\), Maria Grosovski\(^2\), Smadar Dabush\(^1\), Patia Erdstein\(^1\), Bayan Hino\(^1,3\)
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#1346
THE RESULT OF LIVER STIFFNESS MEASUREMENT IS INFLUENCED BY THE SERUM BILIRUBIN LEVEL
Yeon Seok Seo\(^1\), Sang Jun Suh\(^1\), Yong Dae Kwon\(^1\), Sanghun Park\(^1\), Bora Keum\(^1\), Beom Jin Park\(^2\), Yong Sik Kim\(^1\), Yoon Tae Jeen\(^1\), Hoon Jai Chun\(^1\), Chang Duck Kim\(^1\), Ho Sang Ryu\(^1\), Soon Ho Um\(^1\)
\(^1\)Internal Medicine, Korea University College of Medicine, Seoul, South Korea. \(^2\)Radiology, Korea University College of Medicine, Seoul, South Korea

#1347
OBESITY IS AN INDEPENDENT PREDICTOR OF FIBROSIS PROGRESSION IN PATIENTS WITH CHRONIC HEPATITIS C VIRUS (HCV) USING PAIRED LIVER BIOPSY SPECIMENS
Anjana A. Pillai, Lisa M. Yerian, Rocio Lopez, Ibrahim A. Hanouneh, Nizar N. Zein
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#1348
ACCURATE IDENTIFICATION OF LIVER FIBROSIS USING THE POINT-OF-CARE CONTINUOUS 13C METHACETIN BREATH TEST: A DECISION MAKING TOOL IN THE TREATMENT OF PATIENTS WITH CHRONIC HCV INFECTION
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#1349
IRON OVERLOAD DOES NOT EFFECT THE QUANTIFICATION OF FIBROSIS BY LIVER STIFFNESS MEASUREMENT
Vito Di Marco\(^1\), Fabrizio Bronte\(^1\), Daniela Cabibi\(^2\), Francesco Barbara\(^1\), Zelia Borsellino\(^3\), Giuseppe Alaimo\(^1\), Vincenza Calvaruso\(^1\), Stefania Cimmnisi\(^1\), Marcello Capra\(^2\), Aurelio Maggio\(^4\), Piero Luigi Almasio\(^1\), Antonio Craxì\(^1\)
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#1350
HCV GENOTYPE DETERMINATION IN CLINICAL PRACTICE: WEAKNESSES OF ASSAYS BASED ON THE 5’ NONCODING REGION AND IMPROVEMENT WITH THE CORE-CODING REGION
Stephane Chevaliez, Magali Bouvier-Alias, Claire Vandervenet, Jean-Michel Pawlotsky
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#1351
SERUM LEVELS OF SH2A, A SECRETED FORM OF THE ASIALOGLYCOPROTEIN RECEPTOR, AS A NON-INVASIVE SENSITIVE MARKER FOR LIVER FUNCTION
Elena Veselkin\(^1\), Maria Kondratyev\(^1\), Efrat Ron\(^1\), Moshe Santo\(^2\), Shimon Reif\(^2\), Irma Elashvili\(^1\), Lana Bar\(^2\), Yoav Lurie\(^2\), Ran Oren\(^2\), Gerardo Z. Lederkremer\(^1\)
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#1352
COMPARISON OF A NEW ASSAY FOR HEPATITIS C VIRUS (HCV) GENOTYPING TARGETING BOTH 5’NC AND NSS5B GENOMIC REGIONS, WITH REVERSE HYBRIDIZATION AND SEQUENCING METHODS
Elisa Martro\(^1\), Victoria Gonzalez\(^1\), Andrew Buckton\(^2\), Veronica Sanders\(^1\), Ramon Planas\(^3\), Vicenç Ausina\(^1\)
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#1353

**NATURAL HISTORY OF HEPATITIS C VIRUS INFECTION IN HIV-INFECTED INDIVIDUALS IN THE ERA OF HAART: SYSTEMATIC REVIEW AND META-ANALYSIS**

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#1354

**VALIDATION OF SIMPLE INDEXES (FIB-4, APRI, FORNS INDEX) AND PLATELET COUNT FOR NON INVASIVE PREDICTION OF LIVER FIBROSIS IN HIV-HEV-COINFECTED FRENCH PATIENTS**

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#1355

**CAN PLATELET COUNT OR APRI BE THE POOR MAN’S TRANSIENT ELASTOGRAPHY IN HCV PATIENTS?**

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#1356

**LIVER STIFFNESS MEASUREMENT IN PATIENTS WITH CHRONIC HEPATITIS B IS NOT AS USEFUL AS THAT IN PATIENTS WITH CHRONIC HEPATITIS C FOR THE ASSESSMENT OF LIVER FIBROSIS**

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#1357

**FIB4 USEFULNESS FOR MEASURING FIBROSIS CHANGES FOLLOWING ANTI HCV -VIRAL TREATMENT**

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#1358

**EVOLUTION OF HEPATITIS C VIRUS (HCV) VIREMIA IN PROSPECTIVELY FOLLOWED INTRAVENOUS DRUG USERS (IVDU:S) WITH DOCUMENTED ANTI-HCV ANTIBODY SEROCONVERSION PARTICIPATING IN A SWEDISH NEEDLE-EXCHANGE PROGRAM**

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#1359

**FREQUENCY AND SIGNIFICANCE OF F-ACTIN ANTIBODIES (FAA) IN HIV/HEV CO-INFECTED PATIENTS**

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#1360

**CAUSE OF DEATH IN PATIENTS WITH HEPATITIS C (HCV)-MIXED CRYOGLOBULINEMIA (MC) VASCULITIS**

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#1361

**INCIDENCE OF TYPE 2 DIABETES MELLITUS AND GLUCOSE ABNORMALITIES IN TREATED PATIENTS WITH CHRONIC HEPATITIS C INFECTION: RESULTS OF A CONTROLLED FOLLOW-UP STUDY**

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#1362
**CYP1A1 POLYMORPHISM IS RELATED WITH FIBROSIS IN CHRONIC HEPATITIS C**

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#1363
**COMPARISON OF TWO REAL-TIME PCR BASED ASSAYS (REALTIME HCV, COBAS TAQMAN) WITH A SIGNAL AMPLIFICATION ASSAY (BDNA) FOR HCV RNA DETECTION**

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#1364
**VALIDITY OF HEPATITIS C ANTIBODY TESTING IN UGANDA: A COMPARISON STUDY**

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#1365
**FIBROSIS STAGES DETERMINATION, REPRODUCIBILITY AND ROBUSTNESS OF BLOOD TESTS FOR LIVER FIBROSIS IN CHRONIC HEPATITIS C**

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#1366
**HOST RATHER THAN VIRAL FACTORS REDUCE HEALTH-RELATED QUALITY OF LIFE IN HEPATITIS C VIRUS INFECTION**

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#1367
**THE APRI CAN NEGATIVELY PREDICT CIRRHOSIS IN PATIENTS WITH CHRONIC HEPATITIS C (HCV)**

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#1368
**PROSPECTIVE COMPARISON OF TWO COMMERCIAL NON-INVASIVE FIBROSIS SERUM MARKER PANELS (FIBROSURE AND FIBROSPECT II) DURING INTERFERON-BASED COMBINATION THERAPY IN CHRONIC HCV GENOTYPE 1**

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#1369
**A PRACTICAL INDEX (FIBROINDEX) FOR THE PREDICTION OF SIGNIFICANT FIBROSIS IN PATIENTS WITH CHRONIC HEPATITIS C**

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#1370
**ANTI-ARFP ANTIBODY POSITIVITY IS SIGNIFICANTLY ASSOCIATED WITH INCREASED HCV VIRAL LOAD IN END STAGE LIVER DISEASE**

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#1371
SERUM LEVELS OF ASIALOGLYCOPROTEIN RECEPTOR SH2A CORRELATE WITH SUCCESS OF TREATMENT OF HCV PATIENTS
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#1372
INFLUENCE OF INTERFERON (IFN)-BASED THERAPY ON LIVER FIBROSIS PROGRESSION (LFP) IN HIV/HCV CO-INFECTED PATIENTS
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#1373
HIGHER LEVEL OF ALANINE AMINOTRANSFERASE MASS IN LIVER DISEASE PATIENTS THAN NORMAL POPULATION MEASURED BY SANDWICH ELISA
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#1374
IN VIVO DETECTION OF CASPASE ACTIVITY IN PATIENTS WITH CHRONIC HEPATITIS C AS A NOVEL BIOMARKER OF DISEASE SEVERITY
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#1375
COMMON PATHWAYS IN ENDOGENOUS DEPRESSION AND IN IFN-INDUCED DEPRESSION IN HCV PATIENTS
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#1376
DIFFERENTIAL PATTERN OF HCV-SPECIFIC T CELL RESPONSES DISTINGUISHES SVR AND RELAPSE PATIENTS RECEIVING STANDARD ANTIVIRAL THERAPY PLUS THERAPEUTIC IC41 VACCINATION
Heiner Wedemeyer1, Erich Tauber2, Elisabeth Schuller2, Johannes Wiegand3, Ingolf Schieke4, Wolf-Peter Hofmann5, Stefan Zeuzem6, Holger Hinrichsen7, Mark R. Thursz8, Bernd Jilma9, Rudolf E. Stauber1, Michael P. Manns1, Christoph S. Klade2
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#1377
A PHASE 1 STUDY TO EVALUATE THE SAFETY, TOLERABILITY, PHARMACOKINETICS (PK) AND PHARMACODYNAMICS (PD) OF ESCALATING SINGLE DOSES OF R7025, A NOVEL PEGYLATED INTERFERON α COMPARED TO PLACEBO AND PEGINTERFERON α 2A (40KD) (PEGASYS®) IN HEALTHY VOLUNTEERS
Barbara Brennan1, Richard Robson2, Ignacio Rodriguez3, Julian Symons3, Jane Huang3, Margaret Chan1, Steven Blotner1, Natanya Jennings1, Bina Rawal4, Michael Brunda1, Greg Hooper4, Ashok Rakhi1
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#1378
PHOSPHOROTHIOATE OLIGONUCLEOTIDE IS A POTENT ENTRY INHIBITOR OF HEPATITIS C VIRUS INFECTION IN VITRO AND IN VIVO
Takuya Matsumura1, Takano H. Kato1, Zongyi Hu2, Michio Imai2, Nobuhiko Hiragaw2, Jean-Marc Juteau3, Andrew Vaillant3, Kazuaki Chayama2, T. Jake Liang1
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#1379
IDENTIFICATION OF MODULATORS OF HCV REPLICATION USING A HIGH-THROUGHPUT SCREEN
Lee F. Peng
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#1380

**DENDRITIC CELLS AND REGULATORY T CELLS AS DECISION MAKERS FOR THE DURATION OF PEGYLATED INTERFERON-α AND RIBAVIRIN THERAPY IN CHRONIC HEPATITIS C PATIENTS**

Tatsuya Kanto, Ichiyo Itose, Michiyo Inoue, Naruyasu Kakita, Masanori Miyazaki, Hideki Miyatake, Mitsuru Sakakibara, Takayuki Yokouchi, Naoki Hiramatsu, Tetsuo Takehara, Akinori Kasahara, Norio Hayashi

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#1381

**DEVELOPMENT OF INTERGENOTYPIC CHIMERIC REPLICONS FOR BROAD-SPECTRUM ANTIVIRAL ACTIVITY DETERMINATION OF HEPATITIS C VIRUS POLYMERASE INHIBITORS**

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#1382

**IN VITRO AND IN VIVO CHARACTERIZATION OF R7025, A NOVEL PEGYLATED HUMAN INTERFERON ALPHA MOLECULE, GENERATED BY DNA SHUFFLING FOR THE TREATMENT OF CHRONIC HEPATITIS C**

Julian Symons, Amy D. Brideau-Anderson, Jane Huang, Xiaojian Huang, Anne Vogt, Siu-Chi C. Sun, Sun-Chun Hsieh, Astrid Pappenberger, Glen Dawes, Volker Schmaible, Mariola Ilnicka, Aga Ahene, Ralf Schumacher, Brunda Michael, Nick Camack, Phillip A. Patten, Tom Tarnowski, Sridhar Viswanathan

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#1383

**BOTANICAL MEDICINES FOR HEPATITIS C**

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#1384

**SVRSCORE: A NOVEL AND HIGHLY PREDICTIVE SCORE FOR SVR PREDICTION IN CHRONIC HEPATITIS C GENOTYPE 1 INFECTED PATIENTS**

Philippe Halfon, Guillaume Pénaranda, Marc Bourié, Albert Tran, Daniela Botta-Fridthun, Isabelle Portal, Christophe Renou, Magali Picon, Claire Wartelle, Jean-Pierre Arpurt, Michel Anton, Michel Guiselle, Claude Guyfier, Patrick Delassalle, Denis Ouzan

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#1385

**GS-9190, A NOVEL SUBSTITUTED IMIDAZOPYRIDINE ANALOGUE, IS A POTENT INHIBITOR OF HEPATITIS C VIRUS REPLICATION IN VITRO AND REMAINS ACTIVE AGAINST KNOWN DRUG RESISTANT MUTANTS**

Inge Vliegen, Jan Paeshuyse, Eric Marberry, Betty Peng, Ihung Shih, Laura S. Lehman, Hélène Dutartre, Barbara Selisko, Bruno Canard, Steven Bondy, Winston Teo, Hans Reiser, Erik De Clercq, William A. Lee, Gerhard Puerstinger, Weidong Zhong, Johan Neyts

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#1386

**GENOTYPE COVERAGE OF THE HCV NS3/4A PROTEASE INHIBITOR ITMN-191 (R7227): BIOCHEMICAL DATA REVEALS POTENT INHIBITION AND SLOW DISSOCIATION WITH GENOTYPE 1-6 PROTEASES**

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#1387

**A HEPATITIS C T-CELL VACCINE CANDIDATE COVERING HCV GENOTYPE COMPLEXITY AND HLA TYPE DIVERSITY**

Annegret Van der Aa, Vera Goossens, Koen Allosery, Gert Verheyden, Scott Southwood, Carol Dahlberg, Denise McKinney, Mark Newman, Helmut M. Diepolder, Geert Maertens, Marie-Ange Buyse

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#1388
**USING DENDRITIC CELLS AS THERAPEUTIC OR PROPHYLACTIC VACCINE AGAINST HCV**

Kilian Weigand, Franziska Voigt, Christoph Eisenbach, Birgit Hoyler, Wolfgang Stremmel, Jens Encke
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#1389
**EFFICACY OF INTERFERON \(\beta\) COMBINED CYCLOSPORINE A TREATMENT IN THE RETREATMENT OF CHRONIC HEPATITIS C - PROMISING ASPECT OF HOST FACTOR TARGETING THERAPY**

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#1390
**IN VITRO ACTIVITY AND PRECLINICAL PHARMACOKINETICS OF THE HCV PROTEASE INHIBITOR, TMC435350**

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#1391
**HCV POLYMERASE (NM107) AND PROTEASE (BOCEPREVIR) INHIBITORS IN COMBINATION SHOW ENHANCED ACTIVITY AND SUPPRESSION OF RESISTANCE IN THE REPlicON SYSTEM**

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#1392
**ANA598, A NOVEL NON-NUCLEOSIDE INHIBITOR OF HCV NS5B POLYMERASE, EXHIBITS FAVORABLE PHARMACOKINETIC PROPERTIES IN MULTIPLE PRECLINICAL SPECIES**

Lea Kirskovsky, Yuefen Zhou, Daniel Norris, Ellen Okamoto, Thomas G. Nolan, Darian Barkowski, Julia Khandurina, Maria Sergeeva, Douglas Murphy, Benjamin Ayida, Alan Xiang, David Ellis, Julie Blazel, Zhongxiang Sun

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#1393
**ME3738 INHIBITS HEPATITIS C VIRUS REPLICATION BY ENHANCING INTERFERON-\(\beta\)**

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#1394
**PRECLINICAL EVALUATIONS OF PF-00868554, A POTENT NON-NUCLEOSIDE INHIBITOR OF THE HEPATITIS C VIRUS RNA POLYMERASE**

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#1395
**MECHANISTIC CHARACTERIZATION OF GS-9190, A NOVEL NON-NUCLEOSIDE INHIBITOR OF HCV NS5B POLYMERASE WITH POTENT ANTVIRAL ACTIVITY AND A UNIQUE MECHANISM OF ACTION**


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#1396
**MODELING THE POTENTIAL COST-EFFECTIVENESS OF ADDING A NOVEL STAT-C AGENT TO CURRENT STANDARD THERAPY IN PATIENTS WITH GENOTYPE 1 CHRONIC HEPATITIS C INFECTION**

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#1397
**AM3 INHIBITS HCV REPLICATION THROUGH ACTIVATION OF PERIPHERAL BLOOD MONONUCLEAR CELLS**

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#1398
**PRECLINICAL PHARMACOKINETIC CHARACTERIZATION OF GS-9190, A NOVEL NON-NUCLEOSIDE HCV NS5B POLYMERASE INHIBITOR**

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#1399
SONIC HEDGEHOG IS AN AUTOCRINE VIABILITY FACTOR FOR MYOFIBROBLASTIC HEPATIC STELLATE CELLS
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#1400
AUTOCRINE SIGNALING BY SDF-1α THROUGH ITS RECEPTOR, CXCR4, MEDIATES HEPATIC STELLATE CELL ACTIVATION IN VIVO AND IN VITRO
Feng Hong, Ana Tuyama, Ritu Agarwal, Ritu Gupta, Xin Cheng, Anita Garg, Yukiko Inoue, M. Isabel Fiel, Myron Schwartz, Martina Schwarzkopf, Alison D. Schecter, Meena B. Bansal
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#1401
HUMAN AND RAT HEPATIC STELLATE CELLS EXPRESS OPIOID RECEPTORS AND ARE STIMULATED BY ENDOGENOUS OPIOID PEPTIDES IN A PARACRINE MANNER
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#1402
EFFECT OF SIMULATED MICROGRAVITY ON RAT HEPATIC STELLATE CELLS AND THEIR INTERACTION WITH EXTRACELLULAR MATRIX PROTEINS
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#1403
MOUSE HEPATIC STELLATE CELL INSULIN RESISTANCE IS CAUSED BY DOWNREGULATION OF INSULIN RECEPTOR PROTEIN AND DEFECTIVE DOWNSTREAM SIGNALING
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#1404
A NOVEL ISOLATION METHOD FOR PRIMARY RAT PORTAL FIBROBLASTS BY EXPLANTING BILIARY TRACT TISSUE REVEALS KEY BIOLOGICAL DIFFERENCES COMPARED TO LOBULAR ACTIVATED HEPATIC STELLATE CELLS AS ASSESSED BY CYTOKINE MICROARRAY
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#1405
NOX2 AND RAC1 PLAY IMPORTANT ROLES IN LIVER FIBROGENESIS BY FACILITATING PHAGOCYTOSIS OF APOPTOTIC BODIES AND PRODUCTION OF ROS BY STELLATE CELLS
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#1406
REGULATION OF HEPATIC STELLATE CELL GROWTH BY NUCLEAR AND CYTOSOLIC CALCIUM SIGNALS
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#1407
APOPTOTIC HEPATOCYTE DNA INHIBITS HSC CHEMOTAXIS AND STIMULATES HEPATIC STELLATE CELL DIFFERENTIATION VIA TLR9
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#1408
SYMPATHETIC NERVOUS SYSTEM SIGNALLING IN HUMAN PRIMARY HEPATIC STELLATE CELLS: A NOVEL ANTI-FIBROTIC PATHWAY
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#1409
ENDOGLIN PHOSPHORYLATION BY TGF-β RECEPTORS IN TRANSDIFFERENTIATING HSC
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#1410
OXIDATIVE STRESS INDUCE COLLAGEN SYNTHESIS BY C-ABL ACTIVATION IN HUMAN HEPATIC STELLATE CELLS
Tommaso Mello1, Elisabetta Ceni1, Simone Polvani2, Laura Cioni1, Francesca Lisi1, Barbara Ottanelli1, Francesca Buccoliero1, Mirko Tarocchi1, Stefano Milani1, Andrea Galli1
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#1411
MESENCHYMAL β-CATENIN SIGNALING REGULATES HEPATIC STELLATE CELLS FATE
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#1412
MOUSE HEPATIC STELLATE CELLS EXPRESS THE INTERMEDIATE FILAMENT PROTEIN SYNCOILIN
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#1413
ATRIAL NATRIURETIC PEPTIDE (ANP) PREVENTED LIVER FIBROSIS IN RAT USING NEW TREATMENT SYSTEM
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#1414
THE SYNTHETIC COLLAGEN ANALOG (GPO)10 STIMULATES PROLIFERATION, MIGRATION AND EXPRESSION OF N-CADHERIN IN HEPATIC STELLATE CELLS VIA ACTIVATION OF PROMMP-2
Christian Freise1, Martin Ruehl1, Marion Seja1, Ulrike Erben1, Richard Farndale2, Detlef Schuppan3, Martin Zeitz1, Rajan Somasundaram1
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#1415
CD95 LIGAND INDUCES PROLIFERATION AND CD95-TYROSINE NITRATION IN QUIESCENT RAT HEPATIC STELLATE CELLS
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#1416
INHIBITION AND IDENTIFICATION OF NONMUSCLE MYOSIN II ISOFORMS IN HEPATIC STELLATE CELLS
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#1417
DIFFERENT REGULATION OF THE CONNECTIVE TISSUE GROWTH FACTOR IN HEPATIC STELLATE CELLS AND HEPATOCYTES
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#1418
RELAXIN INCREASES THE EXPRESSION AND ACTIVITY OF PPARG IN ACTIVATED HEPATIC STELLATE CELLS
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#1419
HSC EXPRESS NALP3 AND ASC, AND UNDERGO DIFFERENTIATION IN RESPONSE TO URIC ACID CRYSTALS
Azuma Watanabe1, Ardeshir Hashmi1, Muhammad N. Jhandier1, Zeeshan Khan1, Fayyaz Sutterwala2, Mohammad A. Sohail1, Shamail Mahmood1, Richard A. Flavell2, Wajahat Z. Mehal1
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#1420
L-THYROXINE (T4) INDUCES PROLIFERATION OF HEPATIC STELLATE CELLS VIA CELL SURFACE INTEGRIN αVβ3 AND ENHANCES EXPRESSION OF α-SMOOTH MUSCLE ACTIN AND COLLAGEN I TRANSCRIPTION
Isabel Zvibel1, Ella Barlev1, Adam Phillips2, Dikla Atias1, Shirley Abramovitch1, Zamir Halpern1, Ran Oren1
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#1421
GLUTATHIONE, BUT NOT CATALASE, IS IMPORTANT IN THE PROTECTION OF HEPATIC STELLATE CELLS AGAINST OXIDATIVE STRESS
Sandra Dunning, Rebekka Hannivoort, Laura Conde de la Rosa, Manon Buist-Homan, Klaas Nico Faber, Han Moshage
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THE ADENINE RECEPTOR IS PRESENT IN RAT HEPATIC STELLATE CELLS (HSC), INDUCES HSC DIFFERENTIATION AND INHIBITS HSC CHEMOTAXIS

Azuma Watanabe, Ardeshir Hashmi, Muhammad N. Jhandier, Samir Gautam, Muhammad A. Sohail, Shamail Mahmood, Zeeshan Khan, Wajahat Z. Mehal
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TARGETED BINDING OF FLUORESCENCE-LABELED CYCLIC RGD PEPTIDES TO ACTIVATED HEPATIC STELLATE CELLS VIA INTEGRIN AVB3 RECEPTOR

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OSTEOPONTIN, AN OXIDANT STRESS-SENSOR IN HEPATOCYTES AND KUPFFER CELLS, TRIGGERS A FIBROGENIC RESPONSE IN STELLATE CELLS

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UPREGULATION OF SMOOTH MUSCLE MARKER GENES IN ACTIVATED HEPATIC STELLATE CELLS IS MEDIATED BY TRANSFORMING GROWTH FACTOR-β INDUCED EXPRESSION OF SERUM RESPONSE FACTOR

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HBV INFECT HEPATIC STELLATE CELLS AND AFFECT THEIR PROLIFERATION AND EXPRESSION OF EXTRACELLULAR MATRIX

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REACTIVE NITROGEN SPECIES MODULATE EARLY EXTRACELLULAR MATRIX REMODELING VIA INDUCTION OF MATRIX METALLOPROTEINASE-1 AND TUMOR NECROSIS FACTOR α

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THY-1 DISTINGUISHES BETWEEN HEPATIC STELLATE CELLS AND LIVER MYOFIBROBLASTS IN VITRO AND IN VIVO

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T3 ENHANCES EXPRESSION OF α-SMOOTH MUSCLE ACTIN AND OF CYTOKINES TNFα AND SDF1α IN PRIMARY STELLATE CELLS, YET INHIBITS COLLAGEN I TRANSCRIPTION

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PRIMARY MOUSE HEPATIC STELLATE CELLS ARE LEPTIN AND ADIPONECTIN RESISTANT

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NOTCH SIGNALING IN NODULAR REGENERATIVE HYPERPLASIA AND HEPATIC MICROCIRCULATION

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EFFECT OF ACUTE AND CHRONIC LIVER INJURY ON HEPATOTROPIC TARGETING PROPERTIES OF ERYTHROCYTE GHOSTS

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ACTIVATION OF KUPFFER CELLS IS INVOLVED IN ALCOHOL-INDUCED DOWN-REGULATION OF HEPcidIN EXPRESSION IN THE LIVER

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DIFFERENTIAL MODULATION OF PRO- AND ANTI-REGENERATIVE CYTOKINES BY TLR STIMULATION IN NON-PARENCHYMAL LIVER CELLS

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CAUSAL KUPFFER CELL ACTIVATION BY FREE FATTY ACIDS SUPPRESSED THROUGH PEROxisome PROLIFERATOR-ACTIVATED RECEPTORS (PPAR) δ

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### #1436

**VARIANT ERYTHROPOIETIC PROTOPORPHYRIA (EPP): A DISORDER WITH SEVERE EPP PHENOTYPE, NO MUTATIONS IN FERROCHELATASE DNA, AND ABNORMAL MITOFERRIN EXPRESSION.**

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### #1437

**DONOR HEPATOCYTES HAVE A SELECTIVE REPOPULATION ADVANTAGE OVER HOST HEPATOCYTES IN ALPHA 1-ANTI-TRYPSIN DEFICIENCY**

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### #1438

**FUNCTIONAL LOSS OF TRANSFERRIN RECEPTOR 2 DECREASES BASAL HEPcidIN EXPRESSION, BUT DOES NOT ABOLISH REGULATION BY IRON OR ERYTHROPOIETIN**

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### #1439

**HEPATOSPLENOMEGALY AND BLOOD PARAMETERS IN GAUCHER DISEASE - ANALYSIS OF DOSE-RESPONSE RELATIONSHIPS IN ENZYME REPLACEMENT THERAPY**

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### #1440

**DIVERGENT PATHOGENESIS FOR HEPATOCYSTIN AND SEC63P ASSOCIATED POLYCYSTIC LIVER DISEASE?**

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### #1441

**EX VIVO GENE THERAPY REVISITED: COMBINATION OF LENTIVIRUS-MEDIATED GENE TRANSFER AND PREPARATIVE IRRADIATION OF HOST LIVER RESULTED IN LONG-TERM AMELIORATION OF JAUNDICE IN A RAT MODEL OF CRIGLER-NAJJAR SYNDROME TYPE 1**

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### #1442

**PULSATILE, PRO-AUTOPHAGIC THERAPY WITH RAPAMYCIN REDUCES THE INTRAHEPATIC ACCUMULATION OF ALPHA-1-ANTITRYPSIN MUTANT Z PROTEIN AND REDUCES LIVER INJURY IN AN IN VIVO MODEL**

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### #1443

**ALCOHOL CONSUMPTION FURTHER MODULATES HEPcidIN GENE EXPRESSION IN AN ANIMAL MODEL OF HEMOCHROMATOSIS**

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### #1444

**REVISITATION OF A COHORT OF 482 ITALIAN PATIENTS WITH HEREDITARY HEMOCHROMATOSIS: CHANGES IN THE LAST THREE DECADES**

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#1445
UPREGULATION OF FERROPORTIN AND DMT1 EXPRESSION IN CIRRHOTIC HUMAN LIVERS: A LINK TO HEMOSIDEROSIS?

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#1446
13C-METHACETIN BREATH TEST: A NEW TOOL TO PREDICT THE PRESENCE OF LIVER VASCULAR MALFORMATIONS IN PATIENTS WITH HEREDITARY HAEMORRHAGIC TELEANGIECTASIA

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#1447
HEPATIC COPPER, IRON AND ZINC CONCENTRATIONS IN A NEAR HISTOLOGICAL RESOLUTION IN WILSON DISEASE DETERMINED BY MICROSCOPIC SYNCHROTRON RADIATION X-RAY FLUORESCENCE

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#1448
LIVER DIRECTED AAV-MEDIATED HOMOLOGOUS RECOMBINATION IS LARGELY INDEPENDENT OF SEROTYPE

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#1449
LONG-TERM FVIII EXPRESSION VIA SLEEPING BEAUTY IN LIVER SINUSOIDAL ENDOTHELIAL CELLS OF HEMOPHILIA A TRANSGENIC MICE

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#1450
BENCHMARK ANALYSIS OF THE ACHIEVEMENT OF THERAPEUTIC GOALS FOR PATIENTS WITH TYPE 1 GAUCHER DISEASE

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Other Viral Hepatitis

#1452
REPLICATION OF HEPATITIS E VIRUS (HEV): LOCALIZATION OF INTRACELLULAR SITE

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#1453
DURATION OF HEPATITIS A IMMUNITY 10 YEARS FOLLOWING VACCINATION

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1454
SCREENING FOR HEPATITIS B VIRUS (HBV) INFECTION BY PRIMARY CARE PHYSICIANS IN NEW YORK CITY: ARE SCREENING RECOMMENDATIONS FOR PERSONS BORN IN ENDEMIC COUNTRIES BEING FOLLOWED?
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Maricruz Velez1, David N. Assis2, Marta Alvarez-Posadilla3, Steven K. Herrine1, Simona Rossi1, Victor J. Navarro1
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#1472
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Denotes AASLD Presidential Poster of Distinction
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HEPATITIS D (HDV) AND B (HBV) VIRUSES GENOTYPES IN CHRONIC LIVER DISEASE PATIENTS FROM EASTERN AMAZON BASIN

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#1479

PRIMARY CARE PROVIDER ADHERENCE TO HCV TESTING RECOMMENDATIONS

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#1480

LIVER DISEASE PROGRESSION AMONG HIV-INFECTED PATIENTS WITH VIRAL HEPATITIS

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#1481

DIAGNOSTIC ACCURACY OF BLOOD TESTS OF LIVER FIBROSIS IN CHRONIC HEPATITIS B: COMPARISON WITH HEPATITIS C

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Late-Breaking Posters

#LB6


E. Jenny Heathcote1, Ed Gane2, Robert DeMan3, Sam Lee4, Robert Flisack5, Michael P. Manns6, Konstantin Tchernev7, Oya Kürdas8, Mitchell L. Shiffman9, Jeff Sorbel10, Jane Anderson10, Elsa Mondu10, Franck Rousseau10

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#LB7

ACTIVATION OF TLR4-TRAF6-TAK1 PATHWAY INDUCES HEPATOCELLULAR CARCINOMAS BY SYNERGISTIC INTERACTIONS BETWEEN ALCOHOL AND HEPATITIS C VIRUS NS5A

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#LB8

EVALUATION OF VIRAL VARIANTS DURING A PHASE 2 STUDY (PROVE2) OF TELAPREVIR WITH PEGINTERFERON ALFA-2A AND RIBAVIRIN IN TREATMENT-NAIVE HCV GENOTYPE 1-INFECTED PATIENTS

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#LB9
ANTIVIRAL ACTIVITY, PHARMACOKINETICS, SAFETY, AND TOLERABILITY OF R7128, A NOVEL NUCLEOSIDE HCV RNA POLYMERASE INHIBITOR, FOLLOWING MULTIPLE, ASCENDING, ORAL DOSES IN PATIENTS WITH HCV GENOTYPE 1 INFECTION WHO HAVE FAILED PRIOR INTERFERON THERAPY

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#LB10
PHASE 2A STUDY TO EVALUATE THE SAFETY AND TOLERABILITY AND ANTI-VIRAL OF 4 DOSES OF A NOVEL, CONTROLLED-RELEASE INTERFERON-ALFA 2B (LOCTERON) GIVEN EVERY 2 WEEKS FOR 12 WEEKS IN TREATMENT-NAIVE PATIENTS WITH CHRONIC HEPATITIS C (GENOTYPE 1)

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#LB11
ANTIVIRAL ACTIVITY OF THE NON-NUCLEOSIDE POLYMERASE INHIBITOR, VCH-759, IN CHRONIC HEPATITIS C PATIENTS: RESULTS FROM A RANDOMIZED, DOUBLE-BLIND, PLACEBO-CONTROLLED, ASCENDING MULTIPLE DOSE STUDY.

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#LB12
RESTORATION OF THE DEFECTIVE INNATE IMMUNE SYSTEM FOLLOWING TREATMENT WITH THE PROBIOTIC LACTOBACILLUS CASEI SHIROTA IN PATIENTS WITH ALCOHOLIC CIRRHOSIS: A PROOF OF CONCEPT STUDY

Vanessa Stadlbauer, Rajeshwar P. Mookerjee, Stephen Hodges, Gavin A. Wright, Nathan Davies, Rajiv Jalan

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#LB13
CONTINUOUS TERLIPRESSIN (TER) INFUSION IMPROVES RENAL FUNCTION IN PATIENTS WITH HEPATORENAL SYNDROME (HRS)

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#LB14
FIRST EVIDENCE THAT ALBUMIN CAN DIRECTLY IMPROVE CARDIAC CONTRACTILITY IN CIRRHOTIC RATS

Giulio Ceolotto, Italia Papparella, Maurizio Cavalli, Antonietta Sirica, Lorenzo Franco, Sergio Bova, Andrea Semplicini, Angelo Gatta, Paolo Angeli

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#LB15
EFFICACY AND SAFETY OF VALOPTICITABINE IN COMBINATION WITH PEGYLATED INTERFERON-α (PEG IFN) AND RIBAVIRIN (RBV) IN PATIENTS WITH CHRONIC HEPATITIS C

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1 DELAYED AND REDUCED DOSE TACROLIMUS WITH MYCOPHENOLATE MOFETIL AND DACLIZUMAB REDUCES RENAL IMPAIRMENT AFTER LIVER TRANSPLANTATION: RESULTS OF A 1 YEAR PROSPECTIVE, RANDOMISED INTERNATIONAL TRIAL

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Introduction: Late onset renal impairment after liver transplantation is a major cause of end-stage renal disease and a risk factor for death. One of the risk factors for late-onset renal impairment is the dose and level of the calcineurin inhibitor during the early months after transplantation. Renal function at one year predicts longer-term renal function. Aim: we did a prospective randomised study to determine whether lower levels with or without delayed introduction of tacrolimus was associated with less renal impairment (as assessed by the Cockcroft-Gault formula) and no adverse effect on patient or graft. Methods: 525 adult recipients of a first graft were randomised to one of three groups in 30 European centers: group A: tacrolimus at the recommended dose (to achieve target levels ≥10ng/mL for the first month); group B: tacrolimus at a lower dose (trough target levels <8ng/mL for the first month and mycophenolate mofetil 2g/day) and group C: daclizumab on day 1 and 7, mycophenolate mofetil 2g/day and tacrolimus introduced on day 5 with trough target levels <8ng/mL. Corticosteroids were given according to local practice in each group. Results: Calculated GFR was 103, 107 and 98 mL/min in groups A, B and C at the start of the study and fell by 25, 24 and 17 mL/min at one year; the difference between A and C was statistically significant (p=0.003). Withdrawals because of adverse events occurred in 29, 18 and 21% and, as a sub-category, withdrawal related to renal impairment occurred in 12, 2 and 1% of the patients. At one year, patient death occurred in 9.2, 10 and 5% respectively and graft loss was 6.0, 5.9 and 6.6% respectively; dialysis was required in 17, 10 and 9% respectively during the first year: there was no difference in the incidence of biopsy-proven acute rejection (31, 30 and 25%). Diarrhoea occurred in 21, 31 and 25%. Conclusions: this study suggests that lower and delayed introduction of tacrolimus together with mycophenolate mofetil and daclizumab is associated with better preservation of renal function at one year without any significant adverse impact on patient or graft.

Disclosures:
James M. Neuberger - Speaker: Astellas; Speaker: Novartis; Speaker: Roche
A. David Mayer - Speaker: Astellas; Speaker: Novartis; Speaker: Roche

2 MYCOPHENOLATE MOFETIL (MMF) IN COMBINATION WITH LOW-DOSES OF CALCINEURIN INHIBITORS (CNI) FOR CHRONIC RENAL DYSFUNCTION AFTER LIVER TRANSPLANTATION (LT): 2-YEAR RESULTS OF A PROSPECTIVE, MULTICENTER, RANDOMIZED STUDY

Georges-Philippe Pageaux1, Lionel Rostaing2, Yvon Calmus3, Christophe Duvoux4, Claire Vanlemmens5, Jean Hardgwissen6, Paul-Henri Bernard7, Francis Navarro8, Dominique Larrey9

The following people have nothing to disclose: Lionel Rostaing, Yvon Calmus, Christophe Duvoux, Claire Vanlemmens, Jean Hardgwissen, Paul-Henri Bernard, Francis Navarro, Dominique Larrey

Chronic renal dysfunction has a major impact on long-term morbidity and mortality after LT. CNI nephrotoxicity represents the main cause. Aim: To introduce MMF in liver transplant recipients with chronic renal dysfunction to decrease CNI dosages without increasing rejection risk. Methods: 2-year prospective, multicenter, randomized study including liver transplant recipients with minimum interval of one year from LT. Chronic CNI-related renal dysfunction was defined as follows: increase in serum creatinin ≥140 µmol/l, proteinuria <1g/24h, absence of hematuria, absence of renal arteries stenosis or urinar tract disease. In all patients, a liver biopsy was performed during the 6 previous months before inclusion and at the end of the study. Patients were randomized in two groups. Group I combination of MMF (2 to 3 g/day) and reduced dose of CNI ≥50% of initial dose; group II: no MMF and reduced dose <25% of initial dose. Results: 56 patients were included in the study and we present the results of 46 patients, 24 in group I et 22 in group II. The two groups were comparable in terms of age, sex, etiology, indication for LT, interval from LT to inclusion, initial dose of CNI, initial serum creatinine and creatinine clairance. In group I, the mean daily doses of cyclosporine and tacrolimus were 163.2 mg and 4.4 mg at day 0, and 62.2 mg and 1.85 mg at month 24, respectively. In group II, there were 145.4 mg and 2.75 mg at day 0, and 102 mg and 1.5 mg at month 24, respectively. In group I, the mean serum creatinine and creatinine clairance were 171.7 µmol/l and 42.6 ml/mn at day 0, 142.8 µmol/l and 51.7 ml/mn at month 12, and 139.6 µmol/l and 48.3 ml/mn at month 24, respectively. In group II, they were 175.4 mg and 42.8 mg at day 0, 170.5 µmol/l and 44.8 ml/mn at month 12, and 166 µmol/l and 37.2 ml/mn at month 24, respectively (p=0.004 for Δ creatinine and 0.006 for Δ clairance between D0 and M12 and M12 and M24 ; no significant difference between M12 and M24). None biological rejection episode was observed in group I. There was no significant difference between the two groups in terms of secondary effects. Graft biopsy was performed in 7 patients of group 1: there were rejection-compatible lesions in 1 patient. Conclusion: In this 2-year prospective, randomized study, the reduction of at least 50 % of CNI dose combined to MMF, allowed to significantly improve the renal function of liver transplant recipients, without any biological rejection episode and without significant secondary effects. However, improvement was achieved at month 12, and there was no supplementary improvement between month 12 and month24.

Disclosures:
Georges-Philippe Pageaux - Consultant/Adviser: Roche
The following people have nothing to disclose: Lionel Rostaing, Yvon Calmus, Christophe Duvoux, Claire Vanlemmens, Jean Hardgwissen, Paul-Henri Bernard, Francis Navarro, Dominique Larrey
3 HOSPITALIZATION RATES BEFORE AND AFTER ADULT-TO-ADULT LIVING DONOR OR DECASED DONOR LIVER TRANSPLANTATION

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Background: Adult-to-adult living donor liver transplant (LDLT) recipients are reported to have higher complication rates than deceased donor (DDLT) recipients. This study examined resource utilization using hospitalization rates before and after transplant for LDLT and DDLT recipients. Methods: Data from the 9-center Adult-to-Adult Living Donor Liver Transplantation (A2ALL) retrospective cohort study were analyzed to determine pre-transplant (PRE), transplant (TXP), and post-transplant (POST) hospitalizations among LDLT candidates (potential living donor was evaluated) who received LDLT or DDLT. Hospital days and admission rates for LDLT and DDLT patients were calculated per patient-year at risk, starting from the date of initial potential donor history and physical examination. Rates were compared using Poisson regression. Results: Among 806 candidates, 384 received LDLT and 216 received DDLT. In addition to the transplants, there were 1678 recipient hospitalizations (252 PRE; 1426 POST). Mean PRE, TXP, and POST length of stay (LOS) was 5.7±6.2, 28.6±36.6, and 9.1±14.3 days for LDLT recipients and 4.1±3.7, 23.4±31.9, and 8.9±27.9 days for DDLT recipients, respectively. Compared to DDLT, LDLT recipients had significantly lower PRE hospital day and admission rates, but significantly higher POST rates (Table). Higher LDLT admission rates were observed for non-biliary reasons during the first post-transplant year and for biliary tract morbidity throughout follow-up. Patients were discharged to destinations other than home in 9.7% and 6.0% of POST hospitalizations for DDLT and LDLT, respectively (P=0.02). Conclusions: After transplantation, recipients of LDLT have significantly and persistently higher hospitalization rates than DDLT recipients. Higher hospitalization rates after LDLT in the first year were due to biliary and non-biliary reasons. During later follow-up, persistently higher rates of LDLT biliary tract morbidity requiring hospitalization were observed. LDLT candidates should be informed about the likelihood of higher hospitalization rates.

<table>
<thead>
<tr>
<th>Total hospital days per patient-year</th>
<th>Hospital admissions per patient-year</th>
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<td>1.72±0.10</td>
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4 FACTORS INFLUENCING LIVER TRANSPLANT LENGTH OF STAY AND RESOURCE UTILIZATION AT TWO LARGE VOLUME TRANSPLANT CENTERS

Sandy Feng1, John P. Roberts1, Glenn A. Halff2, Kenneth Washburn3; 1Surgery, UCSF, San Francisco, CA; 2Transplant Center, Univ of Texas HSC, San Antonio, TX

Background: Length of stay (LOS) following liver transplantation (OLT) has been shown to be a reliable surrogate for costs associated with OLT. Little information exists regarding the influence of donor and recipient variables on LOS. Methods: Extensive data for adult non-status 1 OLTs from 1998-2005 were collected from two institutions (A, n=745; B, n=710) geographically removed from one another. The Donor Risk Index (DRI) and laboratory MELD scores were calculated to represent donor quality and recipient disease severity, respectively. Cox models were constructed to evaluate variables most likely to influence LOS for each institution's cohort and the combined cohorts. Results: The two OLT cohorts significantly differed in recipient diagnosis, race, gender, BMI (A:26.9; B:28.7;p<.0001), need for pre-tx HD (A:12.5%; B:8.5%; p<.0001), and donor race, gender (female; A:33.4%; B:40.1%; p=.0079), age (A:40.8 yrs; B:39.3 yrs; p=.03), % local donors (A:78.9%; B:82.1%; p=.0007), CIT (A:9.5 hrs; B:6.2 hrs; p<.0001) and DRI (A:1.46; B:1.40; p=.0013). LOS was comparable (A: 13.7 days; B:13.3 days; p=.052) although MELD was higher at A (A:22.4; B:20.4; p=.046). Cox models showed that increased R age, R MELD and non-local donor were associated with longer LOS for Institution A. For Institution B, increased R MELD and donor age, longer CIT, Re-Tx, R female, and decreased R INR and D weight were associated with a longer LOS (Table). For the combined cohort, non-local donor, R female gender, Re-tx, increased R MELD, R Age, D Age and decreased D weight were risk factors for increased LOS. Conclusions: The influence of recipient and donor factors on LOS varies by institution. This may reflect programmatic differences in practice patterns, maturity, philosophy, and geography. Donor age and non-local donors influence LOS for the combined cohort, yet DRI, encompassing multiple donor variables did not impact LOS. Recipient MELD has a consistent and significant impact on LOS for both institutions emphasizing the dominant role of recipient disease severity on LOS and presumably resource use following OLT.
Multivariate Cox Model for OLT LOS (HR=Hazard Ratio)

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Disclosures:
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6

REGIONAL DIFFERENCES IN DECEASED DONOR LIVER UTILIZATION AND ORGAN WASTAGE

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Background: There is significant regional variation in liver utilization criteria. Aim: To estimate the number of livers left untransplanted due to regional differences in organ use. Methods: We selected deceased persons successfully donating at least one solid organ between 7/15/00 and 7/17/05 from the UNOS database. Within each region, we performed logistic regression analysis for liver exported or not transplanted vs. local or regional transplant. Age, obesity, donation after cardiac death, donor ALT, bilirubin, anti-HCV (+), anti-HBc (+) and AB blood type were examined as predictors of export or liver not transplanted. With intent for best case scenario, we identified the most 'lenient' region(s) for regional liver use based on smallest predictor adjusted odds ratios (OR) and fewest significant predictors. We identified untransplanted livers from regions with stricter criteria for organ use, and determined the odds of being used by the more lenient region(s). For those with > 1.5 odds of being used by the more lenient region(s), we calculated donor risk indices (DRI's) for 6, 8 and 12 hour cold ischemia times. Results: Of 30,474 livers, 23,535 were transplanted within their region of procurement, while 1883 were exported and transplanted and 5056 were not transplanted. Older donor age was a significant predictor of non-use for all regions except Region 9 (age 50-60, OR=1.29, 95% CI: 0.8-2.2, p=0.34). Similarly, only Region 9 had predictors with smaller and fewer significant OR’s for non-use compared to all other regions (median OR: 1.4 vs 3.6; p<0.01; median number of significant ORs: 6 vs. 16; p=0.01). 4798 livers were left untransplanted from regions 1-8 and 10-11. 4,504 had >1.5 odds for being transplanted by the more lenient region(s).

Summary: Regional differences in criteria for liver use exist with the most lenient centers. Conclusions: Under the present system of regional differences in organ use, a small number of potentially usable livers are left untransplanted presumably due to fears of extended cold ischemia time or other non-captured variables (e.g. liver histology). Improved communication through a national, electronic sharing system that improves logistics and lowers cold ischemia time may increase transplants by only 2-3% in the best case scenario.
7

HEPATITIS C (HCV): 3 STUDY: DOES IMMUNOSUPPRESSION (IS) AFFECT THE PROGRESSION OF FIBROSIS OF HCV RECURRENCE AFTER LIVER TRANSPLANTATION (OLT)?


Aims: To assess the efficacy and the safety of a steroid (Pred)-free IS regimen using daclizumab (DAC), tacrolimus (TAC) and mycophenolate mofetil (MMF) to minimize the incidences of acute cellular rejection (ACR), HCV recurrence and adverse events post OLT. Methods: Open-label, prospective, multicenter study (n=18) involving 312 adult HCV-OLT recipients randomized, pre-OLT (1:1:2), to 3 IS arms (see Results). Laboratory data and liver histology were obtained when clinically indicated and, by protocol, at days 90, 365 and 730. Protocol biopsies were successfully accomplished in: 81%, 69% and 54%, respectively. ACR was graded by Banff schema. HCV recurrence was staged by Batts and Ludwig classification. Clinically significant primary endpoints: ACR (Grade ≥2 + RAI ≥4) and/or HCV recurrence (Stage ≥2 or Grade ≥3 at any time). Significant statistics: p ≤ 0.05. Results: 312 patients were studied. Arm 1 (n=80): TAC + Pred; Arm 2 (n=79): TAC + Pred + MMF; Arm 3 (n=153): DAC (3 doses, post op. days: 1, 3 and 8th) + TAC + MMF. All data (intention to treat [ITT]; central protocol [PP]/CP and PP/LP). No differences across arms were found for HCV RNA levels at any time point. Similar death causes: HCV recurrence (5, 2, 4), respiratory (1, 5, 4), malignancy (2, 4, 1), sepsis (2, 1, 3); others (5, 2, 6). Conclusions: This 2-year report suggests that recurrence of HCV is not affected by immunosuppression. However, the rate of progression to stage ≥3 fibrosis, in year 2, may be influenced by the presence of MMF and steroids. Longer-term follow-up is indispensable to confirm these initial findings on HCV recurrence.


8

SCHOOL OUTCOMES IN CHILDREN REGISTERED IN THE STUDIES FOR PEDIATRIC LIVER TRANSPLANT (SPLIT)


Aims: To assess the efficacy and the safety of a steroid (Pred)-free IS regimen using daclizumab (DAC), tacrolimus (TAC) and mycophenolate mofetil (MMF) to minimize the incidences of acute cellular rejection (ACR), HCV recurrence and adverse events post OLT. Methods: Open-label, prospective, multicenter study (n=18) involving 312 adult HCV-OLT recipients randomized, pre-OLT (1:1:2), to 3 IS arms (see Results). Laboratory data and liver histology were obtained when clinically indicated and, by protocol, at days 90, 365 and 730. Protocol biopsies were successfully accomplished in: 81%, 69% and 54%, respectively. ACR was graded by Banff schema. HCV recurrence was staged by Batts and Ludwig classification. Clinically significant primary endpoints: ACR (Grade ≥2 + RAI ≥4) and/or HCV recurrence (Stage ≥2 or Grade ≥3 at any time). Significant statistics: p ≤ 0.05. Results: 312 patients were studied. Arm 1 (n=80): TAC + Pred; Arm 2 (n=79): TAC + Pred + MMF; Arm 3 (n=153): DAC (3 doses, post op. days: 1, 3 and 8th) + TAC + MMF. All data (intention to treat [ITT]; central protocol [PP]/CP and PP/LP). No differences across arms were found for HCV RNA levels at any time point. Similar death causes: HCV recurrence (5, 2, 4), respiratory (1, 5, 4), malignancy (2, 4, 1), sepsis (2, 1, 3); others (5, 2, 6). Conclusions: This 2-year report suggests that recurrence of HCV is not affected by immunosuppression. However, the rate of progression to stage ≥3 fibrosis, in year 2, may be influenced by the presence of MMF and steroids. Longer-term follow-up is indispensable to confirm these initial findings on HCV recurrence.


The following people have nothing to disclose: Paul H. Hayashi, Paolo R. Salvalaggio, David A. Axelrod, Schnitzler Mark.
9 ERYTHROPOETIN IMPROVES LIVER REGENERATION OF THE DONOR AND RECIPIENT IN A RAT MODEL OF LIVING RELATED LIVER TRANSPLANTATION

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Introduction: Living donor liver transplantation (LDLT) has become an important procedure to overcome the growing organ shortage. In this setting, the unique regenerative capacity of the liver is vital for the clinical outcome of donor and recipient. Insufficient liver regeneration is still a major problem in LDLT. Recently, it has been stated that erythropoietin (EPO) has a wide range of non-erythropoietic functions. Aim of our study was to investigate the potential role of EPO as an exogenous stimulator for liver regeneration in the clinical context of LDLT. Material and Methods: Donor male Lewis rats were preconditioned with a dose of 1 I.E. EPO/g/BW i.p. at the 12th, 11th and 10th preoperative day. Recipient or resected rats received EPO at a dose of 5 I.E./g/BW i.v. perioperatively when undergoing partial liver transplantation (pLTx), 70% or 90% partial hepatectomy (PH). Controls were treated with heat-inactivated EPO in the same vehicle at the same timepoints. Samples were taken to investigate liver serum and functional parameters, liver body weight ratio (LBWR), hepato-sinusoid index (4.6 ± 0.3 vs. 3.56 ± 0.15; p<0.05), which occurred subsequently to the increase in Ki-67 proliferation index (4.6 ± 1.3 vs. 1.1 ± 0.5; p<0.001) in the intact, non-resected liver. Exogenous administration led to an improved liver regeneration as shown by increased Ki-67 proliferation index, LBWR and serum parameters such as AST, ALT, GLDH and bilirubin as well as PTT and INR 24h and 5 days after pLTx, 70% or 90% PH compared to control rats. Mechanistically, EPO markedly prevented the apoptosis of hepatocytes 24h after pLTx. Treatment with EPO led to a significantly improved overall survival after 90% PH (88% vs. 58%, p<0.05). Furthermore we demonstrated an 88% (7/8) survival in rats receiving EPO compared to only 38% (3/8) in rats with vehicle 28 days after pLTx. (p=0.03). Discussion: We have shown that exogenous administration of EPO induces a proliferative response in hepatocytes resulting in an improved liver regeneration following 70% PH and 90% PH. Furthermore, using this dose regimen, EPO-treated rats had a statistically significant improved overall survival after pLTx.

10 OUTCOMES AFTER HEART TRANSPLANTATION AMONG RECIPIENTS WITH CHRONIC HEPATITIS C INFECTION

Tse-Ling Fong1, James Cicciarelli2, Yong-Won Cho3; 1University of Southern California, Los Angeles, CA; 2National Institute of Transplantation, Los Angeles, CA

BACKGROUND: Prior studies examining the impact of hepatitis C infection (HCV) after heart transplant (OHT) do not differentiate between pre-existing chronic HCV infection (prior to OHT) or de-novo acquisition of HCV during or following OHT. Despite the paucity of data regarding the outcomes of heart transplant recipients who are HCV-positive prior to transplant, many transplant centers are declining to perform OHT on HCV-positive patients based on the experience of using HCV-negative allografts in HCV-negative recipients which is associated with decreased survival. AIM: This study was undertaken, utilizing the OPTN/UNOS database, to assess the clinical outcome of HCV-positive compared to HCV-negative heart transplant recipients. METHODS: Outcomes for OHT recipients are affected by the era of transplantation and immunosuppression. Therefore, analysis was limited to the five-year period between January 1, 2000 and December 31, 2005. 224 HCV-positive and 10,406 HCV-negative recipients who received HCV-negative donor organs were identified. Since co-morbid factors significantly influence early mortality rate after transplantation, logistic regression analysis was performed for early phase (6 months) and Cox regression analysis for late phase (beyond 6 months post-transplant). Outcome variables compared between the two groups included patient survival and acute rejection rates. RESULTS: Overall patient survival rates of HCV-positive recipients were significantly lower than those of HCV-negative recipients (88.0% at 1-yr, 83.4 3-yr, 74.2% 5-yr vs. 87.1% at 1-yr, 79.2% 2-yr and 72.6% 3-yr HCV-negative recipients, p<0.01). However, after adjusting for potential confounding factors including donor age, recipient sex, age, race, obesity, congenital heart disease, peak PRA, re-transplant, HBV core antibody positive, renal insufficiency, ICU and status 1A at time of transplantation), recipient HCV positive status was no longer a significant risk factor in either early or late phase (OR=1.22, p=0.356 for early phase and RR=1.36, p=0.091 for late phase (Table). Causes of death among HCV-positive and HCV-negative groups were similar. Cumulative incidence of acute rejection episode among HCV-positive recipients was 37.5% vs. 32.6% HCV-negative recipients (p=0.32). CONCLUSIONS: A more rational approach should be developed for the management of HCV-positive heart transplant candidates. Carefully selected HCV-positive patients should not be excluded from OHT.
A RANDOMIZED STUDY TO ASSESS THE SAFETY AND EFFICACY OF ADEFOVIR DIPIVOXIL SUBSTITUTION FOR HEPATITIS B IMMUNE GLOBULIN IN LIVER TRANSPLANTATION PATIENTS RECEIVING LONG-TERM LOW DOSE IM HBIG AND LAMIVUDINE PROPHYLAXIS

Peter W. Angus1, Simone I. Strasser2, Scott Patterson1, Geoff W. McCaughan2, Ed Gane3; 1Liver Transplant Unit, Austin Health, Melbourne, VIC, Australia; 2AW Morrow GE and Liver Centre, Royal Prince Alfred Hospital, Sydney, NSW, Australia; 3NZ Liver Transplant Unit, Auckland Hospital, Auckland, New Zealand

Combination hepatitis B (HBV) immune globulin (HBIG) and lamivudine (LAM) provides effective prophylaxis against graft re-infection following liver transplant (OLTx) but HBIG is expensive and inconvenient, necessitating long-term monthly clinic follow-up. The aims of this study were to determine the safety, efficacy and economics of substituting adefovir dipivoxil (ADV) for HBIG, whilst continuing LAM, in patients transplanted for HBV related liver disease. This was a randomized, prospective, multi-centre, open label study of adults at least 12 months post-OLTx for HBV disease who were receiving HBIG and LAM prophylaxis without recurrence of HBV infection in the graft. No OLTx grafts had been obtained from executed prisoners or other institutionalized persons. Patients were randomized (1:1) to receive ADV 10mg daily or to continue HBIG. LAM therapy was continued in both groups. Lactating or pregnant females as well as patients with HIV or a creatinine >180 µmol/L were excluded. All direct costs of antiviral prophylaxis were recorded (LAM, ADV, HBIG, clinic visits). Patients were reviewed at least 3 monthly until study completion. 34 patients were randomized: 16 to ADV/LAM (one withdrew consent after 2 months and is not included in the analysis) and 18 to continue HBIG/LAM. Patient groups at baseline did not differ significantly in time since OLTx, ALT, renal function, HBsAb titre, HBeAg status, Hepatitis C or D status, duration of prior antiviral therapy, or HBV DNA load at OLTx. One patient on ADV became HBsAg positive after 5 months but is persistently HBV PCR undetectable with a further 20 months of follow-up. All remaining patients are HBsAg and HBV DNA undetectable at a median of 17.2 months (range 7-31) from randomization. One patient on ADV had deterioration in renal function (creatinine 198 µmol/L from baseline 136 µmol/L in setting of diabetic nephropathy) requiring dose adjustment and ultimately ADV cessation after 15 months (peak creatinine 211 µmol/L). No other patient required dose adjustment (mean change in creatinine for other ADV patients +6 µmol/L). There were no further adverse events related to study therapy. Cost effectiveness analysis (using costs from the largest centre) is in favor of combination ADV/LAM therapy: $US 7,277 vs. $US 12,505 per treatment-year for LAM/HBIG (not including other societal costs). In adults more than one year post-OLTx for HBV disease who are without evidence of graft re-infection, switching from HBIG/LAM to ADV/LAM provides safe, effective and convenient protection against recurrent HBV. This strategy significantly reduces costs of long-term antiviral prophylaxis.

Disclosures:
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Geoff W. McCaughan - Consultant/Adviser: Gilead

The following people have nothing to disclose: Simone I. Strasser, Scott Patterson, Ed Gane

DECLINE IN THE NEED FOR LIVER TRANSPLANTATION FOR END STAGE LIVER DISEASE SECONDARY TO HEPATITIS B IN THE US

W. Ray Kim1, Joanne T. Benson1, Andrew Hindman2, Carol Bros- gart2, Carolyn Fortner-Burton2; 1Mayo Clinic College of Medicine, Rochester, MN; 2Gilead Sciences, Foster City, CA; 3United Network for Organ Sharing, Richmond, VA

Background/Aims: Liver transplant (LTx) physicians have anecdotally noticed that fewer patients seem to come to LTx for hepatitis B (HBV)-related end stage liver disease, coincident with widespread application of effective antiviral agents. We analyzed nationwide data on patients who were waitlisted for HBV-related liver disease in the US. Methods: All LTx candidates registered to the Organ Procurement and Transplantation Network for LTx between 1994 and 2006 were identified. All patients whose primary diagnosis included hepatitis B and/or hepatitis C (HCV) were included in the analysis. The outcome of waiting on the list was categorized into LTx, death and improvement. Results: The figure compares the number of registrants with HBV and HCV between 1994 and 2006. There was a rapid increase in the number of registrants with HBV and HCV in the 1990s. However, the number of registrants for HBV peaked in 2000 (n=586), which was followed by a 30% reduction in the ensuing 6 years (n=409 for 2006). This was in contrast to the number of registrants with HCV, which dipped in 2002 following implementation of MELD then regained its previous level. Among HBV patients, the proportion of registrants of Asian race increased from 12% to 35% and African race from 5% to 15% between 1994 and 2006. The proportion of HBV patients with HCC at the time of LTx increased from 8% to 12% over time. Conclusions: The number of patients registered for LTx in the US for decompensated liver disease secondary to HBV decreased substantially since 2000, a trend not mirrored in HCV. Many other epidemiologic explanations for this trend are feasible, but widespread application of antiviral agents may have contributed to the decreased incidence of decompensated liver disease.

Disclosures:
W. Ray Kim - Consultant/Adviser: Gilead; Consultant/Adviser: Bristol-Myers Squibb; Consultant/Adviser: Indenix
Andrew Hindman - Employee: Gilead
Carol Brosgart - Employee: Gilead

The following people have nothing to disclose: Joanne T. Benson, Carolyn Fortner-Burton
MECHANISMS OF IMMUNE EVASION BY TRANSITION VARIANTS OF HEPATITIS C VIRUS

Jane H. Wang1,2, Matthew J. Pianko1, Xiaogang Ke2, Scott Cotler2, Peter F. Whilington1; 1Pediatrics, Division of GI, Hepatology & Nutrition, Northwestern University, Chicago, IL; 2Medicine, Section of Hepatology, University of Illinois at Chicago, Chicago, IL

Introduction: Antigenic variation is an effective way by which viruses evade host immune defence leading to viral persistence. Little is known about the inhibitory mechanisms of viral variants on CD4 T cell functions. Here we show evidence that naturally occurring antigenic variants of a CD4 epitope derived from hepatitis C virus (HCV) transform a protective peripheral Th1 immune response into an inhibitory Th3 and/or Tr1 response.

Methods: Peripheral blood mononuclear cells (PBMCs) from patients infected with HCV for less than 5 years were cultured with synthetic peptides of a CD4 epitope derived from the NS3 protein of HCV and its variant peptides with single or double amino acid substitutions. The experiments were evaluated by proliferation assay and flow cytometry with the cultured cells and cytokine ELISA with the cultured supernatant. Results: HCV variants resulting from single or double amino acid substitutions at the center of the core region of the Th1 peptide not only induce failed T cell activation and proliferation but also simultaneously up-regulate inhibitory cytokine IL-10, CD25-TGF-beta+ Th3 and CD4+IL-10+ Tr1 cells compared to wild type peptide. Other HCV variants tend to induce higher levels of TGF-beta that promote differentiation of CD25+ Th3 suppressors. Either CD25-TGF-beta+ or CD25+TGF-beta+ Th3 suppressors play a critical role in inhibition of T cell proliferation and cell contact is necessary for the inhibitory function of those Th3 cells. In addition, CD25+ cells stimulated by HCV variants showed characteristics of regulatory T cells supported by their expression of higher levels of the transcription factor forkhead box P3 (Foxp3) and cytotoxic T lymphocyte-associated antigen 4 (CTLA-4). In contrast, the wild type peptide down-regulated the expression of Foxp3 and CTLA-4 on CD25+ cells compared to cells cultured with medium alone. Discussion: Our data reveal multiple mechanisms of peripheral immune modulation initiated or enhanced by naturally occurring HCV variants of a CD4 epitope in the early course of HCV infection in humans. Because these CD4 variants can transform a protective peripheral Th1 immune response into an inhibitory Th3 and/or Tr1 response, we call them "transition variants". The modulation of transition variants on CD4 response is efficient and extensive, and is likely critical in viral persistence in HCV infection.

Disclosures:
The following people have nothing to disclose: Jane H. Wang, Matthew J. Pianko, Xiaogang Ke, Scott Cotler, Peter F. Whilington

ENDOPLASMIC RETICULUM (ER) STRESS ASSOCIATED WITH DOWNREGULATION OF THE UNFOLDED PROTEIN RESPONSE (UPR) IN LIVERS OF PATIENTS WITH CHRONIC HEPATITIS C

Tarik Asselah1, Ivan Bièche2, Ingrid Laurendeau2, Dominique Cazals-Hatem3, Gérard Feldmann4, Pierre Bedossa5, Valérie Paradis1, Didier Lebrec1, Eric Ogier-Denis1, Abdel Mansouri1, Michel Vidaud5, Patrick Marcellin, Richard Moreau

In hepatocytes, the accumulation of unfolded proteins in the ER lumen causes ER stress that induces the UPR. The UPR is mediated by the activation of 3 ER-membrane proteins: ATF6, IRE1 and PERK. ATF6 induces XBP1 mRNA and IRE1 cuts XBP1 mRNA to produce spliced XBP1 mRNA which codes for the transcription factor XBP1[S] inducing EDEM mRNA (involved in degradation of unfolded proteins). PERK attenuates global protein synthesis. Since in vitro studies have shown that cells carrying hepatitis C virus (HCV) subgenomic replicons exhibit ER stress and an altered UPR, we aimed to investigate in vivo ER stress and the UPR in liver samples from patients with chronic hepatitis C (CHC). We used electron microscopy to study hepatocyte ER morphology in livers with mild CHC (n=6). Real-time RT-PCR was used to study the mRNA expression of 24 selected genes involved in the UPR in 34 individual liver samples (normal (n=6); HCV-related fibrosis (mild, n=13; advanced, n=15). Livers with mild hepatitis B virus (HBV)-related fibrosis (n=10) were also studied. In mild CHC, electron microscopy showed that most hepatocytes had ER stress because their granular ER was slightly dilated, disorganized, and scattered. In keeping with this, the ratio of spliced to unspliced XBP1 mRNA, a marker of ER stress-elicited IRE1 activity, was significantly higher in liver samples with mild fibrosis than in normal livers. However, the relative levels of EDEM mRNA, a prototypical XBP1[S] target, were not induced in mild fibrosis suggesting a defect of the IRE1 pathway downstream of XBP1 mRNA splicing. The levels of ATF6-dependent mRNAs, i.e., XBP1, GRP78, GRP94, calnexin, calreticulin, SERCA2 were not significantly altered in livers with mild fibrosis, indicating that ER stress did not result in ATF6 activation. Moreover, in these livers, we found evidence of a defect in the PERK pathway because there was a significant decrease in the relative levels of GADD34 mRNA and no induction of the proapoptotic CHOP mRNA. In HCV-related advanced cirrhosis, we found a more marked defect in the IRE1 and ATF6 pathways. In livers with HBV-related mild fibrosis, we only found a decrease in XBP1 mRNA levels. Interestingly, CREB4, an hepatocyte-specific gene, which links ER stress to the hepatic acute-phase response, was induced in HCV-related fibrosis (mild and advanced) but not in HBV. In both HCV- and HBV-related fibrosis, NF-kB and AP1 target mRNAs were activated. In conclusion, livers from patients with CHC exhibit ER stress and downregulation of the 3 arms of the UPR. These results which seem specific for HCV, suggest that UPR downregulation allows unrestricted synthesis of HCV proteins in hepatocytes.

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PP2A OVEREXPRESSION IN CHRONIC HEPATITIS C INHIBITS INSULIN SIGNALING IN THE LIVER

Christine Bensmeeer1, Francois Duong1, Verena Christen1, Luigi Terracciano2, Markus Heim1, 1Gastroenterology and Hepatology, University Hospital Basel, Basel, Switzerland; 2Institute of Pathology, University Hospital Basel, Basel, Switzerland

Background: Epidemiologic studies suggest a direct role of HCV in insulin resistance development and type 2 diabetes. Moreover presence of insulin resistance and diabetes are risk factors for fibrosis progression and nonresponse to antiviral therapy in chronic hepatitis C (CHC). In previous publications we have shown that protein phosphatase 2A (PP2A) is overexpressed in liver biopsies from patients with chronic hepatitis C and inhibits interferon alpha signaling. Interestingly, PP2A has been described to inhibit also insulin signaling in adipocytes. Moreover, some experimental data suggest that HCV interferes with the insulin signaling cascade. We therefore tested if HCV...
induced hepatic PP2A overexpression not only inhibits interferon signaling but also insulin signaling. Methods: We studied insulin signaling in UPP2AC8 and UHCv57.3 cells, that allow the inducible overexpression of PP2Ac and HCV proteins, respectively. Insulin signaling was further studied in the liver of HCV transgenic mice and in human liver biopsies. Results: In cell culture we observed inhibition of insulin signaling by overexpression of PP2A at the level of PKB/Akt phosphorylation. Inhibition of PKB/Akt phosphorylation in response to insulin was also observed in HCV transgenic mice. In liver biopsies from HCV patients we confirmed PP2A overexpression and found inhibition of PKB/Akt phosphorylation in response to insulin treatment. Furthermore, AMPK (adenosine monophosphate-activated protein kinase) phosphorylation was also regulated by PP2A cells and was reduced in HCV transgenic mice. Conclusion: HCV induced PP2A interferes with insulin and AMPK signaling promoting insulin resistance in hepatocytes. This mechanism could explain the high prevalence of type 2 diabetes in patients with CHC.

Disclosures: The following people have nothing to disclose: Christine Bernsmeier, Francois Duong, Verena Christen, Luigi Terracciano, Markus Heim

16 IFN-α AND TGF-BETA MODULATE THE CELLULAR UPTAKE OF HEPATITIS C VIRUS
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Background. Although IFN-α has been used for the treatment of HCV for more than 15 years, its antiviral mechanism of action is still largely hypothetical. Preliminary data utilizing genome-wide expression profiling by microarray analysis, suggested an influence of IFN-α on the expression of potential HCV binding cell surface molecules. Therefore, the aim of this study was to determine the influence of IFN-α on HCV uptake into liver cells and whether this process could be further modulated by the anti-inflamatory cytokines such as TGF-β. Materials and Methods. Huh-7 human hepatoma cells were treated with IFN-α for 8, 12, 24 or 48 h, respectively, and then incubated with MLV (mouse leukemia virus)-HCV pseudoparticles (HCVpp) expressing the viral envelope glycoproteins for 4 h to achieve complete infection. Infection was measured by luciferase assay 48 h later as well as by quantification of early reverse-transcribed long terminal repeats (LTR) of MLV by quantitative real-time PCR assay after 4 h of contact with target cells. Results. Pretreatment of Huh-7 with IFN-α for 24 or 48 h resulted, in a dose dependent manner, in a significantly decreased luciferase activity and LTR detection, initially leading to the suggestion that HCV binding or uptake is reduced by the cytokine. However, after 8 or 12 h of IFN-α pretreatment, LTR detection revealed a strong transient increase in the pseudoparticles. To elucidate the possible underlying molecular mechanisms, IFN-α induced modulation of the expression of cell surface molecules involved in HCV uptake (CD 81, SR-BI, Claudin-1) was studied. Consistent with the findings above, SR-BI expression was elevated during the first 12 hours of IFN treatment followed by a marked suppression thereafter. In contrast, Claudin-1 expression was not altered by IFN treatment. Pretreatment of hepatoma cells with the anti-inflammatory cytokine TGF-beta prior to addition of IFN-α counteracted the IFN-induced decrease of HCVpp uptake and showed a 4 fold increase of HCVpp after 24 h. Conclusions. These results show, for the first time, that IFN-α and other anti-inflammatory cytokines such as TGF-beta might deploy their antiviral activity not only by inhibition of HCV replication, but have the potential to additionally modulate the initial steps of infection, thereby contributing to disruption of the infection-reinfection cycle of the virus.

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17 DEFECTIVE T HELPER 1 RESPONSE BY HEPATOCE- STIMULATED CD4 T CELLS IMPAIRS ANTI-VIRAL CD8 RESPONSE AND VIRAL CLEARANCE
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Background: During hepatitis, hepatocytes gain the ability to express major histocompatibility complex (MHC) class II molecules and to present antigen to CD4 T cells. Here, we investigated whether MHC class II-expressing hepatocytes influence virion the differentiation of CD4 T cells, and in vivo the T cell response to and control of viral infection. Methods: Class II transactivator-transgenic hepatocytes, which constitutively express MHC class II molecules (Hepatology 37:1079-85), were used to stimulate CD4 T cells in vitro, and the effector response type of the stimulated CD4 T cells was determined. In vivo relevance of the obtained findings was confirmed by infecting non-transgenic or class II transactivator-transgenic mice with lymphocytic choriomeningitis virus (LCMV). Results: MHC II-expressing hepatocytes induced T helper (Th) 2 differentiation of uncommitted CD4 T cells and abrogated the ability of previously differentiated Th1 cells to secrete interferon-gamma, even in the presence of pro-inflammatory microbial signals. The suppression of Th1 responses by hepatocytes was associated with poor expression levels of Th1 promoting Delta-like Notch-ligands. In vivo, MHC II-expression by hepatocytes impaired the interferon-gamma production by LCMV-specific CD4 and CD8 T cells and prolonged viral persistence. Conclusions: By instructing infiltrating CD4 T cells to differentiate into a less inflammatory phenotype, MHC II-expressing hepatocytes seem to impair anti-viral CD8 T cell responses and viral clearance. Thus, hepatocytes may contribute to chronicity of hepatitis virus infection.

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called these ‘exposed uninfected’ (EU) cases and have studied them for evidence of HCV-specific T cell responses as a marker of HCV exposure and potential resistance to infection. Methods: 40 EU cases were studied. All tested negative for HCV antibodies and HCV RNA. Details of injecting behaviour were ascertained by structured questionnaire. Peripheral blood mononuclear cells (PBMC’s) were isolated and interferon-gamma (IFN-g) production in response to a range of HCV proteins (Core, NS3, NS4, NS5) was assessed by Enzyme Linked Immunospot Assay (ELISpot) and compared with 21 healthy controls. Flow cytometry was used to characterise circulating CD4 memory subsets in a proportion of EU cases and compared with chronic HCV cases. Results: All EU cases reported sharing needles or other injecting paraphernalia. The mean duration of drug injecting was 9.3 years (0.5 - 26 years) with a mean estimated number of injection episodes of 9310 (range 156 to 28,470). Of the 40 EU cases 22 (55%) produced IFN-g in response to HCV proteins compared to 3 (19%) of the 21 controls (p = 0.002). 8 EU cases responded with IFN-g production to two or more HCV antigens compared to none of the controls (p=0.02). IFN-g production was found most frequently in response to NS3. Core (p = 0.01), NS3 (p=0.04) and NS5 (p=0.03) elicited stronger responses in EU cases than controls. Characterisation of CD4 memory subsets showed that, EU cases, like healthy controls, had a higher proportion of circulating naïve CD4 cells (CD45RA +ve, CD62L +ve)(p=0.03) and a lower proportion of effector cells (CD45RA +ve, CD62L -ve) (p=0.007) than chronically infected HCV cases. Conclusion: The majority of long-term IDU’s who remain uninfected by HCV have demonstrable HCV-specific T cell responses. This contrasts with controls where such responses are rarely seen and weaker. CD4 phenotyping in EU cases is distinct from chronically infected HCV cases, suggesting that these individuals are truly naïve for HCV infection. Whilst cross-reactivity to other commonly encountered antigens could be an explanation for finding these T cell responses, the frequent demonstration of responses to two or more HCV proteins makes this unlikely. We think that these responses represent an immunological footprint of HCV exposure that has not resulted in viraemia or HCV antibody seroconversion. The potential role of these responses in protecting from HCV infection is of great interest.

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19 PREDICTORS OF DROPOUT FROM TRANSPLANT WAITING LIST AMONG PATIENTS LISTED FOR HEPATOCELLULAR CARCINOMA USING THE UNOS/OPTN DATABASE
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Background: Liver transplantation (OLT) is an excellent treatment for selected patients with hepatocellular carcinoma (HCC) with 5-year survival rates between 60%-75%. Previous single center studies have studied predictors of dropping out from the waiting list. The aim of this study was to determine the risk factors for dropout among HCC candidates in the transplant waiting list utilizing the entire US transplant database. Methods: We analyzed the UNOS/OPTN database from 1998-2006 for all patients listed for a primary, secondary or tertiary diagnosis of HCC. Demographic, laboratory, clinical, and tumor character-istics were obtained at the time of listing. Among dropouts, only patients withdrawn from the waiting list due to tumor progression and/or death were evaluated. Kaplan-Meier and Cox regression was utilized to determine independent factors of dropout. Results: A total of 4482 patients have been listed for HCC since 1998. Of these, 766 (17.1%) patients dropped out due to tumor progression or death. The median time before removal from waiting list for death or tumor progression was 140 days (range 1-1928 days), shorter in the post-MELD era (176 days pre-MELD vs. 74 days post-MELD, p<0.0001). In the univariate analysis, age > 55 years, AFP (alpha-fetoprotein) > 20 ng/ml, non-viral etiology, Child class, MELD score > 10, tumor size > 2.5 cm, Region 9 (New York, Western Vermont), portal vein thrombosis, and pre-MELD liver allocation era increased the risk of dropping out; while Region 3 (Alabama, Arkansas, Florida, Georgia, Louisiana, Mississippi and Puerto Rico) and meeting Milan criteria were protective against dropping out from the transplant waiting list. The multivariate analysis is shown in the table below. Conclusion: In the largest study to date, approximately 17% of patients with HCC dropped out from the liver transplant waiting list in the US due to death or tumor progression. Advanced liver failure, non-viral etiology and region 9 were associated with increase risk of drop out, while meeting Milan criteria at listing and Region 3 were associated with decrease risk of drop out.

Independent risk factors of dropout in multivariate analysis

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20 PROSPECTIVE VALIDATION OF AASLD GUIDELINES FOR THE EARLY DIAGNOSIS OF HEPATOCELLULAR CARCINOMA IN CIRRHOTIC PATIENTS
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Background: Confident diagnosis of hepatocellular carcinoma (HCC) is currently based on both invasive and non invasive criteria with dynamic imaging. Aim: We prospectively evaluated the diagnostic accuracy of contrast-enhanced ultrasound (CE US), dynamic computed tomography (CT) and dynamic magnetic resonance (MR) in cirrhotic patients with de-novo liver nodes (LN) detected during surveillance. Patients/Methods: 38 patients with Child-Pugh A cirrhosis (44 to 76 yr), 26 (68%) males, under US surveillance at 6-month intervals who developed a LN underwent CE US, spiral TC, MR and fine-needle biopsy (FNB). FNB was the diagnostic gold standard. Results:
40 LN were identified: 1 (2.5%) <1cm, 35 (87.5%) 1-2cm, 4 (10%) >2cm. 20 (50%) hepatocellular carcinoma (HCC), 1 (5%) <1cm, 16 (80%) 1-2cm, 3 (15%) >2cm; 1 (2.5%) cholangiocarcinoma; 16 (40%) macroregenerative nodules; 3 (7.5%) low grade dysplastic nodes. Among 1-2cm LN, a typical vascular pattern for HCC, defined by contrast uptake in arterial phase and wash-out in the portal/venous phase, was detected in 5 (14%) by CE US, in 7 (20%) by CT, in 6 (18%) by MR, all techniques showing absolute specificity for HCC. 10 HCC patients (62%) had typical vascular pattern for HCC with one of these techniques whereas 6 (38%) showed a coincidental typical vascular pattern with two techniques (AASLD criteria). Conclusion: Among patients with de-novo LN of 1-2 cm, one technique may confidently allow for non invasive diagnosis of HCC in patients under surveillance, thus sparing liver biopsy in a quarter of patients.

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The following people have nothing to disclose: Nathalie Guedj, Perigny Martine, Françoise Degos, Qian Zhan, Dominique Valla, Jacques Belghiti, Olivier Farges, Pierre Bedossa, Valérie Paradis

22. ONCOCGENIC PATHWAYS AND NEW THERAPEUTIC TARGETS ARE SPECIFICALLY ACTIVATED IN THE DIFFERENT HEPATOCELLULAR ADENOMA SUBTYPES OF TUMORS

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Recent path-molecular classification of hepatocellular adenoma (HCA) identified 3 groups of lesions defined by either HNF1α inactivating mutations, β-catenin activation or the presence of inflammatory infiltrates. Genotype-phenotype analysis showed that each group of HCA was related to a specific tumor phenotype and malignant transformation was more frequent in β-catenin activated HCA. To better understand the physiopathology of the three defined subtypes of HCA, we performed a transcriptomic analysis. We compared the expression profile of more than 15,000 genes in normal livers with those of HNF1α-mutated (n=8), β-catenin mutated (n=3) and inflammatory (n=7) HCA. The most relevant deregulated pathways identified were further validated by quantitative RT-PCR and western-blotting experiments in an additional series of 40 tumors. In HNF1α-mutated HCA, we identified a strong over-activation of mTOR, a key pathway for cell survival. Phosphorylated mTOR and its targeted proteins, were over-expressed leading to the activation of the translational machinery. In addition, these tumors showed an over-expression of several onco genes such as cyclinD1 and EEF1A2 that may contribute to cell proliferation. We also found an aberrant induction of glycolysis and lipogenesis, that may explain the severe steatosis constantly observed in HNF1α inactivated HCA. In β-catenin mutated HCA, transcriptional deregulations affected mainly amino acid metabolism predicting impaired amino acid catabolism and an increased rate of glutamine synthesis. Most of the known β-catenin targeted genes were found over expressed in these particular HCA and interestingly, we also found a recurrent overexpression of the mTOR pathway. In contrast, inflammatory HCA showed a dramatic deregulation of the inflammatory response that may contribute to cell proliferation. We found a constitutive activation of the IL-6, type 1 and 2 interferon pathways, a downstream induction of the acute phase response and of the antigen presentation machinery. Using immunohistochemistry, we found the over-expression of SAA, a target of the acute phase response, restricted in tumor hepatocytes without reinforcement beside the lymphocytic infiltrates. In conclusion, in each HCA subtype, we identified a specific combination of alterations disturbing the energetic metabolism, cell survival and cell proliferation. While inhibitors of mTOR, β-
catenin and inflammatory pathways are already available or under development, our findings could open the way to a future “à la carte” management of HCA, particularly useful in case of multiple adenomas and adenomatosis.

Disclosures:
The following people have nothing to disclose: Sandra Rebouissou, Sandrine Imbeaud, Cristel Thomas, Charles P. Balbaud, Paulette Bioulac-Sage, Jessica Zucman-Rossi

23 CLINICAL SAFETY AND BIOACTIVITY OF OK432-STIMULATED DENDRITIC CELL TRANSFER INTO HEPATOCELULAR CARCINOMA FOLLOWING TRANSCATHETER HEPATIC ARTERIAL EMBOLIZATION

Yasunari Nakamoto1, Eishiro Mizukoshi2, Masaaki Kitahara1, Yoshio Sakai1, Kaeheita Kakinoki3, Kuniaki Arai1, Tatsuya Yamashita1, Naofumi Mukaida1, Kouji Matsushima2, Osamu Matsui1, Shuichi Kaneko3; 1Kanazawa University, Kanazawa, Japan; 2University of Tokyo, Tokyo, Japan; 3University of Tokyo, Tokyo, Japan

BACKGROUND: The curative treatments for hepatocellular carcinoma (HCC) including surgical resection and radiofrequency ablation (RFA) do not effectively prevent tumor recurrence. Dendritic cell (DC)-based immunotherapies are believed to contribute to the eradication of the residual and recurrent tumor cells. Recently, we have developed the combination therapy of transcatheter hepatic arterial embolization (TAE) with immature DC infusion (n=13) in comparison with the historical control group (n=10) monitored clinically and biochemically after the OK432-stimulated DC infusion for HCC (Clin. Exp. Immunol. 147:296-297, 2007). The current study was designed to assess the safety and bioactivity of infusion of DCs stimulated with OK432, a Streptococcus-derived anticancer immunotherapeutic agent, to tumor tissues following TAE treatment for patients with cirrhosis and HCC.

METHODS: Adherent cells isolated from peripheral blood mononuclear cells (PBMCs) of patients with hepatitis C virus-related cirrhosis and HCC were cultured and differentiated into DCs in the presence of IL-4 and GM-CSF. OK432 was added at a concentration of 1.0 KE/ml on day 5. Subsequently, 5 x 10^6 of DCs were administered through arterial catheter during the procedures of TAE treatment on day 7. The adverse events were monitored clinically and biochemically after the OK432-stimulated DC infusion (n=13) in comparison with the historical controls that were treated with immature DC infusion (n=10) or TAE alone (n=11). To evaluate the immunomodulatory effects of DCs, PBMCs were pulsed with 42 of defined tumor antigenic peptide epitopes, and the epitope-specific T lymphocyte responses were quantitated in an IFN-γ ELISPOT assay.

RESULTS: DCs obtained from each study patient were shown to express MHC class II (HLA-DR) and B7-2 (CD86) in the absence of mature monocytes (CD14) in flow cytometric analysis. In contrast to immature DCs, OK432-stimulated cells highly expressed the activation markers CD83 and CD86. There was no clinical or serological evidence of the adverse events including hepatic failure and autoimmune responses in any patients in addition to those due to TAE. Interestingly, the T lymphocyte responses were induced against peptides derived from tumor antigens, AFP, hTERT, SART2 and MRP3, after the infusion in some patients. Furthermore, the time course analysis indicated that these T cell responses were elevated four weeks after DC infusion and minimized three months later.

CONCLUSIONS: Transcatheter arterial infusion of OK432-stimulated DCs following TAE treatment is feasible and safe for patients with cirrhosis and HCC. Furthermore, the antigen-nonspecific DC infusion into HCC tissues may induce the immune responses to unprimed tumor antigens, providing a plausible strategy to enhance tumor immunity.

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24 DIFFUSION-WEIGHTED MRI FOR DETECTION OF HEPATOCELLULAR CARCINOMA IN PATIENTS WITH HEPATIC CIRRHOSIS: A CORRELATION TO HISTOLOGY AND IMAGING FOLLOW-UP

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Background/aim. Survival of patients with hepatocellular carcinoma (HCC) depends on the number and size of lesions, requiring detection at early stage of disease. The aim of the study was to evaluate diffusion-weighted magnetic resonance imaging (DW-MRI) for detection of HCC and/or evolutive lesions. Materials and methods. A 1.5 Tesla MRI was performed in 54 patients. An echo-planar DW-MRI with b-values ranging from b0 to b1000 was added to the conventional sequences. Signal intensity (SI) of solid liver lesions was measured for b0, b300, b600 and b1000 on native DW-MRI images. Apparent diffusion coefficient (ADC) maps were calculated for the entire b-value range (ADCavg) and for the high b-values [b>500 sec/mm2, ADChigh]. In 29 patients SI for b0, b300, b600 and b1000 and ADC-values of identified lesions were correlated to histopathology (50% explant livers; 50% resection or biopsy). In 23 additional patients, the imaging findings were correlated to lesion evolution on imaging follow-up (without performing histopathology). Sensitivity and specificity of DW-MRI was calculated using an optimal threshold and compared to T2- and contrast-enhanced T1-weighted sequences. Results. 72% of lesions were 2 cm or smaller: 153 old and compared to T2- and contrast-enhanced T1-weighted sequences. In comparison, T2-weighted imaging had a sensitivity of 76% and specificity of 94%, Contrast-enhanced T1-weighted imaging had a sensitivity of 87% and specificity of 76%. The SI on b0, ADCavg- and ADChigh-value showed no significant difference for any lesion type (P>0.05). Conclusions. DW-MRI using SI at b600 showed the highest sensitivity and specificity for detection of HGDN and HCC and correlated significantly with early lesion progression. This higher accuracy for detection of HGDN and HCC and progressive lesions may help in treatment or transplant planning.

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Introduction: Recurrent HCV infection after OLT can cause graft failure and death, but safety concerns have limited prophylactic/early treatment with pegylated interferon/ribavirin. The Phoenix trial was designed to compare prophylactic administration of antiviral therapy before histological recurrence of HCV (Prophylaxis arm) with initiation of antiviral therapy only at the time of histologically apparent recurrence of HCV (Observation arm). Methods: Patients were randomized 10:26 wks after OLT. Patients in the Prophylaxis arm received 135 µg peginterferon alfa-2a/wk for 4 wks and 180 µg/wk for 44 wks; plus 400 mg/d ribavirin (initial dose), escalating to 1200 mg/d, for 48 wks, followed by 72 weeks of treatment free follow-up. Patients in the Observation arm were treated with the same regimen only upon histologic recurrence of HCV (HAI >3 and/or FS >2), followed by 24-72 weeks of treatment free follow-up. The primary efficacy assessment is the proportion of patients in each group who experienced histological evidence of HCV recurrence at 120-weeks post-randomization. Here we report 24-wk interim data. Results: To date, 55 patients have been randomized to the Prophylaxis arm and 60 to the Observation arm; of the latter, 12 (20%) have started treatment due to meeting criteria for histological recurrence and have completed 12 weeks of treatment. At weeks 4, 12, and 24, respectively, 4 (7.3%), 12 (21.8%), and 21 (38.2%) patients in the Prophylaxis arm were HCV negative, as were 0 (Week 4) and 4 (33.3%; Week 12) patients in the Observation arm who started delayed treatment. Of patients in the Prophylaxis and Observation arms, 53 (98.1%) and 55 (91.7%), respectively, have experienced at least 1 adverse event (AE); and 23 (42.6%) and 14 (23.3%), respectively, have experienced at least 1 serious AE. ACR was experienced by 2/54 (3.7%) patients in the Prophylaxis arm and 3/48 (6.3%) untreated patients in the Observation arm, and significant infections by 4/54 (7.4%) and 1/48 (2.1%), respectively; clinically significant depression was not observed in either arm. Although anemia (16.7% vs 2.1%) and grade 3/4 neutropenia (16.7% vs 8.3%) were higher in the Prophylaxis arm than in untreated patients in the Observation arm, these AEs were likely related to treatment. Conclusion: After 24 weeks of treatment, 38.2% of patients in the Prophylaxis arm were HCV RNA negative. Although AEs were common during prophylaxis for post-OLT HCV recurrence, the incidence of clinically significant ACR was not increased by prophylaxis. These findings suggest that peginterferon alfa-2a/ribavirin prophylaxis is relatively safe and effective in OLT recipients.

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Stephen Rossi - Employee: Roche

David R. Nelson - Grant/Research Support: Roche; Grant/Research Support: Roche
Intensive Dietary Treatment in Living Donor Liver Transplantation in Adults

Riccardo Volpes1,2, Lisa Randisi1,2, Salvatore Gruttadauria1,2, Marcello Castellese1,2, Maria Rubino1,2, Marida Minervini1,2, Giovanni Vizzini1,2, Bruno Gridelli1,2, 1ISMETT, Palermo, Italy; 2UPMC, Pittsburgh, PA

A living donor liver transplant (LDLT) program has been developed in our Center as an alternative for recipients who would have limited or delayed access to a cadaveric organ. Around 20% of our total liver transplant activity currently comes from living donors. A comprehensive step-by-step work-up protocol for donor evaluation is designed to ensure donor safety and to guarantee donor capability to provide a suitable graft. The usefulness of steatotic livers depends on the fat percentage, being graft and patient survival decreased by donor steatosis >30%. Body Mass Index (BMI) >30 may reliably predict higher degree of steatosis in most donors. In order to enlarge the pool of living donors and may be therefore useful to increase the organ pool without affecting the donor and recipient safety.

Results:
Successful completion of the 3-month treatment (BMI <30), a liver biopsy was performed. In all 7 treated donors steatosis <30% was found, and they were considered eligible for donation. After LDLT, none of such donors experienced life-threatening complications or died. Liver function of both resected donors and transplanted graft showed a good outcome, with no differences (in term of hospital LOS, liver functional parameter normalization after resection, and liver regeneration) between them and other LDLT without hepatic steatosis. Treated fat donors showed no long-term clinical impairment, and all of them returned to previous activity after donation. In conclusion, donor livers with <30% steatosis are usable and anticipated to have good function. A short-term dietitian intensive regimen for fat donors seems to be effective in lowering hepatic steatosis by reaching such acceptable histological cutoff. This approach may provide a way for persons with steatosis to be successful liver donors and may be therefore useful to increase the organ pool without affecting the donor and recipient safety.

Disclosures:
The following people have nothing to disclose: Riccardo Volpes, Lisa Randisi, Salvatore Gruttadauria, Marcello Castellese, Maria Rubino, Marida Minervini, Giovanni Vizzini, Bruno Gridelli

28 SURVIVAL AND RISK OF HEPATITIS B VIRUS (HBV) RECURRENCE IN HIV-HBV COINFECTED LIVER TRANSPLANT RECIPIENTS: PRELIMINARY FINDINGS FROM THE HIV-TR STUDY

Carla S. Coffin1, Carl L. Berg2, Lorna M. Dove3, Fred Poordad4, Michael P. Curry5, Fredric G. Regenstein6, Kenneth E. Sherman7, Michelle E. Rolan8, Peter G. Stock9, Norah Terrault1,2, 1Medicine, University of California San Francisco, San Francisco, CA; 2Surgery, University of California San Francisco, San Francisco, CA; 3Medicine, University of Virginia, Charlottesville, VA; 4Medicine, Columbia University, New York, NY; 5Medicine, Cedars-Sinai, Los Angeles, CA; 6Medicine, Harvard School of Medicine, Boston, MA; 7Medicine, Tulane University, New Orleans, LA; 8Medicine, University of Cincinnati, Cincinnati, OH

Background: Liver transplantation (LT) is the treatment of choice for HBV-infected patients with end stage liver disease, but the outcome of LT in HIV+ patients may be complicated by HIV disease progression and/or recurrent liver disease post-LT. Aims: To evaluate the safety and efficacy of LT for HBV-infected patients with end stage liver disease, but the outcome of LT in HIV+ patients may be complicated by HIV disease progression and/or recurrent liver disease post-LT.

Methods: Multicenter prospective cohort study of HIV-HBV coinfected patients in the “Solid Organ Transplantation in HIV Study” (HIV-TR) undergoing LT from 2003-2007. HIV-specific criteria included undetectable HIV viral load if on antiretrovirals (ARV) or prediction of full suppression post-LT if unable to tolerate ARV and CD4 cells >100/mm3, or >200/mm3 if there was a history of opportunistic infections or neoplasms. Standardized post-LT HBV prophylaxis included hepatitis B immunoglobulin (HBIG) & antivirals: tenofovir with lamivudine (LAM) or emtricitabine. Sera were collected pre-transplant, 12, 26, 52 wks & yrs post-LT. We assessed patient and graft survival and HBV recurrence (defined by positive HBV DNA and hepatitis B surface antigen, HBsAg, in serum). Ultra-sensitive real-time PCR was used to detect HBV genomes using S-gene specific primers and fluorescent-labeled Taq-man based DNA probes (range 20 – > 107 IU/ml). A contemporary cohort of 60 HBV monoinfected LT recipients with median follow-up of 21.2 mo (0 – 42.0) served as controls for survival analysis. Results: 16 coinfected patients (all Caucasian males, median age 46 yrs, pre-LT CD4 count median 362/mm3 was considered about 2-4 kg, with a final gained BMI <30kg/m2. 7/14 donors didn’t complete the 3-mo dietician follow-up for donor unrelated reasons. In all 7/14 donors who successfully completed the 3-mo treatment (BMI <30), a liver biopsy was performed. In all 7 treated donors steatosis <30% was found, and they were considered eligible for donation. After LDLT, none of such donors experienced life-threatening complications or died. Liver function of both resected donors and transplanted graft showed a good outcome, with no differences (in term of hospital LOS, liver functional parameter normalization after resection, and liver regeneration) between them and other LDLT without hepatic steatosis. Treated fat donors showed no long-term clinical impairment, and all of them returned to previous activity after donation. In conclusion, donor livers with <30% steatosis are usable and anticipated to have good function. A short-term dietitian intensive regimen for fat donors seems to be effective in lowering hepatic steatosis by reaching such acceptable histological cutoff. This approach may provide a way for persons with steatosis to be successful liver donors and may be therefore useful to increase the organ pool without affecting the donor and recipient safety.

Disclosures:
The following people have nothing to disclose: Carla S. Coffin, Carl L. Berg, Lorna M. Dove, Fred Poordad, Michael P. Curry, Fredric G. Regenstein, Kenneth E. Sherman, Michelle E. Rolan, Peter G. Stock, Norah Terrault, 1Medicine, University of California San Francisco, San Francisco, CA; 2Surgery, University of California San Francisco, San Francisco, CA; 3Medicine, University of Virginia, Charlottesville, VA; 4Medicine, Columbia University, New York, NY; 5Medicine, Cedars-Sinai, Los Angeles, CA; 6Medicine, Harvard School of Medicine, Boston, MA; 7Medicine, Tulane University, New Orleans, LA; 8Medicine, University of Cincinnati, Cincinnati, OH.

PedsQL 4.0™ Generic Core Scales for Child Self-Report and Parent Proxy-Report and Comparison with Healthy Children Controls

<table>
<thead>
<tr>
<th>Scale</th>
<th>Liver Transplant Mean (±SD) n=259</th>
<th>Healthy Sample Mean (±SD) n=1844</th>
<th>P value</th>
<th>Effect Size</th>
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<tr>
<td>Child Self-Report</td>
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<tr>
<td>Total Score</td>
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<td>83.7 (±12.3)</td>
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<td>82.0 (±5.6)</td>
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<td>0.49</td>
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<td>Psychosocial Health</td>
<td>74.3 (±6.0)</td>
<td>81.3 (±14.0)</td>
<td>&lt; 0.001</td>
<td>0.50</td>
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<tr>
<td>Emotional Functioning</td>
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<td>78.6 (±18.3)</td>
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<td>Social Functioning</td>
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<td>School Functioning</td>
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<td>Social Functioning</td>
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BACKGROUND: The outcome of liver transplantation (LT) in patients infected with Human Immunodeficiency Virus (HIV) has been a matter of controversy. Patients coinfected with HIV and hepatitis C virus (HCV) appeared in several small studies to have poorer outcome after LT compared to patients with HCV monoinfection. Methods: A retrospective cohort study was performed to assess the impact of HIV on LT survival by using UNOS Standard Transplant Analysis and Research (STAR) files. Results: A total of 138 HIV(+) and 30,520 HIV(-) patients who underwent LT were included in the analysis. Of 138 HIV(+) patients, 58 were HCV antibody positive (HCVAb)(+) and 21 hepatitis B surface antigen (HBsAg)(+). Among all HIV(+) patients, the estimated 2-year survival probability was lower (70%) than among non-HIV patients (81%). This excess risk appeared entirely among those with coinfections, e.g. HIV with HBV or HCV, as none of the 24 HIV infected patients who did not have HBV or HCV died during an average of 1.2 years of follow-up per person. HIV/HCV coinfected patients had significantly lower survival rate compared to non-HIV patients with HCV (p=0.006) and without HCV (p=0.003) after LT (Figure 1). In contrast to HCV/HIV coinfected patients, there was no significant difference in survival rates between HIV/HBV coinfected patients and HBV patients without HIV coinfection. Controlling for age, coinfection, MELD scores and other potential confounders in a Cox proportional hazards regression analysis, HIV(+) patients had a hazard ratio (HR) of 1.41 (P= 0.14, %95 CI: 0.90-2.22) for mortality post LT. Conclusion: Patients who had HIV/HCV coinfection had lower survival rates after LT compared to HCV patients without HIV coinfection. Such difference was not observed in HIV/HBV coinfected patients compared to HBV monoinfected patients undergoing LT.

Disclosures: Fred Poordad - Grant/Research Support: Bristol-Myers Squibb; Speakers Bureau: GlaxoSmithKline, Grant/Research Support: Gilead
Fredric G. Regenstein - Speakers Bureau: Roche; Speakers Bureau: Schering-Plough; Speakers Bureau: Gilead
Kenneth E. Sherman - Consultant/Adviser: Human Genome Sciences
Norah Terrault - Grant/Research Support: Gilead; Consultant/Adviser: Indexx, Inc; Consultant/Adviser: Bristol-Myers Squibb
Laurence S. Magder

The following people have nothing to disclose: Ayse L. Mindikoglu, Arie Regev, Laurence S. Magder

IMPACT OF HIV ON SURVIVAL AFTER LIVER TRANSPLANTATION: ANALYSIS OF UNITED NETWORK FOR ORGAN SHARING (UNOS) DATABASE

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Background: The outcome of liver transplantation (LT) in patients infected with Human Immunodeficiency Virus (HIV) has been a matter of controversy. Patients coinfected with HIV and hepatitis C virus (HCV) appeared in several small studies to have poorer outcome after LT compared to patients with HCV monoinfection. Methods: A retrospective cohort study was performed to assess the impact of HIV on LT survival by using UNOS Standard Transplant Analysis and Research (STAR) files. Results: A total of 138 HIV(+) and 30,520 HIV(-) patients who were ≥ 18 years old and underwent LT during the HAART era (starting from January 1, 1997) in the US were included in the analysis. Of 138 HIV(+) patients, 58 were HCV antibody positive (HCVAb)(+) and 21 hepatitis B surface antigen (HBsAg)(+). Twenty four HIV(+) patients were neither HCVAb(+) nor HBsAg(+). Among all HIV(+) patients, the estimated 2-year survival probability was lower (70%) than among non-HIV patients (81%). This excess risk appeared entirely among those with coinfections, e.g. HIV with HBV or HCV, as none of the 24 HIV infected patients who did not have HBV or HCV died during an average of 1.2 years of follow-up per person. HIV/HCV coinfected patients had significantly lower survival rate compared to non-HIV patients with HCV (p=0.006) and without HCV (p=0.003) after LT (Figure 1). In contrast to HCV/HIV coinfected patients, there was no significant difference in survival rates between HIV/HBV coinfected patients and HBV patients without HIV coinfection. Controlling for age, coinfection, MELD scores and other potential confounders in a Cox proportional hazards regression analysis, HIV(+) patients had a hazard ratio (HR) of 1.41 (P= 0.14, %95 CI: 0.90-2.22) for mortality post LT. Conclusion: Patients who had HIV/HCV coinfection had lower survival rates after LT compared to HCV patients without HIV coinfection. Such difference was not observed in HIV/HBV coinfected patients compared to HBV monoinfected patients undergoing LT.

LONG TIME FOLLOW UP FOR THE PATIENT OF AUTOLOGOUS BONE MARROW CELL INFUSION (ABMI) THERAPY FOR LIVER CIRRHOSIS

Shuji Terai, Makoto Segawa, Kaoru Omori, Takuya Iwamoto, Yuko Mizunaga, Toshihiko Matsumoto, Yohei Urata, Yoshio Marumoto, Tsuyoshi Ishikawa, Naoki Yamamoto, Koichi Uchida, Takahiro Yamazaki, Isao Sakaida; Department of Gastroenterology & Hepatology, Yamaguchi University Graduate School of Medicine, Ube, Yamaguchi, Japan

We previously reported the results of clinical study: Autologous bone marrow cell infusion (ABMI) therapy for liver cirrhosis patient. In the study, we reported 9 liver cirrhosis (LC) cases that underwent ABMI from the peripheral vein and followed up to 6 months. Subjects were LC patients with T.B. of <3.0 mg/dl, Plt of >50 (10^{10}/l) and no viable hepatocellular carcinoma on diagnostic imaging. Autologous bone marrow (BM: 400 ml) was isolated from the ilium under general anesthesia. Mononuclear cells (MNCs) were separated by cell washing and were infused via the peripheral vein. After ABMI therapy, liver function was monitored by blood examination for 6 months. Serum albumin and Child-Pugh Score were significantly improved at 1 month and 6 months (p<0.05) (Stem Cells 2006). Until now we did total 16 cases of ABMI therapy for LC patients. No major adverse effects were noted to do ABMI therapy. After clinical study, we could follow up 9 cases (Age: 61.6±5.0, HBV:3 cases, HCV:6 cases, Male: Female=8:1) up to 15 months after ABMI therapy. Serum albumin and total protein level was significantly improved at 6 months (p<0.05). In conclusion, ABMI therapy should be considered as a novel treatment for LC patients.

Disclosures: The following people have nothing to disclose: Ayse L. Mindikoglu, Arie Regev, Laurence S. Magder
Serum Albumin and Total Protein after ABMI therapy

<table>
<thead>
<tr>
<th></th>
<th>Before-ABMI</th>
<th>1 month</th>
<th>3 months</th>
<th>6 months</th>
<th>9 months</th>
<th>12 months</th>
<th>15 months</th>
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<tr>
<td>Serum Albumin (g/dL)</td>
<td>2.6±0.1</td>
<td>2.9±0.1</td>
<td>3.0±0.1*</td>
<td>3.1±0.1*</td>
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<td>3.1±0.2*</td>
<td>3.0±0.2*</td>
</tr>
<tr>
<td>Total Protein (g/dL)</td>
<td>6.3±0.2</td>
<td>7.1±0.3*</td>
<td>7.2±0.3**</td>
<td>7.5±0.4**</td>
<td>7.3±0.2**</td>
<td>7.3±0.2**</td>
<td>7.1±0.3**</td>
</tr>
</tbody>
</table>

Values are shown as mean ± SE.

Data was analyzed of variance with Fisher’s PLSD test. * and ** showed significantly different compared with the baseline value of before-ABMI.

therapy.

Disclosures:
The following people have nothing to disclose: Shuji Terai, Makoto Segawa, Kaoru Omori, Takuya Iwamoto, Yuka Mizunaga, Toshikiko Matsumoto, Yohei Urita, Yosio Marumoto, Tsuyoshi Ishikawa, Naoki Yamamoto, Koichi Uchida, Takahiro Yamasaki, Isao Sakaida

31 METRON FACTOR-1, AN HGF-MSP CHIMERA, PREVENTS LIVER INJURY WITHOUT PROMOTING TUMOR GROWTH AND METASTASIS

Terumi Takahara1, Feng Xue1,2, Yutaka Yata1, Kazunobu Nonome1, Masami Kanayama1, Kengo Kawai1, Toshiro Sugiyama1, Paolo Michieli2; 1Third Department of Internal Medicine, University of Toyama, Toyama City, Japan; 2Organ Transplantation Center, Renji Hospital Affiliated to Shanghai Jiao-tong University, Shanghai, China; 3Institute for Cancer Research and Treatment, University of Torino Medical School, Turin, Italy

BACKGROUND & AIMS: Hepatocyte Growth Factor (HGF) is the most powerful hepatotrophic factor identified so far. However, the ability of HGF to promote tumor cell ‘scattering’ and invasion raises some concern about its therapeutic safety. Recently, we engineered an HGF-MSP chimera named Metron Factor-1 (MF-1) that separates the favorable effects of HGF (prevention of apoptosis and tissue regeneration) from its adverse properties (invasion and metastasis; Michieli et al., Nature Biotechnol. 2002). We already reported that MF-1 stimulates hepatocyte proliferation and protects hepatocytes against apoptosis in vitro. In this study we compare the therapeutic efficacy of MF-1 with that of HGF in mouse models of acute and chronic liver injury. At the same time, we test the ability of MF-1 and HGF to promote tumor growth and metastasis in several mouse models of cancer. METHODS: MF-1 protein produced by lentiviral technology was purified by affinity chromatography. Acute liver injury was induced in mice by CCl4 injection. Chronic liver injury was induced by continuous CCl4 intoxication. Recombinant MF-1 or HGF was delivered using a micro-osmotic pump. For tumorigenesis analysis, MF-1 or HGF was delivered to human and mouse tumor cells by lentiviral technology, and cells were injected subcutaneously into CD-1 nu/nu mice. RESULTS: In acute liver injury, serum ALT levels were significantly suppressed by MF-1 or HGF treatment compared to the control group (MF-1, 5734 IU/L; HGF, 8430 IU/L; control, 17420 IU/L at 24 hours, p<0.001). TUNEL-positive apoptotic hepatocytes were significantly fewer in MF-1 (49.6%) or HGF (50.7%) treated mice relative to controls. Phospho-histone-3 positive hepatocytes were increased 4.5 times in MF-1- or HGF-treated mice. In the chronic injury model, hepatic fibrosis was prominent in control mice. In both MF-1- and HGF-treated mice, fibrotic area was significantly reduced. Hepatocyte proliferation index determined by Ki67 expression raised 2.1 times in both the MF-1 and HGF groups. In the subcutaneous tumor model, HGF significantly promoted xenograft growth of both A549 lung carcinoma cells (2.3 times, p<0.05) and MDA-MB-435 breast carcinoma cells (5.9 times, p<0.01). In contrast, tumors expressing MF-1 were slightly larger than control tumors (A549, 1.1 times; MDA-MB-435, 1.7 times). Analysis of lung metastasis revealed that HGF increased both metastasis incidence and number in both cell systems, while MF-1 had no effect. CONCLUSION: These data indicate that MF-1 is as effective as HGF at preventing liver injury and at promoting hepatocyte regeneration, but therapeutically safer than HGF as it lacks pro-metastatic activity.

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The following people have nothing to disclose: Terumi Takahara, Feng Xue, Yutaka Yata, Kazunobu Nonome, Masami Kanayama, Kengo Kawai, Toshiro Sugiyama, Paolo Michieli

32 DEGRADATION OF THE EGF RECEPTOR IN HEPATOCYTES IS MEDIATED BY A DYNAMIN-ASSOCIATED UBIQUITIN-BASED ENDOCYTIC MACHINERY

Barbara Schroeder1, Jing Chen1, Mark A. McNiven1,2; 1Division of Gastroenterology and Hepatology, Mayo Clinic, Rochester, MN; 2Biochemistry and Molecular Biology, Mayo Clinic, Rochester, MN

Internalization and degradation of receptor tyrosine kinases (RTKs) such as the EGFR is an essential process in hepatocytes that helps regulate growth factor concentrations in the serum as well as overall liver growth and size. This important process is mediated mainly by clathrin-coated vesicles (CCVs) and associated adaptor proteins that work together to internalize and target activated ubiquitinated EGFR to the lysosome for degradation. The large GTPase Dynamin 2 (Dyn2) is well known for its role in the sequestration of CCVs from the plasma membrane during receptor-mediated endocytosis (RME); however, it is unclear whether Dyn2 also participates in the sorting and degradation of the EGFR. Therefore, the GOAL of this study was to test for a potential interaction between Dyn2 and the ubiquitin-based endocytic machinery; define the molecular basis of this interaction; and test for a functional significance in EGFR sorting and degradation. Using GST-pulldown and immunoprecipitation assays, we detected a direct interaction between Dyn2 and CIN85 (cbl-interacting protein of 85 kDa), an adaptor protein that is ubiquitinated after EGFR stimulation and participates in EGFR degradation. CIN85 contains three SH3 domains, two of which can interact with the proline-rich domain of Dyn2. Interestingly, using two distinct assays employing either fluorescence microscopy or immunoprecipitation, we observed that the formation of this Dyn2-CIN85 complex is inducible and occurs 5-30 minutes following EGFR stimulation of hepatocytes (Clone 9, HuH7). To test for a functional role of the Dyn2-CIN85 interaction in EGFR trafficking in hepatocytes, we expressed a mutant CIN85 protein that cannot bind to Dyn2 and observed the fate of Rhodamine-labeled EGFR by fluorescence microscopy or the EGFR by Western blot analysis. Importantly, CIN85 mutant-expressing cells showed a marked increase in the time needed to degrade the EGFR. Further, these cells retained the labeled EGFR in a perinuclear Rab7-positive vesicular compartment for about 1 hour longer than control cells. Rab7 is known to associate with late endosomes, suggesting that cells expressing the CIN85 mutant are unable to traffic the receptor-ligand complex from late endosomes to lysosomes. In SUMMARY, these findings demonstrate a novel EGFR-induced association between the mechanoenzyme Dyn2 and the ubiquitin-based endocytic machinery. Further, this interaction appears to mediate the sorting and trafficking of EGFR in hepatocytes and, thus, is likely to have profound effects on the regulation of EGFR levels and subsequently cell growth.
33 PREVENTION AND TREATMENT OF ACUTE LIVER INJURY IN ANIMAL MODELS BY ENDOTHELIAL PROGENITOR CELLS

Verónica Fernández-Ruiz, Milosz P. Kawa, María Ilíiguez, Jesús Prieto, Cheng Qian; Hepatology and Gene Therapy, Center for Applied Medical Research-University of Navarra, Pamplona, Spain

Background and Aims: It has been demonstrated that the vascular endothelium plays an important role in tissue homeostasis and selective activation of VEGF receptor-1 reduced liver damage in animals exposed to a hepatotoxic compound via angiogenesis independent mechanism. Transplantation of endothelial progenitor cells (EPC) following acute liver injury induced by carbon tetrachloride improved survival of the mice, accompanied by increased hepatocellular proliferation. In order to explore therapeutic potency of EPC, we have established optimal protocols for prevention and therapy of acute liver damage induced concanavalin A (ConA) and adenovirus-mediated transfer of CD40 ligand (CD40L). Methods: Murine EPC were obtained by culturing mononuclear cells from bone marrow in endothelial growth factor enriched medium for 7 days. Two different models of acute liver failure were applied in C57BL/6 mice; intravenous administration of ConA at 12 mg/Kg (sub-lethal dose) and of adenovirus encoding CD40L (AdCD40L) at 10^10 pfu/mouse (lethal dose). The liver-damaged animals were treated by intravenous administration of 2x10^6 EPC either before (prevention) or after liver damage (therapy). The animal survival was observed and liver transaminases levels and histology were analyzed. Results: Our data showed intravenous administration of EPC at 24 hours before liver damage or intrasplenic administration (IS) at 1 hour before liver injury could significantly prevent increased serum level of transaminases in ConA model. The intravenous administration of EPC at 3 hours after liver damage could also significantly reduce serum level of transaminases, as compared with control animals without therapy. In model of CD40L, all animals died before 132 hours after intravenous injection of AdCD40L. Treatment of liver-damaged animals with a single dose of EPC by intravenous administration of EPC at 24, 48 and 72 hours after liver injury could induce 17%, 50% and 67% of animal survival, respectively. Treatment with two doses of EPC at 48 and 72 hours resulted in 100% of animal survival. Significant inhibition of increased transaminase was also observed in those animals after therapy with EPC. In our in vitro data we have shown that EPC produced VEGF and IGF-I at high level. Conclusion: Our data indicated that bone marrow derived-EPC exerted preventive and therapeutic activity in animals with acute liver damage. These effects may be due to that EPC produced hepatoprotective factors facilitating regenerative process of injured liver parenchyma.

Disclosures:
The following people have nothing to disclose: Barbara Schroeder, Jing Chen, Mark A. McNiven

34 DYNAMIC MICROTUBULES ARE REQUIRED FOR CONSTITUTIVE CANALICULAR TRAFFICKING OF BSEP (ABCB11) IN POLARIZED WIF-B9 CELLS AND PROVIDE THE "MISSING LINK" TO THE CANALICULAR ACTIN NETWORK

Yoshiyuki Wakabayashi, Jennifer Lippincott-Schwartz, Irwin M. Arias; CBMB, NICHD, NIH, Bethesda, MD

In WIF-B9 cells, endosomes containing the bile salt export pump (BSEP, ABCB11) traffic along microtubules (MTs) throughout the cytosol but only target the canalicular membrane from which they constitutively cycle to rab11a-positive endosomes (1, 2). In differentiated cells, including hepatocytes, there are at least two types of MTs: (a) dynamic MTs which are unstable (t 1/2 ~10 min), and (b) MTs which are stable for several hours. We investigated the role of dynamic MTs on BSEP trafficking and their linkage to the canalicular actin network. Using polarized WIF-B9 cells, we performed 3D reconstitution of MT distribution, and quantified live cell imaging of BSEP-YFP trafficking combined with fluorescence recovery after photobleaching. To visualize MT distribution, immunostaining was performed with alpha-tubulin and gamma-tubulin, a marker of the microtubular organizing center (MTOC). 3D reconstruction revealed that the MTOC is proximate to the bile canalculus which is surrounded by a MT web. EB1, a dynamic MT plus-end marker, also surrounded the canalculus. To determine the role of dynamic MTs in apical trafficking of BSEP, WIF-B9 cells were treated with 201F, a marine sponge product, which specifically disassembled dynamic MTs and not stable MTs. BSEP-YFP trafficking remained, presumably along stable MTs, and did not reach the canalicular membrane. In contrast, nocodazole disrupted all MTs, and all intracellular movement and canalicular trafficking of BSEP-YFP ceased. These studies reveal that apical BSEP trafficking requires dynamic MTs. To explore the relation between dynamic MTs plus-end and the actin network, we performed immunostaining of IQGAP, Rac, APC and EB1, which link dynamic MT plus-end and actin. These proteins all surrounded the bile canalicus in association with actin. Conclusion: Dynamic MTs are required for canalicular targeting of BSEP. Proteins known to link dynamic MTs to the actin network localize in the bile canalicular domain. Dynamic plus-end MTs may provide the long sought link between the MT and actin-based endosome trafficking systems which surround the bile canaliculus. (1) Wakabayashi Y et al, MBC 15: 3485, 2004 (2) Wakabayashi Y et al, PNAS 102: 15087, 2005

Disclosures:
The following people have nothing to disclose: Yoshiyuki Wakabayashi, Jennifer Lippincott-Schwartz, Irwin M. Arias

35 ENDOCYTIC INTERNALIZATION OF THE TRANSFERRIN RECEPTOR 1 (TFR1) IN HEPATOCYTES IS MEDIATED BY ACTIVATION OF SRC

Hong Cao1, Jing Chen1, Mark A. McNiven1,2; 1Division of Gastroenterology and Hepatology, Mayo Clinic, Rochester, MN; 2Biochemistry and Molecular Biology, Mayo Clinic, Rochester, MN

Hepatocytes internalize numerous receptors and trophic factors from the sinusoidal plasma membrane via a clathrin-mediated endocytic process. Ligand-induced activation of receptor tyrosine kinases such as the epidermal growth factor (EGF) receptor is thought to stimulate receptor internalization in part via activation of Src kinase family members. However, the uptake of other trophic ligand-receptor systems such as transferrin (Tf) has been assumed to be constitutive and unregulated. Therefore, the Goal of this study was to test whether ligand-induced
internalization of the transferrin receptor (TfR1) in hepatocytes is indeed via a Src-regulated, clathrin-based endocytic process. We observed that Src is activated (8-fold) in cultured cells upon addition of Tf, as assessed by Western blot analysis and immunofluorescence cell staining with a widely used antibody that recognizes activated Src. To test whether Tf uptake could be attenuated by direct inhibition of Src kinase we utilized several different approaches, including pharmacological inhibition of cells (PP2 and SU6656); expression of a dominant-negative Src mutant (Y419F/K297M); and the use of embryonic fibroblasts from Src-Yes-Fyn (SYF) knock-out mice. The drugs PP2 and SU6656 reduced uptake of Tf in cultured hepatocytes (Clone 9) by 60-70% as did expression of mutant Src. Further, Tf internalization was reduced by over 80% in SYF cells compared to control fibroblasts. Importantly, Tf uptake could be rescued to near normal levels in SYF cells that were transfected to express active Src (Y530F). Together, these results indicate that Src is activated by the TfR1. Next we asked whether Src is activating the endocytic machinery to support Tf internalization. Here, we tested whether the endocytic proteins dynamin 2 (Dyn2) and cortactin (Cort) were phosphorylated during Tf addition in 32P-labeled Clone 9 cells. We observed a marked increase (2.5-fold) in phosphorylation of both proteins that unexpectedly exceeded that exhibited by cells treated with either 10% FBS or 30 ng/ml EGF. Accordingly, expression of Dyn2 or Cort mutant proteins that could not be phosphorylated at key tyrosine residues (Dyn2 Y231F, Y597F; Cort Y384, 429, 445F) significantly attenuated Tf internalization in Clone 9 cells. In Conclusion, the observations described above provide the first evidence that internalization of the TfR1 in hepatocytes is potentiated by ligand-induced Src activation of the Dyn2 and Cort endocytic machinery rather than occurring through a constitutive endocytic mechanism as previously assumed. Supported by NIH R01 DK44650 to MAM.

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The following people have nothing to disclose: Hong Cao, Jing Chen, Mark A. McNiven

36
EPIGENETIC GENE EXPRESSION PROFILING IDENTIFIED HGF ACTIVATOR INHIBITOR 2 (HAI-2) AS A FREQUENTLY SILENCED CANDIDATE TUMOR SUPPRESSOR GENE IN HUMAN HEPATOCELLULAR CARCINOMA
Edmund K. Tung, Chun M. Wong, Irene O. Ng; Pathology, The University of Hong Kong, Hong Kong, China

Epigenetic alteration is an important mechanism in the inactivation of tumor suppressor genes. We employed epigenetic profiling with pharmacological unmasking of DNA methylation to identify silenced candidate tumor suppressor genes in hepatocellular carcinoma (HCC). Following treatment with 5-aza-2’-deoxycytidine (5-aza-dC) in high-throughput cDNA microarray analysis, the expression of HGF activator inhibitor, HAI-2, was found to be significantly upregulated in two of three HCC cell lines (Hep3B, SMMC7721, and BEL7402). HAI-2 belongs to Kunitz-type serine protease inhibitor family and inhibit the generation of biologically active hepatocyte growth factor (HGF) through their interaction with HGF activator (HGFA). In this study, we validated the epigenetic silencing by DNA methylation of the HAI-2 gene and characterized its tumor suppressive function. Results: The mRNA expression of HAI-2 was absent or low in 12 HCC cell lines, but 5-Aza-dC treatment significantly upregulated HAI-2 expression in 9 of these cell lines in a dose- and time-dependent manner. The mRNA levels of HAI-2 were significantly underexpressed in ~80% of human HCCs. Using bisulfite DNA sequencing, we confirmed that the promoter of HAI-2 gene was frequently hypermethylated in HCC cell lines and human HCCs. In addition, using methylation-specific PCR, HAI-2 promoter methylation was detected in 71% of human HCCs, and HAI-2 promoter hypermethylation was associated with low mRNA expression levels. These results showed that HAI-2 was frequently hypermethylated and underexpressed in human HCCs. Ectopic expression of HAI-2 in SMMC cells significantly suppressed the growth of subcutaneous tumors in nude mice. In addition, HAI-2 significantly inhibited cell motility and cell invasion. To further elucidate the mechanisms of HAI-2's effects in HCC, we performed knockdown and overexpression experiments. Our results provided evidence that HAI-2 was capable of inhibiting cell growth and invasion, and that this effect was mediated through the inhibition of invasion. The findings of this study have important implications for the development of new therapeutic strategies for the treatment of HCC.
placebo group. Analysis conducted in patients without HCC at enrollment did not show any significant difference in 2- and 6-month survival between the 2 groups. In the subgroup of patients with severe acute alcoholic hepatitis (n=132), pentoxifylline did not affect mortality at 2 months (14% vs. 16%, p=0.77) or at 6 months (27% vs. 31%, p=0.30). In the subgroup of patients with renal dysfunction at enrollment (n=39), pentoxifylline did not affect mortality at 2 months (35% vs. 31%, p=0.82) or at 6 months (52% vs. 62%, p=0.52). There was no significant difference between pentoxifylline and placebo group in the incidence of adverse events. In multivariate analysis, factors significantly associated with mortality were older age, greater MELD score and the presence of early HCC. In conclusion, treatment with pentoxifylline does not improve short-term survival in patients with advanced cirrhosis, even in the subgroup of patients with severe acute alcoholic hepatitis or with renal dysfunction.

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**GLOMERULAR FILTRATION RATE AS AN IMPROVED PROGNOSTIC INDICATOR IN PATIENTS WITH END STAGE LIVER DISEASE**

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**Background:** Renal function is an important prognostic indicator in patients with end stage liver disease (ESLD). One of the advantages of MELD is its inclusion of serum creatinine, although its accuracy as a measure of renal function may be suboptimal. Recently, serum sodium (Na), which also reflects renal function in patients with ESLD, has been shown to improve MELD. **Aim:** We evaluated the degree to which directly measured renal function (glomerular filtration rate, GFR) adds to MELD. **Methods:** Adult (age>18 years) patients with ESLD underwent GFR measurement at the time of initial evaluation for liver transplantation. GFR was measured by standard non-radioabeled iothalamate clearance. Proportional hazards regression analysis was performed to evaluate GFR in addition to MELD variables in predicting survival. **Results:** We collected data on 861 Mayo Clinic patients listed for primary liver transplantation from 1990 through 1999. Of 650 patients with complete data, 35 died without receiving LTx. The mean MELD at baseline was 15.6 (standard deviation [SD]: 7.1). The mean GFR was 77.9 mL/min/1.73m2 (SD:31.8). The mean creatinine was 1.14 mg/dl (SD: 0.86). There was significant, yet modest correlation serum creatinine and GFR (R=-0.57, p<0.01). The table summarizes Cox models incorporating MELD variables, Na and GFR. Model 1 includes only MELD, whereas Models 2 and 3 incorporate GFR in addition to MELD variables. Adding GFR clearly improves the accuracy of MELD. In Model 3, coefficients were refit for the MELD variables and Na. Once GFR is included, serum creatinine and Na were no longer significant. The final model consisting of total bilirubin [HR=2.31, p<0.01], INR [HR=3.14, p<0.01] and GFR [HR=0.26, p<0.01] remained superior to other models (Chi-square=53.0). **Conclusions:** Directly measured GFR is superior to serum creatinine in assessing mortality risk in patients with ESLD awaiting liver transplantation. These data suggest that incorporating measures of renal function that are more accurate than serum creatinine may improve the accuracy of MELD.

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**ENDOSCOPIC VARICEAL LIGATION (EVL) PLUS PROPRANOLOL (P) AND ISOSORBIDE MONONITRATE (ISMN) VERSUS EVL ALONE IN SECONDARY PROPHYLAXIS OF VARICEAL BLEEDING: A PROSPECTIVE RCT**

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**Background:** Both EVL and propranolol are valuable methods for secondary prophylaxis of variceal bleeding. Addition of ISMN to propranolol enhances reduction of the hepatic venous pressure gradient (HVPG) and improves the efficacy of drug therapy. It is hypothesized that a combination of EVL with the two portal pressure reducing drugs should significantly be better than EVL alone. Patients and Methods: Patients with history of variceal bleed were randomized into either EVL alone (Group A) or EVL plus propranolol and ISMN (Gr. B) HVPG was measured at baseline in everyone. EVL was repeated every 2 wk until varices were eradicated. Propranolol dose was adjusted to reduce the resting heart rate <55 bpm. Dose of ISMN was 40 mg/d. Primary end points were rebleeding or death. Secondary end-points were complications of cirrhosis (encephalopathy, hepatorenal syndrome, spontaneous bacterial peritonitis, and jaundice) Results: A total 171 patients were randomized into EVL alone (Gr. A, n=92) and EVL plus propranolol and ISMN (Gr. B, n=79). Demographic, clinical, and biochemical profiles were comparable. Mean follow-up was 16±8 mo. Primary end points were achieved in 19 patients (21%) in group A and 20 (25.3%) in group B (p=0.46). The bleeding rates were 21.7% in Gr. A and 24% in Gr. B patients (p=0.71). Five patients (5.4%) of group A and 9 (11.3%) of group B died (P=0.17). Twenty four patients (26.1%) of group A and 23 (29.1%) of group B achieved secondary end-points. Five (36%) (Group A, 1; Group B, 4) died as a result of rebleeding. HVPG [19.3±5.7 vs 15.9± 4.8mmHg (P<0.001)] Child score [9.5±2.15 vs. 7.9± 2.07 (P<0.001)], MELD score 17.1±6.9 vs 13.1± 5.4(P<0.001), S. bilirubin [3.2±3.4mg/dl vs 1.9±2.7(P=0.003) and prothrombin time [25±10 vs20±6second (P<0.001)] were higher in patients who reached the primary end points. However, on multivariate analysis only HVPG and PT were found to be independently significant. **Abbreviations:**

GFR: glomerular filtration rate

INR: international normalization ratio

Na: serum sodium concentration
associated with primary end-points. Baseline and follow-up HVPG (n=33) after 12 months were 15.8±5.6 and 16.1±4.0 (p=0.78) respectively. Conclusions: Addition of propranolol and ISMN to EVL does not further reduce the incidence of variceal rebleeding as achieved by EVL therapy given alone. There is little benefit of identifying non-responders by doing serial HVPG measurements as Child’s and MELD scores also significantly influence the probability of variceal rebleeding.

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40 PRESENCE OF BACTERIAL DNA IS A NEW SURVIVAL INDICATOR IN PATIENTS WITH CIRRHOSIS AND NON-INFECTED ASCITIC FLUID

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Introduction: We have previously reported that roughly 40% of patients with cirrhosis and non-infected ascitic fluid (AF) show the presence of bacterial DNA (bactDNA) in blood and AF. In this work, we tested the hypothesis that the presence of bactDNA in AF and serum is associated to decreased survival in patients with cirrhosis and non-infected ascites. Material and Methods: In a prospective, multicenter study, we analyzed the clinical evolution of 156 patients with cirrhosis and sterile AF according to the presence or absence of bactDNA fragments in both AF and serum at admission. BactDNA was detected by polymerase-chain reaction-based method and species identified by an automated nucleotide sequencing process. Survival was determined during a follow-up of 12 months and analyzed using the Kaplan-Meier life-table and Log-rank test. Data were censored at 365 days or liver transplantation or TIPS procedures. Results: Age of patients was 61±12 years and 67% were male. The aetiology of cirrhosis was alcoholic in 64% and chronic infection by hepatitis C virus in 35% patients. Sixty percent were Child-Pugh B and 39% were Child-Pugh C, and 43% of patients were included in the study during their first episode of ascitic decompensation. Serum/ascitic bactDNA was detected in 48 patients (bactDNA(+)). The most prevalent bacteria identified was E. Coli (72%). Mean age was significantly higher in bactDNA(+) than bactDNA(-) patients (64±12 vs 60±11 years respectively; p=0.03). No other statistical significant differences between groups were found. Eighteen out of 48 bactDNA(+) patients (38%) died during the 12 months of follow-up compared with 15 out of 108 bactDNA(-) patients (14%; p=0.001). Eight out of 17 patients (47%) with previous episodes of encephalopathy died compared with 25 patients in the group without antecedents of encephalopathy (18% p=0.006). In a Cox proportional hazards multiple regression including bactDNA status, age and previous encephalopathy, the only significant relationship was established between bactDNA status and death (hazard ratio of death: 2.2, 95%CI: 1.1-4.6, p=0.03). Spontaneous bacterial peritonitis developed in 1 bactDNA(+) (2%) and in bactDNA(-) patients (8%; p=0.18) during the year of follow-up. Conclusions: The presence of bactDNA in serum/AF in a patient with cirrhosis and sterile AF during an ascitic decompensation episode is an indicator of poor prognosis, and does not predispose patients to the development of spontaneous bacterial peritonitis episodes.

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41 A MULTI-CENTER CASE-CONTROL STUDY OF GENETIC PREDICTORS FOR HEPATOPULMONARY SYNDROME (HPS)

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Introduction: The hepatopulmonary syndrome (HPS) occurs in 10-30% of patients with cirrhosis and adversely affects survival with or without liver transplantation (OLT) outcomes. There are no established predictors of HPS. We aimed to determine whether genetic variation was associated with the risk of HPS in patients being evaluated for OLT. Methods: We performed a case-control study at six US academic medical centers between 2004-2006. HPS was defined as an abnormal alveo-arterial oxygen gradient and intrapulmonary vascular shunting on contrast enhanced transthoracic echocardiography (CE) in the absence of intrinsic restrictive or obstructive lung disease. Controls included those with negative CEs or with positive CEs and normal alveolar-arterial oxygen gradients and no lung disease. We performed a genetic analysis association study using >1000 single nucleotide polymorphisms (SNPs) in 94 candidate genes per subject. Results: 53 patients with HPS were compared to 139 controls. HPS and controls had similar ages (53 ± 10 vs 52 ± 10), sex (53% vs 66% male) and MELD scores (13.8 ± 3.8 vs 12.8 ± 5.6) respectively. HPS patients had significantly higher median [IQR] alveolar-arterial oxygen gradients (22 [19-33] vs 8.5 [3-13], p < 0.0001) and lower PaO2 (76±12 mmHg vs 93±12 mmHg, p<0.0001) values. Using an additive genetic model, SNPs in endothostatin (COL18A1, angiogenesis inhibitor), Elastin (ELN, elastic extracellular matrix fiber) and Von Willebrand factor (VWF, endothelial derived coagulation and inflammatory mediator)were associated with an increased risk of HPS (p<0.005). SNPs in Tie-1 (vascular-specific receptor tyrosine kinase involved in angiogenesis) and caveolin3 (CAV3, muscle specific caveolin isoform) were also associated with an increased risk of HPS. (p<0.006). All of the SNPs associated with HPS were in Hardy-Weinberg equilibrium (p>0.05). Conclusions: Genetic variation in several systems, including those involved in endothelial cell signaling, angiogenesis, and activation as well as vascular matrix production and smooth muscle development are associated with an increased risk of HPS in cirrhosis. Future studies will examine these pathways in mechanisms of HPS.

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42 ELTROMBOPAG RAISES PLATELET COUNTS IN TWO WEEKS IN PATIENTS WITH HCV AND SIGNIFICANT THROMBOCYTOPENIA

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**Background**

Thrombocytopenia is observed in patients with advanced hepatic fibrosis and cirrhosis and is considered a surrogate marker for the severity of fibrosis. These patients require medical care that often includes invasive medical procedures, such as liver biopsy, dental extractions, gastrointestinal endoscopy with biopsy or coronary angiography. For patients with severe thrombocytopenia (<50,000/µL), platelet transfusions are often used to manage the bleeding risk associated with such invasive procedures.\(^1,2\) Eltrombopag is an oral non-peptide thrombopoietin receptor agonist which has been shown to increase platelet counts in healthy volunteers and subjects with idiopathic thrombocytopenic purpura. **Methods**

In a double-blind, randomized, multi-center clinical trial, 74 subjects with advanced hepatic fibrosis and cirrhosis due to chronic hepatitis C virus (HCV) with baseline platelet counts between 20,000-70,000/µL received placebo or eltrombopag tablets 30, 50 or 75 mg once daily for 4 weeks. The primary endpoint was increase in platelet count to ≥100,000/µL within 4 weeks. Patients could then initiate pegylated interferon and ribavirin combination therapy and continue eltrombopag or placebo for an additional 12 weeks. Data from a subgroup analysis of 26 patients with baseline platelet count <50,000/µL are reported here.

**Results**

After 2 weeks of treatment with eltrombopag, 20%, 71% and 63% of patients in the 30, 50, and 75 mg groups, respectively, achieved platelet count ≥100,000/µL. No patient on placebo achieved platelet count of ≥100,000/µL. The mean platelet count after 2 weeks of treatment was 74,000/µL, 129,000/µL, and 146,000/µL for the 30, 50 and 75 mg groups, respectively. The mean platelet count after 3 weeks of eltrombopag was 112,000/µL, 193,000/µL, and 192,000/µL for the 30, 50, and 75 mg groups, respectively. At 3 weeks, no patient had platelet count >400,000/µL.

**Conclusions**

Within 2 weeks, eltrombopag increased platelet counts in this patient population with advanced liver disease to levels that could reduce the need for platelet transfusion. Confirmation of these data and evaluation of the effect of increased platelet counts on platelet transfusion rate in a larger patient population is warranted.

References:


### Table

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<th>Week 3</th>
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<td>% with platelet counts ≥100,000/µL</td>
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43 ANALYSIS OF HEPATOCYTE-SPECIFIC CASPASE-8 KNOCKOUT MICE IN EXPERIMENTAL MODELS OF LIVER INJURY

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The pro-apoptotic factor caspase-8 is the most upstream-located protease (caspase) involved in apoptosis mediated by Fas, TNF and related death receptors of the TNF superfamily. Induction of apoptosis in the liver is related to liver injury potentially resulting in liver failure. The aim of this study was to investigate the effect of caspase-8 depletion in different models of experimental liver injury. To investigate the molecular function of caspase-8, conditional hepatocyte-specific knockout (KO) mice were generated by flanking exons 3-4 with loxP sites and introducing a cre-recombinase transgene under the control of the hepatocyte-specific albumin promoter. Deletion of exons 3 and 4 introduced a frame shift mutation within the caspase-8 mRNA resulting in a premature stop of protein translation after 107 amino acids. The efficiency of the caspase-8 knockout was analysed in primary hepatocytes on DNA, RNA and protein levels revealing a deletion efficiency of >90%. To study the role of caspase-8 for Fas-mediated liver injury, KO mice and control animals (WT) were treated with a Fas-activating antibody (JO2). WT mice showed highly enhanced transaminases beginning 5 hours after treatment and a mortality of 80% within 24 hours due to massive apoptosis and liver failure as shown by TUNEL analysis and liver histology. In contrast, 100% of caspase-8 KO mice survived JO2 treatment and displayed normal transaminase levels and liver histology. Next, we investigated caspase-8 KO mice and controls in the lipopolysaccharide/galactosamine (LPS/GalN) model of TNF-dependent hepatotoxicity. WT mice showed increased serum transaminases beginning 6 hours after LPS/GalN treatment which was correlated with enhanced caspase-3 activity, strong liver apoptosis and 30% mortality within 24 hours whereas all KO mice survived the treatment without any signs of liver damage. Finally, we applied the Concanavalin A (ConA) model of liver injury on caspase-8 KO mice and controls. ConA-mediated liver injury is also TNF dependent and involves massive
recruitment of activated T cells to the liver. Unexpectedly, caspase-8 KO mice were not protected from ConA-induced liver injury. On the contrary, KO mice showed enhanced serum transaminase levels and decreased survival (85% compared to 100% in WT animals) following ConA treatment. In conclusion, our data shows that inactivation of caspase-8 in hepatocytes protects from Fas- and LPS-mediated liver injury but not from ConA-induced hepatotoxicity implicating that caspase-8 dependent apoptosis is not a major signaling pathway during T-cell induced hepatitis.

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44 APOPTOSIS-INDUCING FACTOR CAUSES MITCHONDRIAL OXIDANT STRESS AND NUCLEAR DNA FRAGMENTATION IN ACETAMINOPHEN-INDUCED LIVER INJURY

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Acetaminophen (APAP) overdose causes centrilobular necrosis in human and mouse livers. DNA fragmentation is a hallmark of APAP-induced cell death. Recent evidence showed the nuclear translocation of apoptosis-inducing factor (AIF), which correlated with DNA fragmentation, after APAP overdose in mice (Bajt et al., Tox Sci 94: 217-225, 2006). When released from mitochondria, AIF combines with cyclophilin A and forms an active DNase. On the other hand, mitochondrial AIF is critical for oxidative phosphorylation and can function as superoxide-generating NADH oxidase. To address the hypothesis that AIF may be a critical mediator in APAP-induced cell death, fasted male AIF-deficient mice (Hq) and respective wildtype animals were treated with 200 mg/kg APAP. Liver injury (plasma alanine aminotransferase (ALT) activity, necrosis by histology) and nuclear DNA damage (TUNEL assay and anti-histone ELISA) were evaluated at 6 h after APAP. In wildtype animals, APAP induced severe liver injury as indicated by the increase of plasma ALT activities (8600 ± 1870 U/L) and 61 ± 8% necrosis. This injury was accompanied by massive DNA strand-breaks in centrilobular hepatocytes (TUNEL assay) and release of DNA fragments into the cytosol (anti-histone ELISA). In contrast, Hq mice had significantly less liver injury (ALT: 330 ± 130 U/L; necrosis: 4 ± 2%) and minimal nuclear DNA damage at 6 h. Both wildtype and Hq mice had the same baseline levels of glutathione (GSH) and glutathione disulfide (GSSG), showed the same (75%) depletion of hepatic GSH 20 min after APAP and had a similar recovery of liver GSH levels at 6 h. This suggests that there was no relevant difference in metabolic activation of APAP. However, hepatic levels of GSSG and immunohistochemical staining for nitrotyrosine protein adducts, which reflect a mitochondrial oxidant stress and peroxynitrite formation, resp., were only increased in APAP-treated wildtype animals and remained near baseline in Hq mice. These data indicate an absence of APAP-induced mitochondrial oxidant stress in Hq mice. Conclusion: Our data suggest that AIF has a critical dual function in APAP hepatotoxicity. AIF is important for the generation of reactive oxygen in mitochondria (NADH oxidase function) and, upon release from mitochondria, is involved in DNA fragmentation (DNase function). Supported by NIH grant DK070195.

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45 ETHANOL SUPPRESSES MHC CLASS I-RESTRICTED ANTIGEN PRESENTATION IN LIVER CELLS: ROLE OF PROTEASOME

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Background: To clear hepatitis B or C viruses, infected hepatocytes are targeted by cytotoxic T lymphocytes (CTLs), which recognize viral peptide-MHC class I complexes. The proteasome is the major enzyme that cleaves viral proteins to peptides for antigen presentation. Proteasome function is compromised by ethanol metabolism. Heavy alcohol consumption contributes to disease progression, possibly by impaired recognition of infected hepatocytes by CTLs. The aim of this study was to investigate the effect of ethanol on processing and presentation of the ovalbumin peptide, SIINFEKL, a CTL epitope generated by the proteasome and presented in the H2Kb context. Methods: HepB5 cells, a H2Kb+ hepatocyte-derived cell line, which possess low alcohol dehydrogenase (ADH) and high cytochrome P450E1 (CYP2E1) activities. H2Kb-expression was induced by IFNγ treatment. Cells were exposed to 50 mM ethanol for 48 hr and then C-extended peptide, SIINFEKL-TE was delivered to the cells via the Chariot® reagent. Cleaved SIINFEKL peptide-H2Kb complex were quantified on the cell surface by flow cytometry. Results: SIINFEKL-H2Kb surface expression, following extended peptide delivery to the cell was blocked by the ER trafficking inhibitor, brefeldin A. Prior exposure to ethanol caused a 35% reduction of SIINFEKL-H2Kb presentation, which was partially reversed by treatment with 4-methylpyrazole. The proteasome inhibitor, MG132 also reduced SIINFEKL presentation. Ethanol-elicted reduction in presentation can be attributed to impaired IFNγ signaling, which regulates antigen presentation machinery and stimulates H2Kb expression and/or to impaired generation of SIINFEKL from SIINFEKL-TE by the proteasome. In fact, induction of immunoproteasome subunits and PA28, but not H2Kb expression by IFNγ was partially suppressed by ethanol exposure. However, we observed no changes in IFNγ-induced STAT1 phosphorylation. In addition, proteasome activity was suppressed by 25-40% by ethanol treatment, which caused stabilization of uncleaved SIINFEKL-TE peptide detected by HPLC. Suppressive effects of ethanol on proteasome function were mimicked by the SAH hydrolase inhibitor, tubericidin, indicating that ethanol-impaired methylation may be partially responsible for the reduced proteasome activity. We conclude that ethanol metabolism impairs MHC class I-restricted antigen presentation on hepatocytes by interfering with IFNγ signaling and by blocking proteasome activity, thereby preventing subsequent peptide cleavage.

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46 ACUTE ETHANOL CAUSES LIVER MITOCHONDRIAL DEPOLARIZATION IN VIVO INDEPENDENT OF THE MITOCHONDRIAL PERMEABILITY TRANSITION (MPT) AND POTASSIUM CHANNEL OPENING

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BACKGROUND: Acute ethanol treatment increases oxygen uptake and decreases ATP production in isolated-perfused
rodent livers, suggesting an alteration of mitochondrial function. It is unclear, however, if ethanol causes mitochondrial dysfunction in vivo. Accordingly, the aim of this study was to investigate changes of mitochondrial polarization and permeability in vivo after acute alcohol treatment. **Methods:** Mice were gavaged with one inebriating dose of ethanol (6 g/kg). Mitochondrial polarization, cell death and mitochondrial inner membrane permeability were assessed by intravital multiphoton microscopy of rhodamine 123 (Rh123), propidium iodide (PI) and calcine, respectively, at 1-24 h after ethanol treatment. **Results:** In livers of saline-treated mice, hepatocytes exhibited punctate green Rh123 fluorescence. By contrast after ethanol treatment, mitochondria of many hepatocytes did not take up Rh123, indicating mitochondrial depolarization. Mitochondrial depolarization occurred in 16%, 55% and 94% of hepatocytes at 1, 4 and 6 h after ethanol treatment, respectively, and remained >85% at 12 h, which indicated widespread, severe mitochondrial dysfunction. Subsequently, mitochondria repolarized such that only 16% of hepatocytes contained depolarized mitochondria 24 h after treatment. Virtually all hepatocytes not taking up Rh123 excluded PI at 1-24 h, indicating that loss of cell viability was minimal after acute ethanol treatment. Calcine, a 623 Da fluorophore that loads into the cytosol and can gain entrance to the mitochondrial matrix space when MPT pores open, outlined mitochondria as dark voids in the hepatocytes of saline-treated mice. Importantly, these voids remained 6 h following ethanol treatment despite mitochondrial depolarization in >90% of hepatocytes. Moreover, nim811 (20 mg/kg, i.g. or i.p.), a non-immunosuppressive cyclosporin A derivative that inhibits the MPT, did not prevent mitochondrial depolarization after acute ethanol. These findings suggest that acute ethanol-induced mitochondrial depolarization in vivo was not due to onset of the MPT. \( K_{ATP} \) channel blockers glybenclamide (5-20 mg/kg, i.p.) and 5-hydroxydecanoic acid (10-100 mg/kg, i.p.) also did not prevent mitochondrial depolarization caused by ethanol. **Conclusion:** Acute ethanol causes widespread, reversible mitochondrial depolarization in the liver. This mitochondrial depolarization is not due to onset of the MPT or opening of potassium channels in the mitochondria. Ethanol-induced mitochondrial dysfunction may contribute to steatosis after acute and hepatotoxicity after chronic ethanol exposure (NIDDK).

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The following people have nothing to disclose: Zhi Zhong, Venkat K. Ramshesh, Hasibur Rehman, John J. Lemasters

**47 BILE ACIDS REGULATE HEPATOCYTE CELL DEATH BY DIFFERENTIAL ACTIVATION OF CLASS 1A PHOSPHATIDYL-INOSITIDE-3-KINASE P110 ISOFORMS**

Simon Hohenester, Ulrich Beuers, Sawkat Anwer, Cynthia R. Webster

**Background:** The mechanism by which acute ethanol toxicity impairs liver regeneration is incompletely understood. We have previously shown that Fibroblast Growth Factor 10 (FGF10), expressed by embryonic hepatic stellate cells binds to the receptor FGFR2b expressed by hepatoblasts to promote cell proliferation during hepatogenesis (Berg et al., Hepatology 46: 2007). Also we have shown that expression of Fgf10 is upregulated over twenty-fold after partial hepatectomy (PH) and that expression of the dominant negative form of the soluble FGFR2b leads to impaired liver regeneration. We hypothesize that impaired liver regeneration in the setting of acute ethanol toxicity is in part due to impaired expression of Fgf10 and that upregulation of Fgf10 may rescue such impairment. **Methods:** We have generated triple transgenic mice (CMV-Cre; rtTAflox; tet(O)Fgf10) allowing for doxycycline induced ubiquitination of PI3K-p110 isoforms by GCDC, TLC and TUDC and the effect of PI3K-p110 gamma inhibition on GCDC-induced apoptosis was determined. Methods: Cultured rat hepatocytes were treated with 50 nM GCDC or TUDC or 25 μM TLC for 15 min. After selective immunoprecipitation, Class IA PI3K-p110 isoform specific activity was determined in a lipase kinase assay by monitoring the production of phosphatidylinositol -3,4,5 triphosphate from phosphatidylinositol -4,5 biphosphate by thin layer chromatography. Akt (S473) phosphorylation as a readout of PI3K activity was determined by immunoblot of whole cell lysates. Hepatocyte apoptosis was determined 2 hrs after treatment with 50 μM GCDC in the presence or absence of a selective PI3K gamma inhibitor (Camps M. et al Nat Med 11, 936 2005) by fluorescent microscopy of Hoechest 33258 stained cells. Bile acid uptake was determined by measuring accumulation of [3H] taurocholate after 30 min. All experiments were done 4-6 times and p <0.05 was considered significant. Results: GCDC, TLC and TUDC induced a 2.6 +/- 0.77, 4.1 +/- 0.39- and 3.0 +/- 0.61 -fold phosphorylation of Akt after 30 min, respectively. All 3 bile acids activated the p110 beta isoform 1.5 to 1.6 fold. The p110 alpha isoform was activated 1.5 +/- 0.73 and 1.6 +/- 0.68 fold by TUDC and GCDC, respectively. Both hepatotoxic bile acids, GCDC and TLC, but not TUDC, activated the p110 gamma isoform by 2.0 +/- 0.28 and 1.4 +/- 0.33 fold, respectively. A pro-apoptotic effect of p110 gamma activation was supported by the following results; a) pretreatment (30 min) with 2.5 μM of a selective PI3K gamma inhibitor (Calbiochem S28108) significantly attenuated GCDC induced Akt phosphorylation by 50 +/-15 %, but had no effect on TUDC induced Akt phosphorylation and 2) GCDC induced hepatocyte apoptosis was significantly decreased (60 +/- 19%) in the presence of the PI3K gamma inhibitor. The PI3K gamma inhibitor had no effect on bile acid accumulation. Conclusion: Bile acid-induced activation of PI3K-p110 is isoform-specific. Activation of PI3K-p110 gamma promotes cell death, but is not involved in the activation of Akt by TUDC.

**48 INDUCED OVEREXPRESSION OF FIBROBLAST GROWTH FACTOR 10 PARTIALLY RESCUES ACUTE ETHANOL TOXICITY IMPAIRMENT OF LIVER REGENERATION**

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**Background:** The mechanism by which acute ethanol toxicity impairs liver regeneration is incompletely understood. We have previously shown that Fibroblast Growth Factor 10 (FGF10), expressed by embryonic hepatic stellate cells binds to the receptor FGFR2b expressed by hepatoblasts to promote cell proliferation during hepatogenesis (Berg et al, Hepatology 46: 2007). Also, we have shown that expression of Fgf10 is upregulated over twenty-fold after partial hepatectomy (PH) and that expression of the dominant negative form of the soluble FGFR2b leads to impaired liver regeneration. We hypothesize that impaired liver regeneration in the setting of acute ethanol toxicity is in part due to impaired expression of Fgf10 and that upregulation of Fgf10 may rescue such impairment. **Methods:** We have generated triple transgenic mice (CMV-Cre; rtTAflox; tet(O)Fgf10) allowing for doxycycline induced ubiquitination of PI3K-p110 isoforms by GCDC, TLC and TUDC and the effect of PI3K-p110 gamma inhibition on GCDC-induced apoptosis was determined. Methods: Cultured rat hepatocytes were treated with 50 nM GCDC or TUDC or 25 μM TLC for 15 min. After selective immunoprecipitation, Class IA PI3K-p110 isoform specific activity was determined in a lipase kinase assay by monitoring the production of phosphatidylinositol -3,4,5 triphosphate from phosphatidylinositol -4,5 biphosphate by thin layer chromatography. Akt (S473) phosphorylation as a readout of PI3K activity was determined by immunoblot of whole cell lysates. Hepatocyte apoptosis was determined 2 hrs after treatment with 50 μM GCDC in the presence or absence of a selective PI3K gamma inhibitor (Camps M. et al Nat Med 11, 936 2005) by fluorescent microscopy of Hoechest 33258 stained cells. Bile acid uptake was determined by measuring accumulation of [3H] taurocholate after 30 min. All experiments were done 4-6 times and p <0.05 was considered significant. Results: GCDC, TLC and TUDC induced a 2.6 +/- 0.77, 4.1 +/- 0.39- and 3.0 +/- 0.61 -fold phosphorylation of Akt after 30 min, respectively. All 3 bile acids activated the p110 beta isoform 1.5 to 1.6 fold. The p110 alpha isoform was activated 1.5 +/- 0.73 and 1.6 +/- 0.68 fold by TUDC and GCDC, respectively. Both hepatotoxic bile acids, GCDC and TLC, but not TUDC, activated the p110 gamma isoform by 2.0 +/- 0.28 and 1.4 +/- 0.33 fold, respectively. A pro-apoptotic effect of p110 gamma activation was supported by the following results; a) pretreatment (30 min) with 2.5 μM of a selective PI3K gamma inhibitor (Calbiochem S28108) significantly attenuated GCDC induced Akt phosphorylation by 50 +/-15 %, but had no effect on TUDC induced Akt phosphorylation and 2) GCDC induced hepatocyte apoptosis was significantly decreased (60 +/- 19%) in the presence of the PI3K gamma inhibitor. The PI3K gamma inhibitor had no effect on bile acid accumulation. Conclusion: Bile acid-induced activation of PI3K-p110 is isoform-specific. Activation of PI3K-p110 gamma promotes cell death, but is not involved in the activation of Akt by TUDC.

**Disclosures:**

The following people have nothing to disclose: Simon Hohenester, Ulrich Beuers, Sawkat Anwer, Cynthia R. Webster
tous expression of Fgf10. Triple transgenic mice are considered as experimental while double transgenic (CMV-Cre; rtTAflex) mice are considered as controls. Two-thirds PH were performed in 12-week old wildtype and transgenic mice. Transgenic mice were treated with doxycycline for 48 hours prior to PH. Double and triple transgenic mice were treated with doxycycline alone or with doxycycline and 35% ethanol (3g/kg dose) via orogastric gavage 30 minutes prior to PH and 11 hours after PH. Relative levels of Fgf10 were analyzed by real time PCR at 0 hours and 24 hours after 2/3 PH. Regenerative capacity of the liver was assessed via proliferation (PCNA immunofluorescence staining). Results: Wildtype mice undergoing PH exhibited a 20-fold increase in Fgf10 expression whereas mice treated with EIOH gavage and PH manifested a 43% reduction in Fgf10 expression. Expression of Fgf10 was upregulated over 1000-fold and 600-fold in the experimental mice compared to control mice before and after PH, respectively. This resulted in a three-fold increase in hepatic proliferation prior to PH compared to control. Twenty-four hours after PH, the experimental mice exhibited a six-fold increase in PCNA staining compared to control. Finally, the experimental mice undergoing PH and treated with EIOH exhibited a greater than four-fold increase in hepatic PCNA staining compared to the control mice indicating that impaired liver regeneration can be partially rescued with overexpression of Fgf10. Conclusion: Impaired liver regeneration caused by ethanol ingestion is due in part to the abrogation of Fgf10 upregulation after PH. Induced overexpression of Fgf10 expression partially rescues liver regeneration impaired by acute ethanol toxicity.

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ANTIVIRAL, PHARMACOKINETIC AND SAFETY DATA FOR GS-9190, A NON-NUCLEOSIDE HCV NS5B POLYMERASE INHIBITOR, IN A PHASE-1 TRIAL IN HCV GENOTYPE 1 INFECTED SUBJECTS

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Background & Aims: GS-9190 is a novel non-nucleoside HCV NS5B polymerase inhibitor with potent in vitro antiviral activity (0.6 nM EC50 in Genotype 1b replicon) and a high selectivity index in vitro. Initial results from an ongoing single-dose/multi-dose escalation clinical trial of GS-9190 in HCV-infected volunteers are reported here. Methods: Study GS 196-0101 was a randomized, double-blind, placebo controlled trial designed to evaluate the safety/tolerability, pharmacokinetics and antiviral activity of single (in Part A) and multiple (in Part B) doses of GS-9190 in subjects chronically infected with HCV genotype 1 (GT-1) without cirrhosis. Prospective subjects are 18-60 years of age and are HCV treatment naives. In an already completed Part A, five successive cohorts of 6 subjects were randomized (5:1) to receive single ascending doses of GS-9190 (40, 120, 240, 240-with food, or 480 mg) or placebo. In ongoing Part B, four successive cohorts of 12 subjects are randomized (10:2) to receive multiple ascending doses of GS-9190 (40 mg BID, 120 mg BID, 240 mg QD, 240 mg BID) or placebo, over 8 days. Results: Thirty-one subjects enrolled in Part A were of mean age 43.6 years, predominantly male (20/31), Caucasian (25/31), and infected with either HCV Genotype-1a (24) or 1b (6). Median (range) baseline HCV viral load was 6.6 log10 RNA IU/mL (5.2-7.3). Single doses of GS-9190 were well tolerated, with no serious or treatment-limiting adverse events (AEs) reported. All AEs (except one moderate) were mild in severity, with the most common being headache. There were no Grade 3 or 4 treatment emergent laboratory abnormalities. Median GS-9190 plasma half-life ranged from 10 to 15 hours across cohorts. Systemic exposure was increased approximately 2-fold when GS-9190 was administered with a high fat meal. Mean GS-9190 concentration 24 hours after the 240 mg fasted dose was >7-fold higher than the protein binding adjusted in vitro HCV GT-1b replicon EC50 value. Following single-dose exposure, maximal antiviral effect was observed at 24 hours, with median declines ranging from 0.46 to 1.49 log10 HCV RNA IU/mL across cohorts. Individual HCV RNA declines among all GS-9190 recipients ranged from 0.19 to 2.54 log10 IU/mL following single-dose exposure. Conclusions: This study demonstrates potent dose-dependent antiviral activity of GS-9190, a novel non-nucleoside HCV polymerase inhibitor in HCV infected volunteers. Single dose exposure to GS-9190 was well tolerated and demonstrated favorable PK properties that may support QD or BID dosing. The multiple dose phase of this trial is ongoing, and updated results will be provided.

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TELAPREVI VIR RESISTANCE MUTATIONS IN PATIENTS WITH HEPATITIS C WHO RELAPSED AFTER SEQUENTIAL THERAPY WITH TELAPREVIR, PEG-INFERNER ALFA 2A AND RIBAVIRIN

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Introduction: Telaprevir (TVR) is a highly selective inhibitor of the hepatitis C virus (HCV) NS3/4A protease with highly effective blocking of HCV replication in patients with hepatitis C. Mutations have been identified in the NS3 protease gene at positions 36, 54, 155 and 156 conferring resistance to TVR in vitro and in vivo. For single resistance mutations an inverse correlation of resistance level and viral fitness has been described while combined mutations (i.e. V36/R155) display relative high resistance with compensatory effects on replication efficiency. Little is known about persistence of TVR resistance mutations in patients treated sequentially with TVR, peg-interferon and ribavirin (RBV). Methods: Fifteen patients received either TVR monotherapy or TVR peg-interferon alfa 2a combination therapy for 2 weeks followed by peginterferon alfa 2a plus RBV...
standard combination therapy for 24 or 48 weeks. In the present study, we performed amplification and clonal sequencing (approx. 50 clones per patient and time point) of the HCV NS3 protease gene in 5 patients who relapsed so far. All 5 patients were tested HCV RNA negative during therapy but relapse with increasing HCV RNA concentration was observed after the end of standard combination treatment for 24 or 48 weeks. Results: While in 2 patients no mutations conferring resistance to TVR were detected during different time points after relapse, in 3 patients known TVR resistance mutations within the NS3 protease gene were detected. Patient 1 with initial TVR monotherapy, 48 weeks of standard therapy, and relapse at week 4 after treatment discontinuation (433,000 IU/ml) showed mutations at positions V36 (100% of clones) and R155 (100% of clones). In patient 2 with initial TVR monotherapy, 24 weeks of standard treatment and relapse at week 4 (viral load pending) only single isolates with resistance mutations were observed at position V36 (2%) and A156 (2%). Finally, patient 3 received initially combination therapy followed by 48 weeks of standard treatment. In this patient relapse was detected at follow up week 8 (1,400 IU/ml) and resistance mutations were detected at position V36 (84% of clones) only. In both patients who received TVR monotherapy mutations at positions V36, T54, R155 and A156 were observed during the initial 2 weeks of treatment. Conclusion: In patients treated with TVR with and without peginterferon alfa-2a for 2 weeks followed by standard therapy with peginterferon and RBV for 24 or 48 weeks mutations conferring resistance to TVR can be detected after relapse. The significance of TVR resistance mutations for the probability of relapse has to be investigated in future studies.

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51 SUSTAINED VIROLOGIC RESPONSE WITH ALBINTERFERON ALFA-2B/RIBAVIRIN TREATMENT IN PRIOR INTERFERON THERAPY NON-RESPONDERS

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Background/Aim Albinterferon alfa (alb-IFN) is a novel recombinant protein consisting of IFNa-2b genetically fused to human albumin. This randomized Phase 2 study evaluates the efficacy and safety of alb-IFN/ribavirin treatment in chronic Hepatitis C (CHC) patients who were non-responders (NR) to previous IFNa-based regimens. Methods Subjects were randomized into 3 alb-IFN treatment cohorts (900mcg Q2w, 1200mcg Q2w or 1200mcg Q4w) in combination with weight-based oral ribavirin (RBV) 1000-1200 mg/day. After evaluating safety data, 2 further cohorts were treated with higher doses of alb-IFN 1500mcg Q2w and 1800mcg Q2w. The treatment duration is 48 weeks and the primary efficacy end-point is sustained virologic response (SVR) at 24 weeks posttreatment. The protocol was amended to allow extended treatment (a total of 72 weeks) for slow responders, ie, subjects who became RNA negative after w24. Results The demographics and antiviral response for the 115 patients enrolled are summarized in the table. The 1500 and 1800mcg treatment groups had more patients with baseline characteristics associated with poor response (eg, pretreatment HCV RNA, Peg-IFN+RBV NR, % African-Americans). All doses were well tolerated and the safety profile in the 1500 mcg and 1800 mcg cohorts was comparable to the 900-1200mcg cohorts in incidence and types of adverse events. The overall SVR rate for the first 4 treatment groups was 19% (18/93), though lower in the genotype 1, Peg-IFN+RBV NR group 12% (7/57). Importantly, the 1800mcg group showed robust antiviral response (50% [6/12] achieved EVR12) in genotype 1 “null-responders” to prior Peg+RBV therapy. Conclusions Alb-IFN in combination with oral RBV is safe and effective. Treatment with 1800mcg Q2w demonstrates significant antiviral activity in prior IFNa non-responder patients.

52 VALIDATION OF A GENE SIGNATURE PREDICTING RESPONSE TO TREATMENT IN CHRONIC HEPATITIS C VIRUS INFECTIONS

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Introduction: We previously identified an 18-gene signature that predicts treatment response in chronic hepatitis C (CHC) in a population of 31 patients (Chen, et al. Gastroenterology 2005). In this study we asked whether its predictive capacity held true for a larger cohort. Methods: Liver biopsies were taken from 78 CHC patients prior to initiating treatment with peginterferon/ribavirin. RNA was extracted, amplified, and gene expression was studied using a 19K cDNA microarray. CHC liver biopsies were compared to biopsies from 20 normal, uninfected livers. In the meantime, the expression levels of 10 of the genes from our previous set of 18 were assessed by real-time PCR. Classification accuracy was assessed using 4 different methods (KNN, DQDA, DLDA, CART). Results: All four classification methods yielded similar results. For predicting treatment “responders,” the 18 gene signature had a positive predictive value (PPV) of 92% (sensitivity 80%, specificity 86%).
There was higher variability in the 10 gene real-time PCR dataset, producing a lower PPV of 80% (sensitivity 75%, specificity 59%). In all, 123 genes were differentially expressed in CHC (p <0.001, fold < or > 1.5 compared to normal livers).

As in our preliminary study, pre-treatment expression of a number of interferon-stimulated genes (ISGs) was higher in nonresponder liver tissue. These included the ISGs in our predictive subset of 18 genes. When the 123 genes were analyzed by multivariate analysis, responder status and genotype were the two most important variables contributing to levels of gene expression. When a hierarchical cluster analysis was applied to only the genotype 1 samples using the genes most influenced by genotype, two clearly segregated clusters of patient samples were identified: one consisting of patients who went on to respond to treatment, and the other of patients who did not. Conclusion: The pre-treatment difference between responder and nonresponder liver tissue is robust over large numbers of patients, a finding that has implications for diagnosis and disease pathogenesis.

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53 MULTIPLE DOSE SAFETY AND PHARMACOKINETIC STUDY OF BAVITUXIMAB IN PATIENTS WITH CHRONIC HEPATITIS C VIRUS (HCV) INFECTION
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Background: Bavituximab, an investigational monoclonal antibody targeting phosphatidylinerine (PS) located on the surface of virus infected cells and enveloped viruses, is being developed as an anti-HCV agent. It has immunostimulatory effects in preclinical viral models. Single intravenous (IV) infusions of bavituximab up to 6 mg/kg were well-tolerated and showed transient antiviral activity in chronic HCV patients. Methods: To determine the safety, tolerability and pharmacokinetics of multiple IV infusions of bavituximab, sequential cohorts of 6 patients were given twice weekly 90 min IV infusions for two weeks at 0.3, 1, 3 or 6 mg/kg and followed until week 12. Vital signs, physical exams, safety laboratory parameters, serum bavituximab levels and serum HCV RNA levels were measured. Results: Twenty-four patients (15 male, mean age 49) were enrolled. Eleven were non-responders, 8 were relapsers and 5 were treatment-naive. Mean baseline viral load was 5,000,000 copies/mL and 15 were infected with genotype 1, 8 with genotype 3 and 1 with genotype 2. The infusions were well-tolerated. No serious adverse events (SAEs) or early discontinuations were reported. There was no dose-related increase in incidence or severity of adverse events (AEs). All AEs were mild or moderate and transient, except for grade 3 neck pain and arthralgia (drug-related) in a patient with a history of joint pain at 3 mg/kg bavituximab and grade 3 elevated blood glucose (unrelated) in another patient with diabetes at 0.3 mg/kg bavituximab. All other drug-related AEs were mild: hypertension in 1 patient at 0.3 mg/kg, headache in 1 patient and headache and pruritis in 1 patient at 1 mg/kg and flu-like illness/symptoms in 2 patients at 6 mg/kg after the first infusion. Bavituximab reaches Cmax at 2-3 h postdose with a mean elimination half-life of ~34 h. Bavituximab exhibited dose-proportional increases in Cmax and AUC in single and multiple dosing and did not lead to significant accumulation. All dose levels exhibited on therapy antiviral activity (decline of >0.5 log10 reduction in HCV RNA). The 3 mg/kg cohort had the largest number of patients showing antiviral activity, as 5 of 6 subjects achieved >0.5 log decline in HCV RNA. Conclusions: Twice weekly IV doses of bavituximab up to 6 mg/kg were safe and well-tolerated. Single-dose and multiple dose pharmacokinetics of bavituximab are linear, predictable and no accumulation appears to occur over time. Two weeks of dosing is indicative of antiviral effect in a proportion of patients. Future studies designed to optimize the dosing schedule and investigate combining bavituximab with current standard therapy are planned.

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54 RAPID AND EARLY VIROLOGICAL RESPONSE RATES ARE INCREASED WITH 12 WEEK 360 µG/WK PEGINFERON ALFA-2A (40KD) AND STANDARD RIBAVIRIN IN HCV GENOTYPE 1 TREATMENT NAIVE PATIENTS: EFFICACY AND SAFETY ANALYSIS OF THE INDUCTION PHASE OF THE CHARIOT STUDY
Stuart Roberts1, Martin Weltman2, Darrell Crawford3, Wendy Cheng4, William Sievert5, Geoffrey W. McLaughlan6, Paul V. Desmond7, Motoke Yoshishara8, John E. Miller9, Jean Depamphilis10, Pip Marks10, Gregory J. Dore10, CHARIOT Study Group On Behalf of the1; 1The Alfred Hospital, Melbourne, VIC, Australia; 2Nepean Hospital, Sydney, NSW, Australia; 3Greenslopes Hospital, Brisbane, QLD, Australia; 4Royal Perth Hospital, Perth, WA, Australia; 5Monash Medical Centre, Melbourne, VIC, Australia; 6Royal Prince Alfred Hospital, Sydney, NSW, Australia; 7St. Vincent’s Hospital, Melbourne, VIC, Australia; 8Roche Products, Sydney, NSW, Australia; 9Roche, Nutley, NJ; 10National Centre in HIV Epidemiology and Clinical Research, Sydney, NSW, Australia

Background: The CHARIOT study evaluates the efficacy & safety of a 360 µg/wk dose peginterferon alfa-2a (Peg-IFNα-2a) induction regimen compared to standard 180 µg/wk Peg-IFNα-2a, in combination with standard ribavirin (RBV) in treatment-naive patients with chronic hepatitis C genotype 1. We report results of the planned interim analysis of on-treatment virological responses over the first 12 wks of therapy. Methods: In this international, multi-centre, open label study patients were stratified by baseline HCV RNA and randomised 1:1 to 180 µg or 360 µg Peg-IFNα-2a qw for 12 wks followed by 36 wks of 180 µg Peg-IFNα-2a plus RBV 1000–1200 mg/d for 48 wks. Endpoints include virologic response at wks 4, 8 and 12. Results: 845 patients were evaluable for efficacy & safety analysis. Age, gender, weight, BMI, ethnicity, viral load and fibrosis scores were similar in the two groups. At wks 4, 8 and 12, virological response rates were greater (p<0.001) in the 360 µg/wk arm (Table). Significantly higher response rates (p<0.001) at wk 12 occurred in patients receiving 360 µg dose in all subgroups including baseline HCV RNA ≥800,000 IU/mL (71% vs 55%) and <800,000 IU/mL (84% vs 71%), weight ≥85 kg (74% vs 56%) and <85 kg (74% vs 62%), and age ≥40yrs (70% vs 54%) and ≤40yrs (84% vs 73%). Induction therapy was associated with higher rates of diarrhoea (17% vs 12%), pyrexia (14% vs 8%), chills (14% vs 7%) and weight loss (10% vs 3%). Depression and fatigue reports were similar. Discontinuations were similar in both groups, however induction therapy led to more dose modifications (Table). Use of haematopoetic growth factors was the same in both groups (4 patients [<1%]). Conclusion: Induction dosing with 360 µg/wk Peg-IFNα-2a is more effective than standard 180 µg/wk dosing in achieving early and rapid virological responses and is safe and well tolerated. The effect was independent of baseline viral load, weight, BMI and age.
55 DIET-INDUCED STEATOHEPATITIS IS PREVENTED BY MYD88 KNOCKOUT PHENOTYPE BUT NOT BY SELECTIVE MYD88 DEFICIENCY IN THE BONE MARROW-DERIVED CELLS IN MICE

Arumugam Velayudham, Istvan Hritz, Angela Dolganuc, Evelyn Kurt-Jones, Donna Catalano, Pranoti Mandrekar, Gyongyi Szabo

Background: Activation of inflammation-associated pathways is a major component in the progression of non-alcoholic fatty liver disease (NAFLD). Toll-like receptors (TLRs) expressed on hepatocytes and inflammatory cells in the liver recognize both pathogen-derived and endogenous mediators. A role for lipopolysaccharide (LPS) in the pathogenesis of NAFLD is suggested by the observation that polymorphisms in the components of the LPS receptors, CD14 or TLR4, are risk factors for NASH. Aim: To evaluate the role of TLR4 and its downstream signaling though MyD88 and to dissect the role of bone marrow-derived cells resulted in no protection from steatosis (triglyceride) and oxidative injury (TBARS and CYP2E1). Serum cytokines (TNFa, IL-10, IL-6, and KC) were equally increased in WT and WT/MyD88KO chimeric mice after MCD diet compared to MCS controls suggesting no protection against inflammation. Conclusions: Our results demonstrate a critical role for TLR4 and its MyD88-dependent downstream signaling in development of NASH-associated liver damage (steatosis and inflammation). However, the absence of the TLR4 adaptor, MyD88, in bone marrow-derived cells failed to prevent steatohepatitis pointing to a critical role for MyD88-dependent pathways in liver parenchymal cells. The role of TLR4 and MyD88 activation remains to be investigated in human NASH.

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56 ENHANCED INNATE IMMUNE RESPONSE AND ACCELERATED LIVER REGENERATION IN HEPATOCYTE-SPECIFIC IKK2 DELETED MICE

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Liver regeneration is a complex mechanism involving a plethora of factors, and among them, the transcription factor NF-kB plays an important role. In non-stimulated cells, NF-kB is sequestered in the cytoplasm by IκB. Upon stimulation, IκB is phosphorylated by the IKK complex (consisting of two catalytic subunits IKK1 and IKK2, and the regulatory subunit NEMO), allowing NF-kB activation and translocation into the nucleus. As NF-kB is involved in early events of hepatocyte proliferation, we therefore studied the impact of hepatocyte-specific IKK2 deletion on liver regeneration. IKK2 constitutive knockout (ko) mice die in-utero. Thus, we generated C57BL/6L hepatocyte-specific IKK2 deletion (IKK2Δhepat) mouse using the Cre/LoxP system. 70% partial hepatectomy (PH) was performed on IKK2Δhepa mice (knock-out), and liver regeneration was studied. All mice survived after PH despite a weaker and delayed NF-kB response in IKK2Δhepa mice. In order to understand the role of IKK2 deletion during liver regeneration, we first analysed the priming phase. TNFα-mediated priming of hepatocytes and its ability to induce extracellular matrix remodelling via MMP9, are key events during liver regeneration. TNFα alpha protein expression was immediately up-regulated in IKK2Δhepa mice. 1h after PH, while wt mice exhibited a similar level 6h after PH. Moreover the MMP-9 protein activity was detected earlier in IKK2Δhepa mice, compared to IKK2Δwf mice, suggesting earlier priming in the ko animals. Then, we investigated Serum Amyloid A (SAA), a marker of the Acute Phase Response in mice. Interestingly, ko animals showed inflammatory activation and a mediator of insulin resistance, was significantly lower in MCD diet-fed TLR4KO and MyD88KO mice compared to WT mice. Conversely, the hepatoprotective cytokine, IL-6, and the anti-inflammatory cytokine, IL-10, were significantly higher in the sera of TLR4KO and MyD88KO MCD-fed mice compared to WT. These results suggested that MyD88-dependent pathways were critical in the development of both steatosis and inflammation. To determine whether MyD88 expression was critical in the parenchymal or in the inflammatory, BM-derived cells, we transplanted irradiated WT mice with MyD88KO bone marrow before MCD/MCS diet feeding. Selective MyD88 deficiency in BM-derived cells resulted in no protection from steatosis (triglyceride) and oxidative injury (TBARS and CYP2E1). Serum cytokines (TNFa, IL-10, IL-6, and KC) were equally increased in WT and WT/MyD88KO chimeric mice after MCD diet compared to MCS controls suggesting no protection against inflammation. Conclusions: Our results demonstrate a critical role for TLR4 and MyD88-dependent downstream signaling in development of NASH-associated liver damage (steatosis and inflammation).
a strong upregulation in mRNA expression already at 6h after PH compared to wt. Neutrophil chemoattractant chemokine KC mRNA expression was analysed by real time PCR. KC mRNA expression was enhanced in ko mice with a very significant increase at 6h after PH. This result led us to investigate the involvement of the immune response after PH via flow cytometry. We examined liver and blood cells 12h after PH, and IKK2\(_{\text{ΔIκBα}}\) mice exhibited higher number of neutrophils in both blood and liver, compared to wt animals. As a result of these early events, IKK2\(_{\text{ΔIκBα}}\) mice showed earlier hepatocytes proliferation compared to wt animals confirmed by BrdU analysis and cyclins expression. Our data demonstrate that hepatocyte-specific IKK2 deletion triggered a faster priming phase, an increased acute phase response along with a stronger neutrophils recruitment in the liver leading to an earlier hepatocyte proliferation in IKK2\(_{\text{ΔIκBα}}\) mice during liver regeneration.

Disclosures:
The following people have nothing to disclose: Yann Malato, Naiara Beraza, Leif Sander, Malika Almasaussidi, Manolis Parasarakis, Christian Trautwein

57 DEPENDENCE OF THE ANTIVIRAL RIG-I-MAVS-IKK\(\varepsilon\) SIGNALING PATHWAY ON MITOCHONDRIAL RESPIRATION

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A mitochondrially based signaling pathway, RIG-I - MAVS - IKK\(\varepsilon\), is required for endogenous interferon production in response to HCV infection and is specifically disrupted by the viral NS3/4A protease. However, the reason for the mitochondrial localization of this signaling pathway is unclear. We have previously observed that net signaling requires mitochondrial respiration but the mechanism for this is unknown. The aim of this study was to determine which elements of this signaling cascade require normal mitochondrial function. METHODS: Huh-7 based cell lines were incubated with a series of mitochondrial inhibitors, including rotenone, diphenylene iodonium and oligomycin, and subsequently challenged with Sendai virus (SV). To assess the activation of the signaling pathway, we examined dimerization of IRF-3, activation of interferon-\(\beta\) promoter or ISG-56 promoter, phosphorylation of STAT-1 and expression of ISG15. RESULTS: Inhibition of mitochondrial function prevented SV-induced IRF-3 dimerization, interferon-\(\beta\) promoter activation and ISG56 promoter activation. The inhibitors had no effect on SV infectivity or replication. The site of the block was examined by overexpressing a constitutively active fragment of RIG-I (N-RIG), MAVS, IKK\(\varepsilon\), or the constitutively active IRF-3-5D. Sensitivity to mitochondrial inhibitors was seen for N-RIG, MAVS, and IKK\(\varepsilon\) but not for IRF-3-5D. This approach thus localized the site of block to IKK\(\varepsilon\). We next examined whether the inhibitor effects resulted from changes in redox environment or ATP levels. Manipulation of mitochondrial redox status by either thiols antioxidants or exogenous peroxides did not affect SV induced IRF-3 dimerization. However, the mitochondrial inhibitors did produce an approximately 40% decrease in total cellular ATP. This decrease in ATP did not occur in all cell types and prevention of SV-induced IRF-3 dimerization occurred only in cell types in which an ATP reduction was observed. In CONCLUSION, mitochondrial respiration is required for activation of innate immunity pathways and this requirement appears to be due to strong ATP dependence of the IKK\(\varepsilon\) protein kinase. The need for this kinase to localize in a high ATP microenvironment could be one reason that this signaling complex is closely linked to mitochondria.

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58 HEPATOCYTE CYCLOOXYGENASE-2 MEDIATES ENDOXIN-INDUCED ACUTE LIVER FAILURE

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Bacterial lipopolysaccharide (LPS; endotoxin) is an abundant and essential component of the outer membrane of Gram-negative bacteria. It provokes a generalized proinflammatory response in the infected host that can be associated with multiple organ dysfunctions including acute hepatic failure. LPS derived from intestinal bacteria is also implicated in the pathogenesis of chronic inflammatory liver diseases, such as chronic hepatitis, alcoholic liver disease and cirrhosis. We hypothesized that COX-2 activation in hepatocytes may trigger LPS-induced liver injury and, therefore, inhibition of COX-2 signaling pathway may attenuate LPS-induced tissue damage. To test this hypothesis, we generated transgenic mice with targeted expression of COX-2 in the liver by using the albumin promoter-enhancer driven vector. Although the COX-2 transgenic mice developed normally with no significant liver inflammation or histologic abnormality under normal housing conditions, they exhibited early mortality than wild type mice when the animals were subjected to a standard experimental protocol of LPS-induced acute fulminant hepatic failure (intraperitoneal injection of low dose of LPS in combination with D-galactosamine (GalN)). In COX-2 transgenic group (n=16), mortality became apparent at 5 to 6 h and all mice died by 9 h. In wild type mice (n=16), no death was observed at 6 h; first animal death was observed at 7 h. Under LPS/D-GalN treatment for 4 h, the COX-2 transgenic mice showed significantly higher serum ALT and AST levels and more prominent liver tissue damage (parenchymal hemorrhage, neutrophilic inflammation, hepatocyte apoptosis and necrosis) than wild type mice (p<0.01). Western blot analysis of the liver tissues showed that LPS/GalN treatment for 4 hours induced the cleavage of PARP, caspase-3 and caspase-9 in COX-2 transgenic mice but not in wild type mice. Pretreatment of the COX-2 transgenic mice with ONO-8711 (2.5 μg/mg body weight), a specific antagonist for the prostaglandin receptor EP1, prevented LPS/GalN-induced liver injury and hepatocyte apoptosis. Accordingly, the mice with genetic ablation of EP1 showed less LPS/GalN-induced liver damage and less hepatocyte apoptosis with prolonged survival when compared to the wild type mice (n=15 for each group). These findings demonstrate that COX-2 and its downstream prostaglandin receptor EP1 signaling pathway predispose the liver to LPS-induced injury. Therefore, blocking COX-2/EP1 pathway may represent an effective approach for reversal of LPS-induced liver failure.

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59 LIVER DAMAGE IN A MOUSE MODEL OF FULMINANT AUTOIMMUNE HEPATITIS IS DEPENDENT UPON CD4\(^{+}\) T CELL PRODUCTION OF IFN-γ, BUT INDEPENDENT OF BOTH CD8\(^{+}\) T CELLS AND FAS

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Autoimmune hepatitis (AIH) is thought to result from pathological activities of liver T cells, but mechanisms by which they cause hepatocellular damage remain unclear. Using the
BALB/c-TGF-β1−/− mouse model of fulminant AIH, we focused on the role of IFN-γ, examining both its cellular origin and possible effector mechanisms. Previously, we showed that both CD4+ T cells and IFN-γ are required for the development of liver damage. In the current study, we show that plasma IFN-γ concentration correlates very strongly with AST in twelve TGF-β1−/− mice evaluated ($r^2 = 0.49; p = 0.01$). Among liver lymphocyte subsets, CD4+ T cells are both most numerous and produce the most IFN-γ per cell, with decreasing contributions from CD8+ T and NK cells. Depletion studies using subset-specific monoclonal antibodies definitively show that CD4+ T cells, but not CD8+ T cells or NK cells, are necessary for both elevated plasma IFN-γ and hepatocellular damage. Evaluating potential effector roles for IFN-γ, IFN-γ is not required for TGF-β1−/− CD4+ T cell activation (all TGF-β1−/− or TGF-β1−/−/IFN-γ−/− double knockout liver CD4+ T cells are CD62L−/−, oligoclonal expansion (TGF-β1−/− CD4+ T cells and TGF-β1−/−/IFN-γ−/− CD4+ T cells exhibit non-Gaussian distributions on spectratype analysis), or accumulation in liver. IFN-γ is required for over-expression of class II genes in TGF-β1−/− livers, and likely acts via up-regulation of the co-activator CIITA. Finally, IFN-γ is required for FasL expression in TGF-β1−/− livers, and FasL is strongly expressed by TGF-β1−/− liver CD4+ T cells. However, liver damage developed unabated in fas-deficient TGF-β1−/−/lpr/lpr mice, ruling out a role for fas in the pathogenesis of liver disease in this model system. These data establish that CD4+ T cell production of IFN-γ is the key determinant of liver damage in this model system of AIH, and that liver damage is independent of both CD8+ T cells and Fas.

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60 DELETION OF THE CHEMOKINE RECEPTOR CXCR3 LEADS TO INCREASED ACUTE LIVER INJURY AFTER CCL4 ADMINISTRATION

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Background: Chemokines may play important roles during acute and chronic liver injury through modulation of immune cell recruitment. The chemokine receptor CXCR3 is expressed on activated T-cells, NK cells, monocytes and dendritic cells. CXCR3 and its ligands CXCL9, CXCL10 and CXCL11 have been shown to be up-regulated in acute and chronic viral induced liver damage in mice and humans, but their functional role in liver injury has not yet been elucidated. Therefore, we investigated the function of CXCR3 in acute liver injury after challenge with carbon tetrachloride (CCL4). Material and Methods: Acute liver injury was induced in CCL4−/− mice and wildtype littermates (n = 4-6) through single administration of CCL4 (0.6 mg/kg). The degree of liver injury was assessed by liver histology (H&E staining) and serum transaminases at days 1, 3 and 5 after injury. Immune cell recruitment of T-cells, NK cells and NKT cells was assessed by FACS analysis at the same time points. The m RNA expression of characteristic Th1 (Interferon-gamma, Ifn-gamma) and Th2 (Interleukin-4, IL-4) cytokines within the liver was assessed by quantitative RT-PCR. Results: Compared to their wild type littermates, CXCR3−/− mice displayed significantly elevated AST and ALT levels 24 hours after CCL4 administration (P < 0.002). Increased liver injury in CXCR3−/− mice was also reflected by histology which showed extensive necrosis after 24 hours in the knockout animals, but only moderate necrosis in their wild type littermates. The histological difference between CXCR3−/− and wild-type mice was also evident at days 3 and 5 during the regenerative phase after liver injury. Interestingly, FACS analysis revealed significantly reduced intrahepatic numbers of T-cells, NK cells and NKT cells 24 hours after CCl4 administration in CXCR3−/− mice compared to wild-type mice (all P < 0.05). In CXCR3−/− mice, acute liver injury was associated with an increased hepatic expression of Ifn-gamma, while IL-4 was the predominant cytokine during liver regeneration at day 5. In contrast, level Ifn-gamma was the principal cytokine in wild-type mice during the acute and later phases of liver injury at the mRNA (P < 0.05 compared to CXCR3−/− mice). Discussion: Genetic deletion of CXCR3 leads to increased liver damage after toxic injury. The increased vulnerability of CXCR3−/− mice is associated with decreased numbers of liver infiltrating lymphocytes and a Th2 shift of the Ifn-gamma/IL-4 mRNA ratio during the regenerative phase. These results demonstrate an important role of the chemokine receptor CXCR3 in acute liver diseases.

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61 ORPHAN NUCLEAR RECEPTOR SHP IS A NOVEL TUMOR SUPPRESSOR OF HEPATOCELLULAR CARCINOMA

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Background: The small heterodimer partner (SHP, NROB2), a member of nuclear receptor superfamily, contributes to the biological regulation of several major functions of the liver. The aim of this study was to evaluate the novel role of SHP in cellular proliferation, apoptosis, and tumorigenesis in both mice and humans. Methods: Mouse embryonic fibroblasts (MEFs) derived from SHP+/+ and SHP/-/- mice were used as in vitro models to study the role of SHP on cell cycle regulation, apoptosis, and tumorigenesis. SHPKO and SHPTG mice served as in vivo models to elucidate the role of SHP on hepatoma formation. Human HCC specimens and HCC cell lines were used to determine the tumor suppressor function of SHP in human HCC formation. Results: Immortal mouse fibroblasts lacking SHP exhibited growth advantage, produced increased cyclin D1 mRNA and protein, and cyclin D1 associated kinase activity, and SHP was shown to be a direct negative regulator of cyclin D1 gene transcription. SHP/-/- fibroblasts also displayed characteristics of malignant transformed cells, including loss of contact inhibition, resistance to apoptotic stimuli, formation of more colonies in soft agar, and promoting tumor growth in immune compromised mice. Older SHP/-/- mice developed spontaneous hepatomas, which was strongly associated with an increased expression of cyclin D1 mRNA, enhanced cell proliferation, and decreased apoptosis of hepatocytes. In contrast, overexpressing SHP in hepatocytes of SHPTG mice inhibited proliferation and induced robust apoptosis. The expression of SHP was frequently down-regulated and SHP function was abrogated in human HCC pathological specimens and cell lines by transcriptional silencing due to SHP promoter hypermethylation. Overexpression of SHP inhibited HCC foci formation and cell invasion, increased the sensitivity of HCC cells to apoptotic stimuli, and arrested HCC tumor growth in xenografted nude mice. Conclusions: SHP suppresses tumorigenesis by negatively regulating cellular growth and modulating cell survival through targeting cell cycle regulator and antiapoptotic gene. We con-
62 TUMORIGENIC LIVER STEM CELLS EXPAND DURING AGING IN METHIONINE ADENOSYLTRANSFERASE 1A DEFICIENT MICE

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Background and aim: Methionine adenosyltransferase (MAT) is an essential enzyme that catalyzes S-adenosylmethionine biosynthesis. Of the two genes that encode MAT, MAT1A is expressed in normal liver. Hepatic MAT activity falls in chronic liver diseases and mice lacking MAT1A develop hepatocellular carcinoma (HCC) spontaneously by 18 months. Our current work tests the hypothesis that liver stem cells may be the origin of HCC in this model. Methods: Livers from 6- and 18-month old MAT1A knockout (KO) mice and wild type (WT) littermates were fractionated into CD45- (leukocyte depleted), non-parenchymal (NP) cells and isolated by flow cytometry (FACS). CD45-NP cells were cultured using liver stem cell conditions. Gene expression was analyzed by real-time PCR. Bulk cultured cells and clones from single cells were analyzed using fluorescent immunohistochemistry (FIHC), RT-PCR, and Western Blot. Tumor formation was assessed using 1x106 CD133+CD49f+ cells injected intraperitoneally into immune-deficient mice. Results: The proportion of CD49f+ and CD133+ expressing cells within the CD45-NP fraction increased by 4.5 to 5.5-fold from 6 to 18 months in MAT1A KO mice but not WT mice (CD49f+ = 23±5%; CD133+ = 26±7% in 18-month KO mice; <7% all other groups, p<0.05). Compared to CD49f+ cells from old KO mice, CD49f+ cells from the same animals have marked increase in Kras (8-fold), Nras (4-fold), Survivin (4-fold), and Epidermal growth factor receptor (Egfr) (7-fold) mRNA levels. CD49f+ cells were selected in vitro using laminin coated plates. FACS analysis after 9 months of cultivation showed that 90.3±2.8% of cells have CD49f+ expression. Bulk culture of these CD49f+ cells demonstrated a rapidly proliferating group of small cells with expression of albumin, biliary cytokeratins, a fetoprotein, and c-Met by FIHC. Further sub-sorting of these CD49f+ cells revealed a population (15±7%) with CD133+ expression. Of the CD133+ cells, 99±0.4% have CD49f+ co-expression. Clonal expansion of single CD133+ cells isolated by FACS revealed maintenance of bi-potency, as well as strong expression of Hnf4, c-Met, and Egfr in 100% of expanded clones (N=4). After CD133+CD49f+ cells were injected into immune-deficient mice, 25% formed tumors of homogeneous, primitive, epithelial cells ranging in size from 4-6 mm after 6 weeks. Conclusions: MAT1A KO mice have expansion of liver stem cells as they age. These cells express increased levels of several oncogenes and are tumorigenic in vivo. This is the first demonstration of adult liver stem cells possessing tumorigenic potential without the use of a carcinogen or manipulation of tumor suppressor or oncogene expression.

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63 COX-2-DERIVED PGE2 ACTIVATES β-CATENIN IN HUMAN HEPATOCELLULAR CARCINOMA CELLS: IMPLICATIONS FOR TARGETED CHEMOPREVENTION AND TREATMENT

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Recent evidence suggests that cyclooxygenase-2 (COX-2) and Wnt/β-catenin signaling pathways are implicated in hepatocarcinogenesis. This study was designed to examine the potential interaction between the COX-2-derived prostaglandin E2 (PGE2) and Wnt/β-catenin signaling pathways in human hepatocellular carcinoma (HCC) cells and to evaluate whether these pathways can be concomitantly blocked for effective inhibition of tumor growth. Immunohistochemical stains for COX-2 and β-catenin were performed in twenty paired human HCC and their matched nonneoplastic liver tissues. Increased cytoplasmic staining for COX-2 and nuclear staining for β-catenin were observed in HCC cells when compared with the nonneoplastic hepatocytes (p<0.01, n = 20). Treatment of cultured human HCC cells (Hep3B) with PGE2 induced dissociation of Axin from GSK-3β thus leading to β-catenin accumulation; this result provides novel evidence for activation of β-catenin by COX-2-derived PGE2 in human HCC cells. Furthermore, the levels of COX-2 and β-catenin proteins in Hep3B cells were significantly reduced by two ω-3 PUFAs, docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA). ω-3 PUFAs also inhibited PGE2 level through upregulation of the COX-2 antagonist, 15-hydroxyprostaglandin dehydrogenase (15-PGDH). Accordingly, DHA and EPA induced a dose-dependent apoptosis with cleavage of PARP, caspase-3 and caspase-9 in three human HCC cell lines (Hep3B, Huh-7, HepG2). Consistent with the above in vitro observations, the growth of HCC in vivo was significantly reduced when mouse HCCs (Hepa1-6) were inoculated into the Fat-1 transgenic mice which express a Caeonohabditis elegans desaturase converting ω-6 to ω-3 PUFAs endogenously (n = 10-12 for each group). These findings suggest that concomitant inhibition of COX-2/PGE2 and Wnt/β-catenin signaling pathways may represent a promising therapeutic strategy for effective chemoprevention and treatment of HCC.

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64 LEPTIN IS A GROWTH FACTOR FOR CHOLANGIOCARCINOMA: AN IN VIVO EXPERIMENTAL STUDY

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Background: Few experimental carcinogenesis models of cholangiocarcinoma have been established. Leptin, a protein hormone principally produced by the adipose tissue, plays an important role in the regulation of angiogenesis and growth of...
several tumors. The obese Zucker fa/fa rat is characterized by the lack of leptin function due to the loss of functionality of the leptin receptor. Our recent data demonstrated that leptin enhances the growth of cholangiocarcinoma (CCA) cells in vitro. The Aim of the current study was to evaluate in vivo the effect of the lack of leptin function in regulating the development and growth of cholangiocarcinoma. Methods: Studies were performed in Zucker fa/fa rats and their corresponding littermates. Animals were chronically treated for 24 weeks with or without thiacetamide (TAA, dissolved in tap water at 0.03%), a carcinogenic factor for cholangiocytes. Animal and liver weight were measured and subsequently immunohistochemistry for CK-19 (a marker of biliary cells) and ErbB-2/Neu (overexpressed by malignant cholangiocytes) was performed to assess morphology and tumor growth. Results: After 24 weeks the average body weight showed that controls gained significantly more weight (lean 468.5 g ± 24.5, fa/fa 567.0 g ± 40.0; P < .05) compared to TAA treated rats (lean 299.6 g ± 12.9, fa/fa 417.1 g ± 16.7; P < .05). Liver weight from TAA treated animals also decreased after the ingestion of TAA (control lean 22.7 g ± 3.2 versus TAA-treated lean 16.3 g ± 0.6, P < .05; control fa/fa 26.4 g ± 0.5 versus treated fa/fa 21.2 g ± 1.5, P < .05). Macroscopically, 100% of the TAA treated Zucker lean rats developed multiple white, round and firm nodules over the hepatic surface, including the left, middle and right liver lobes. A reduced number of isolated white, round nodules were present on the liver surface of TAA treated-Zucker fa/fa rats. Morphological evaluation of liver tissue showed invasive intestinal-type CCA with intense stromal desmoplasia. Liver sections of TAA-treated Zucker lean rats showed a higher number of CK-19 and ErbB-2/Neu-positive malignant cholangiocytes compared to TAA-treated Zucker fa/fa rats, indicating that TAA-treated Zucker lean rats showed more tumoral tissue infiltrating their liver with respect to TAA-treated Zucker fa/fa rats. Summary/Conclusion: The lack of leptin-mediated signals reduces cholangiocarcinoma development and growth. Leptin is involved in the cholangiocarcinogenesis process and favors the growth of biliary malignancies. Thus, modulation of the leptin-mediated signal could be useful to counteract the growth of cholangiocarcinoma.

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65 OVER-EXPRESSION OF IL-6 CONTRIBUTES TO CHolangiOCArcinoma INVASION BY MIR-21 DEPENDENT PATHWAYS

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Background: Interleukin-6 (IL-6) is over-expressed and has been implicated in the growth of many cancers, including cholangiocarcinoma. We have shown that over-expression of IL-6 can alter the expression of microRNAs and modulate cell signaling pathways involved in cholangiocarcinoma growth (JBC 2007). In this study, we investigated the involvement of aberrant miRNA expression by IL-6 in tumor cell growth and invasion in human cholangiocarcinoma cells. Methods: IL-6 over-expressing clones of Mz-CH-A-1 and KMCH-1 cholangiocarcinoma cells were generated by transfection with full-length IL-6, and designated as MzIL-6 and KMIL-6 respectively. The expression of miRNA in stable transfectants and parental cells was evaluated using a hybridization based microarray. miR-21 expression was confirmed by real-time PCR. miR-21 expression was modulated using miR-21 specific miRNA precursors or antisense oligonucleotides. Cell proliferation was assessed by a viable cell assay, and invasion was assessed by migration across tumor basement membrane. Expression of downstream targets of miR-21, namely PTEN and MMP-2 was assessed in vitro, and in tumor cell xenografts. Results: The growth of IL-6 over-expressing tumor cell xenografts was significantly increased compared to control. Selected microRNAs such as miR-21, miR-16-1 and members of the let-7 family were increased in both MzIL-6 and KMIL-6 cells. Of these, the greatest change was observed for miR-21 which was increased by 21.6 ± 2.1-fold and by 10.4 ± 0.7-fold in MzIL-6 and in KMIL-6 cells respectively compared to controls. Furthermore, miR-21 expression was increased by 4.5 ± 0.8-fold (p<0.05) in MzIL-6 tumor cell xenografts compared to controls. Cell invasion in vitro was also increased in MzIL-6 cells by 1.9 ± 0.2-fold and by 1.7 ± 0.2-fold in KMIL-6 cells compared to controls. Moreover, IL-6 induced tumor cell invasion was blocked by anti-miR-21 in MzIL-6 cells by 47 ± 9% and in KMIL-6 cells by 39 ± 7%. Anti-miR-21 also decreased IL-6 dependent expression of MMP-2 mRNA expression by 0.4 ± 0.1-fold. RNA interference mediated inhibition of PTEN, a downstream target of miR-21, increased cell invasion by 48 ± 6% in MzIL-6 cells. Summary and Conclusions: Over-expression of IL-6 contributes to cholangiocarcinoma growth and enhances tumor cell invasion by miR-21 dependent pathways. We conclude that targeting IL-6 dependent miR-21 mechanisms involved in tumor growth and spread will be a valuable therapeutic strategy for biliary cancers.

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66 ESTABLISHMENT OF RAT LIVER CANCER STEM CELL LINES EXPRESSING GRANULOCYTE-COLONY STIMULATING FACTOR RECEPTOR (G-CSFR) AND ROLE OF G-CSF/G-CSFR AXIS IN MODULATING THEIR PROLIFERATION AND MIGRATION POTENTIAL

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Background and Aims. Oval cells (OC), putative liver stem cells, may give rise to liver cancers. We have developed a new regimen of carcinogenesis, based upon induction of OC proliferation followed by aflatoxin exposure. Aim of the present study was to establish liver cancer stem cell lines from our animal model. Materials and Methods. F344 rats were subjected to 2-regimen of carcinogenesis, based upon induction of OC proliferation followed by aflatoxin exposure. Animals were then injected with aflatoxin. Cancer cells were isolated from tumors, cultured, and single clones were selected. The established cell lines (LCSCs) were characterized for growth kinetics, morphology, immunophenotype, and karyotype. Tumorigenicity assays were carried out. In vitro proliferation and migration assays were also performed, to establish the effects of granulocyte-colony stimulating factor (G-CSF) and its receptor (G-CSFR) on LCSCs. Results. The primary tumors were well-differentiated hepatocellular carcinomas. Six cancer cell lines, LCSC[1-6], were developed. LCSCs adopted an epithelioid morphology, share with the primary tumors the expression of OC markers, and harbor a subpopulation of small G-CSFR+ cells. Transplantation studies proved the tumorigenicity and metastatic potential of LCSCs. Moreover, G-CSFR

Disclosures:
was expressed by both the primary tumors and all the LCSCs and its blockage inhibited LCSC proliferation and migration in vitro. Conclusions. We established 6 cell lines from our model of hepatocholangiocarcinoma. Phenotype, tumorigenicity, and metastatic potential suggest that LCSCs harbor a population of G-CSFR+/ cancer stem cells of OC origin. In the present study, we have also described the involvement of the G-CSF/G-CSFR axis in modulating LCSCs, and the inhibitory effects of G-CSFR blockage on LCSC proliferation and migration. These results may lead to new treatments against liver tumors, targeting stem cell-mediated cancer growth and preventing the establishment of metastases. Overall, LCSCs may be novel and useful cell lines for further studies of molecular pathways underlying hepatoccholangiocarcinogenesis, as well as for the development of new cancer stem cell-targeted therapies.

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67 VARIABILITY OF THE ABCB4 GENE IN YOUNG ADULT CHOLECYSTECTOMIZED PATIENTS

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Aims and background: The ABCB4 (ATP-binding cassette, subfamily B (MDR/TAP), member 4) transporter expressed in the canalicular membrane of hepatocytes is responsible for phosphatidylcholine secretion into bile. Conceivably, variations of the ABCB4 gene could contribute to cholesterol gallstone formation through lowering biliary phosphatidylcholine secretion. The present study was undertaken to determine the frequency and type of ABCB4 genetic variability amongst patients < 40 years treated surgically for gallstone disease at a regional Norwegian surgical hospital. Materials and methods: One hundred and four patients (mean age 30 years, range 12-39) of Caucasian and Caucasoid ethnic background cholecystectomized on account of symptomatic cholesterol gallstone disease were included in the study. Genomic DNA from peripheral white blood cells was extracted on MagNApure LC from Roche. Coding exons 2 to 28 in the ABCB4 gene, (RefSeq NM_000443.3) including exon-intron boundaries were sequenced using an Applied Biosystem 3730 DNA Analyzer. Predictions of amino-acid substitution matrices (Grantham, BLOSUM62) and computational tools that utilize position-specific evolutionary properties of the protein (SIFT, PolyPhen). Results: 12 exon variations were found, 8 of which were protein-altering: 6 missense, 1 frameshift and 1 nonsense. 11 intron variants were found. Of the 8 protein-altering variations 5 were predicted to have functional consequences (c.523A>G, c.1769G>A, c.3318G>C, c.1399_1400 ins10, c.3136C>T). Conclusion: Substantial genetic variability exists amongst young Caucasian and Caucasoid patients < 40 years of age cholecystectomized at a Norwegian regional hospital. The ABCB4 genetic variations: c.523A>G, c.1769G>A, c.3318G>C, c.1399_1400 ins10, c.3136C>T may contribute to an early debut of cholesterol gallstone disease.

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68 HLA ASSOCIATION IN PRIMARY SCLEROSING CHOLANGITIS: DETECTION AND FINEMAPPING OF AN HLA INDEPENDENT SIGNAL IN THE COMPLEMENT GENE CLUSTER

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Background: An association between the risk of developing primary sclerosing cholangitis (PSC) and genetic variants within the HLA complex on chromosome 6p21 was detected 25 years ago. This genetic region contains more than 250 closely linked genes, the pinpointing of the genetic variants of relevance to PSC has proven extremely difficult. In an attempt to further refine the HLA association in PSC, systematic mapping of the entire HLA complex was performed in a large cohort of Scandinavian PSC patients. Materials and methods: 365 PSC patients and 368 healthy controls were genotyped for all classical HLA loci (HLA-A, -B, -C, DRB1, DRB3 and DQB1) using a sequencing based approach. A two-stage screen using single nucleotide polymorphism (SNP) markers was subsequently performed. In stage one, 420 SNPs were successfully genotyped with SNPlex® technology. Dissection of the association signals was performed using established statistical packages as well as novel statistical approaches. In stage two, saturation of a distinct risk region using additional 130 SNPs was performed to localize causative variants. Results: The SNP screen revealed the presence of a wide and complex association signal blurred by a strong-LD haplotype that may harbor several variants strongly associated with PSC, e.g. at HLA-B (add odds ratio [OR] HLA-B*08 = 3.5, 95% CI [2.6-4.5], p<10^-16) and MICA (OR micas253495_A = 3.5, 95% CI [2.7, 4.5], p<10^-16), which have been also reported in previous studies. When case haplotypes were proactively matched with randomly drawn control haplotypes at markers defining this strong-LD haplotype (i.e. HLA-B*08 and DRB1*0301), a distinct association signal became evident at the complement factor gene cluster within the central HLA class III region. Maximum association in this risk region was observed for a common allele at an intronic SNP (69% vs. 48%, OR=2.5, 95% CI [2.0, 3.1], p=10^-16). Interestingly, this allele maps to all known HLA risk haplotypes in PSC (i.e. DR3, DR6 and DR2), while previously reported protective HLA haplotypes in PSC (i.e. DR4, DR7 and DR11) predominantly carry the opposite allele at this position. Conclusion: The present dataset provides an extensive insight into the complexity of the HLA association in PSC. Multiple risk variants are likely to exist, some of which have been previously reported in other autoimmune diseases (e.g. at HLA-B and MICA), and others which are PSC-specific. Novel alignment strategies with known risk and non-risk HLA haplotypes in PSC helped to dissect a distinct risk locus for PSC in the central HLA class III region. Ongoing analyses aim to identify the exact susceptibility gene within this region.
THE NATURAL HISTORY OF SMALL-DUCT PRIMARY SCLerosing CHOLANGITIS

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Background: Previous studies suggested that patients with small-duct primary sclerosing cholangitis (PSC) have a favorable prognosis in comparison to those with large-duct PSC. Prior studies, however, have included limited numbers of patients with a relatively short follow-up, with minimal or no power for an appropriate analysis of long-term survival. Hence, we aimed at determining the natural history and long-term prognosis of a large number of patients with small-duct PSC and to compare the outcomes and survival to that seen in appropriately matched patients with classic large-duct PSC.

Methods: Data from 83 patients with well-characterized small-duct PSC from several medical institutions in Europe and the United States were combined. Each patient with small-duct PSC was randomly matched to two patients with well-characterized large-duct PSC by age, gender, calendar year of diagnosis, and institution. Outcomes included development of cholangiocarcinoma (CCA), need for liver transplantation, and death. The Kaplan-Meier product limit was used for estimating survival free of liver transplantation.

Results: The median age at diagnosis in both groups was 38 years with 61% being males. Cases of small-duct PSC had a significantly longer survival free of liver transplantation as compared to controls with large-duct PSC (p<0.0001, logrank test). The median follow-up was 13 years (interquartile range 10-17) in the small-duct PSC group and 10 years (interquartile range 6-14) in the large-duct PSC group. Nineteen (22.9%) out of the 83 small-duct PSC patients progressed to large-duct PSC as verified by cholangiography. One patient with small-duct PSC who progressed to large-duct PSC was diagnosed with CCA but after large-duct PSC had been identified; 20 patients in the large-duct PSC group developed CCA during follow up. The number of deaths and liver transplant procedures performed was 11 and 8 respectively in the small-duct PSC group, and 45 and 33 respectively in the large-duct PSC group. The proportion of patients dying and/or undergoing liver transplantation was higher in the large-duct than small-duct PSC group (49.7% vs. 25.7% respectively, p<0.0001). Interestingly two patients originally transplanted for end-stage small-duct PSC developed recurrent small-duct PSC in the graft requiring re-transplantation 9 and 13 years later. Conclusions: Patients with small-duct PSC have significantly better long-term survival than patients with large-duct PSC. Almost a fourth of patients with small-duct PSC progress to large-duct PSC over an average of 13 years of follow-up. Cholangiocarcinoma does not seem to occur in patients with small-duct PSC.

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MULTI-CENTER, DOUBLE BLIND, RANDOMIZED CONTROLLED TRIAL OF ZIDOVUDINE AND LAMIVUDINE (COMBIVIR) THERAPY FOR PATIENTS WITH PRIMARY BILIARY CIRRHOSIS

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A human betaretrovirus resembling the mouse mammary tumor virus has been linked with primary biliary cirrhosis. Biochemical and histological improvement has been reported in uncontrolled pilot studies of PBC patients receiving Combivir. Aim: To conduct a proof of concept trial linking viral infection with PBC.

Methods: 59 PBC patients on 13-15 mg/kg ursodeoxycholic acid for 4 or more months with an alk phos level > 1.5 upper limit of normal were entered into the study. A stratified randomization process using a double blind was employed to match biochemical abnormalities in both study arms. Patients received either 300mg Zidovudine and 150mg Lamivudine (Combivir) BID or placebo BID for 6 months. Serial hepatic biochemistry levels were evaluated from baseline, 1, 3, and 6 months with a 6 month wash out period; virus was detected by RT-PCR using human betaretrovirus env primers. The established endpoints were normalization of alk phos, ALT or AST, normalization of AST and ALT; as well as 50% reduction from baseline to normal range for alk phos, ALT and AST. Data were used for analysis if patients remained on therapy for 3 or more months.

Results: No differences in baseline hepatic biochemistry were observed in the Combivir (n=29) or placebo (n=30) arms; 7 patients were withdrawn from the study (4 Combivir and 3 placebo). A serial reduction in mean alk phos levels was only observed in patients taking Combivir (p<0.0001), where the levels decreased incrementally over the 6 months treatment by > 100 IU/ml from baseline value. Likewise, significant reductions in mean ALT and mean AST were only observed in the Combivir patients (p<0.03). Endpoints were observed in 86% on Combivir and 46% of placebo patients; none achieved normalization of alk phos, 39% Combivir vs. 29% placebo normalized ALT, 32% Combivir vs. 19% placebo normalized AST and 23% Combivir vs. 19% placebo normalized both ALT and AST. Half normalization towards baseline was found in 32% Combivir vs. 19% placebo for alk phos, 69% Combivir vs. 42% placebo for ALT and 68% Combivir vs. 35% placebo for AST. After 6 months, none of the Combivir patients had viremia vs. 19% of placebo patients. Combivir was well tolerated and the 2 serious adverse events occurred in patients randomized to placebo. Conclusions: In this proof of principal study, combination antiviral therapy provided significant but not substantial changes in hepatic biochemistry. This study supports the hypothesis that a human betaretrovirus plays a role in the pathogenesis of PBC. Combivir lacks potency and highly active anti-viral therapy may be required to halt disease in PBC patients with demonstrable retroviral infection.

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71 CONTRIBUTION OF THE VARIANT P.V444A OF ABCB11 (BSEP) TO INTRAHEPATIC CHOLESTASIS OF PREGNANCY

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Background: Intrahepatic cholestasis of pregnancy (ICP) has a complex aetiology with a significant genetic component. Genes mutated in progressive familial intrahepatic cholestasis (PFIC) (ABCC4, ATP8B1, ABCB11) have been implicated in the pathogenesis of the disease in a number of different studies in several populations. Heterozygous mutations of these canalicular transporters occur in a subset of cases, with the aetiology remaining unexplained in the majority of cases. ABCB11 encodes the bile salt export pump (BSEP), which is localized to the canalicular membrane and is a high-affinity ATP-dependent bile salt transporter. Homozygous mutations cause a spectrum of disease ranging from PFIC to benign recurrent intrahepatic cholestasis (BRIC). A polymorphism at position 444 of the protein (valine to alanine, c.1331C>T, rs2287622) has been reported to be associated with reduced expression of BSEP. Two previous studies in small groups of patients have implicated this polymorphism in ICP and drug-induced cholestasis. Patients and Methods: We investigated the role of V444A in ICP by screening a large patient group consisting of 491 Caucasian cases, from a combined resource of UK and European samples. A control group (261 Caucasian women) with uncomplicated pregnancies were also typed for the polymorphism. PCR primers from the UCSF website (http://pharmacogenetics.ucsf.edu/set1/index.html) were used to amplify and sequence patient DNA using an Applied Biosystems 3100 genetic analyser. Electropherograms were analysed using Codoncode Aligner software, and results checked by an independent observer. Results: Analysis of total alleles showed an association of the variant with ICP (OR 1.70, 95%CI 1.37-2.11). A significant difference was observed in genotype frequencies between the case and control groups (Chi-squared 22.778, p<0.001). Comparisons of individuals homozygous for the wild-type (C) or variant (T) allele showed that women with the TT genotype were more likely to develop ICP (OR 2.8 (95%CI 1.8-4.6)). Heterozygotes for the T allele also showed increased risk of ICP (OR 1.8 (95%CI 1.3-2.6)). Conclusion: Our study represents the largest into genetic variation and ICP to date. Our results indicate that the p.V444A polymorphism is a risk factor for ICP in this population.

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72 VARIATION IN THE MDR3 GENE INFLUENCES DISEASE PROGRESSION IN PSC PATIENTS AND DISEASE SUSCEPTIBILITY IN EPISTATIC INTERACTION WITH A POLYMORPHISM IN THE OST-α GENE

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Background and aims: The multi drug resistance 3 gene (MDR3) on chromosome 7q21 encodes a phospholipid transporter of importance to cholangiocyte membrane integrity and bile solubility (ABCB4; ATP-binding cassette, sub-family B, member 4). Knockout mice of the murine MDR3 analogue (mdr2) develop a cholestatic disease resembling primary scarring cholangitis (PSC) in humans. The aim of the present study was to investigate the influence of polymorphisms in the MDR3 gene on disease susceptibility and progression in PSC. Genes encoding other transport proteins in the biliary tract were studied simultaneously to evaluate gene-gene interaction (epistasis) on disease susceptibility. Methods: Single nucleotide polymorphisms (SNPs) covering 10 genes encoding transport proteins for a variety of bile constituents (ABCB4, BSEP, FIC1, MRP2, MRP3, MRP4, ASBT, I-BABP, OST-α and OST-B) were genotyped with SNPlux® technology in 365 Scandinavian PSC patients and 368 healthy controls. Association testing was performed with the Chi-squared test, and epistatic effects were evaluated using logistic regression. Survival was defined as the time from the diagnostic cholangiography to the combined endpoint of death or liver transplantation. Effects from genotypes on disease progression were evaluated with Cox regressions. Results: No associations with PSC susceptibility were seen for any of the individual SNPs. An epistatic interaction between the MDR3 SNP rs8187799 and the OST-α SNP rs11185519 affected PSC risk (p=6.6 x 10^-4). This effect was robust to correction for multiple comparisons using Bonferroni (p<0.01). Two MDR3 SNPs in linkage disequilibrium significantly influenced survival: rs1202283 (AA vs. AG+GG: median 9.8 vs. 14.4 years) and rs4148809 (AA vs. AG+GG: median 9.6 vs. 15.4 years). In Cox regressions, patients homozygous for the A allele at rs1202283 and rs4148809 had an increased risk of death or liver transplantation (hazard ratio (HR)=1.6, p=0.006 and HR=1.7, p=0.002, respectively). To rule out that this effect was caused by a higher age at diagnosis of PSC or concomitant cholangiocarcinoma, patients with cholangiocarcinoma were excluded and age adjusted Cox regressions were performed, with no changes in result (rs1202283 HR=1.7, p=0.007 and rs4148809 HR=1.8, p=0.002). Conclusions: Although no associations with any individual SNPs were seen, our present data provide interesting evidence that polymorphisms in the MDR3 gene influence disease progression in PSC patients, and susceptibility to develop PSC in epistatic interaction with a polymorphism in the OST-α gene.

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THE PROTEIN KINASE G-VASODILATOR STIMULATED PHOSPHOPROTEIN PATHWAY DISRUPTS HEPATIC STELLATE CELL DRIVEN ANGIOGENESIS BY DISRUPTING FOCAL ADHESION FORMATION

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Background. Cirrhosis and portal hypertension are associated with prominent remodeling of the sinusoidal vessels that includes enhanced hepatic stellate cell (HSC) coverage of endothelial cell (EC) lined sinusoids. We have previously demonstrated that nitric oxide [NO] disrupts rac1 dependent HSC migration and ensuing HSC driven angiogenesis. In this study we further examined the signaling intermediates downstream of NO and rac1 that regulate HSC adhesion, migration, and vascular tube formation. Results. Activation of the kinase downstream from NO, protein kinase G (PKG), by overexpression of constitutive active PKG resulted in a reduced capability of HSC to form vascular tube structures in matrigel 6 hrs after seeding (tube length [arbitrary units]; AdGFP: 10180 +/- 847 vs. AdPKGc: 5135 +/- 1025, P<0.05, n=3), suggesting that PKG activation significantly inhibits HSC adhesion and migration. Since focal adhesions are essential for cell adhesion and migration, we explored further how the PKG pathway may regulate focal adhesion function in HSC. The cytoskeletal regulatory protein, vasodilator-stimulated phosphoprotein (VASP), plays a pivotal role in actin dynamics and actin-based cellular structure formation and thus acts as an important regulator of cell-cell adhesion, cell-matrix adhesion and cell migration. Western blot analysis and immunofluorescence microscopy showed that VASP was highly phosphorylated in response to PKG activation, colocalized with the focal adhesion marker, vinculin within focal adhesion complexes, and also co-precipitated with vinculin (n=3). PKG activation induced translocation of phospho-VASP-vinculin complexes from focal adhesions to peripheral plasma membrane, in association with loss of HSC adhesive capacity. Inhibitory effects of PKG activation on HSC adhesion and vascular tube formation appeared to be mediated by VASP because siRNA knockdown of VASP partially reversed this phenotype. Next, to explore the role of VASP in the regulation of Rac1 and its effector protein, IQGAP, we performed immunoprecipitation assay using anti-IQGAP antibody. VASP was found to reside in a complex with vinculin, rac1 and IQGAP in HSC. Conclusion. These studies suggest that VASP may serve as a counter-regulator between NO inhibition of HSC motility and rac dependent activation of HSC motility. Thus, these studies provide mechanistic insights into the regulation of HSC migration and vascular tube formation, which are key steps in the process of HSC driven sinusoidal remodeling.

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ANGIOGENESIS IN EXPERIMENTAL HEPATOPULMONARY SYNDROME (HPS)

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Introduction: HPS occurs in 10-30% of patients with cirrhosis and increases mortality. It is classically thought to result from vasoilatation in the pulmonary microcirculation although whether angiogenesis is also important is unknown. Experimental biliary cirrhosis (CBDL) triggers HPS while non-biliary cirrhosis (thioacetamide, TAA) does not. Aim: To evaluate pulmonary angiogenesis in CBDL and TAA cirrhosis. Methods: Control, 1, 2 and 3 week CBDL and 2 and 8 week TAA [200 mg/kg, TIW, i.p.] treated animals underwent evaluation. IM injection of recombinant adenovirus containing secretable human angiostatin and endostatin (rAAV A+E, 3 x 1011 particles) 2wk prior to CBDL was used to chronically inhibit angiogenesis. HPS was evaluated using lung physiologic (ABG/microsphere shunt) and molecular changes (eNOS, ED1, HO-1). Angiogenesis was evaluated by lung microvessel counts (factor VIII staining) and VWF and Flk-1. Results: CBDL animals, but not TAA animals developed physiologic and molecular alterations of HPS beginning at 2 wk. Microvessel density increased by 2 wk and was maximal at 3 wk in CBDL animals relative to control and TAA (2.9±0.2 fold-control, p < 0.005). These changes
were accompanied by a similar increase in pulmonary VWF (2.6±0.3 fold-control, p<0.001) and Flk-1 (2.4±0.5 fold-control, p<0.05) levels after CBDL relative to control and TAA. Pulmonary endothelial VEGF staining increased in CBDL animals. In contrast, Flk-1 levels decreased after CBDL and were unaltered after TAA. rAAV driven upregulation of circulating angiotatin and endostatin increased early mortality after CBDL (42% rAAV A+E vs 8% CBDL control) but did not influence systemic or portal pressures or liver histology relative to controls in survivors. rAAV A+E treated 3 wk CBDL animals had a significant reduction in pulmonary microvessel density (50% of CBDL control, p<0.005) accompanied by a significant decrease in both VWF and Flk-1 levels relative to CBDL controls (65% for each, p<0.001). The reduction in angiogenin was accompanied by normalization of ABGs and intravascular macrophage accumulation (ED1), a reduction in HO-1 protein levels and no significant change in eNOS levels. Conclusions: Experimental HPS after CBDL is accompanied by pulmonary angiogenesis, not found in TAA cirrhosis where HPS is absent. Delivery of angiogenin and endostatin prevents pulmonary angiogenesis and the gas exchange abnormalities of HPS. Angiogenin appears to be one important pathophysiologic process in experimental HPS and may provide novel therapeutic targets.

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76 FXR BILE ACID RECEPTOR ACTIVATES FOCAL ADHESION KINASE IN ENDOTHELIAL CELLS

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Background. The bile acid receptor, FXR, has recently been demonstrated to promote endothelial cell (EC) migration in a process that contributes to vascular remodeling. Our recent work identified a novel role of FXR activation of MMP9 in the process of endothelial cell migration (JBC 2006). Focal Adhesion Kinase (FAK) is a tyrosine kinase that is implicated in cell migration through effects on actin cytoskeleton. The Aim of the present study was to determine the role of FAK and actin cytoskeleton on FXR regulation of endothelial cell motility. Methods. Endothelial Cells (EC) were incubated with vehicle or the FXR agonist, chenodeoxycholic acid (CDCA), in the presence or absence of MMP9 siRNA, and analyzed for cell motility by Time Lapsed Video Microscopy. FAK phosphorylation was analyzed by Western blot analysis while actin stress fiber formation was analyzed by immunofluorescence microscopy. Results. CDCA significantly increase EC velocity [(µm/sec) 0.0073 ± 0.0018 as compared to vehicle, 0.0044 ± 0.0018, p<0.05, n=3], actin stress fiber formation (Fluorescence intensity: 106.882 ± 11.278 vs. vehicle, 51.504 ± 25.248, p<0.0001), and FAK phosphorylation (n=3). These events were significantly inhibited by MMP9 siRNA (velocity [µm/sec]: 0.0039 ± 0.0021 as compared to CDCA alone; 0.0073 ± 0.0018, p<0.01, n=3). CDCA stimulation of EC was also associated with phosphorylation/activation of a cascade of signaling molecules important for actin remodeling and EC motility including paxillin, vinculin, Rac1, and Rho A kinase (n=3). Conclusion. This study supports a key role for FAK phosphorylation and stress fiber formation in the process of FXR-MMP9 dependent regulation of EC motility and migration, events which may have broad implications for FXR effects on hepatic vascular repair and remodeling.

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77 ISCHEMIA MODIFIED ALBUMIN PREDICTS MORTALITY IN LIVER DISEASE PATIENTS

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Introduction: Albumin is an abundant multifunctional plasma protein, with well documented ligand-binding, transport, antioxidant and enzymatic activities. It is exclusively synthesized by the liver and its circulating concentration is known to decrease in liver disease. We have previously demonstrated that albumin is damaged in patients with liver failure leading to a loss of transport and detoxification functionality (Hepatology(2005), 42(4) suppl.1, 222A). The ischemia modified albumin (IMA) test, based upon protein-metal binding capacity, was used to examine whether this measure of oxidative stress induced albumin damage can be related to outcome in hepatitis patients. This test is now used routinely in cardiac ischemia patients, though as these subjects are norm-albumenic no albumin corrections are employed. We also investigated whether an increase IMA is associated with an alteration in the binding ability of fatty acid site 1 (BS1) as this has been postulated as an alternate cobalt chelating region. Methods: IMA was determined using the albumin cobalt binding test in plasma obtained from healthy control (control, n=6) and biopsy confirmed cirrhotic (n=34) subjects. Albumin was measured using the bromocresol purple assay and the affinity of the fatty acid binding sites was determined using a spin-label (16 doxyl-stearate) titration and ESR spectroscopy. Patients were subsequently divided by 28 day mortality, into survivors (n=12) and non-survivors (n=22). Results: When the IMA levels were normalized to the albumin concentration, significant differences between the groups were observed (control vs survivors, p<0.01; control vs non-survivors, p<0.01, survivors vs non-survivors, p<0.05). No significant differences were observed between the groups when the IMA scores were compared without albumin level correction. Non-survivors were also associated with increased inflammatory indicators (CRP, 56±7 vs 26±10 mg/ml, p<0.05; white cell count 17.1±10±16.6 vs 7.8±10±6.0, p<0.001) A significant negative correlation between the IMA ratio (IMAR) and the fatty acid binding coefficient for site 1 (KB1, r=0.68 p<0.001) was observed. Conclusions: These findings suggest that albumin damage, measured as IMA, expressed as a ratio to the protein, may be a useful tool to evaluate disease severity in cirrhotic patients. In this study IMAR was found to be as sensitive as MELD for predicting outcome (78% sensitivity, 76% selectivity). This indicates that there is increased oxidative stress in liver disease that reduces the functional ability of albumin. We also observe that there is an associated effect at BS1 which may reflect multiple points of albumin damage.

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78 VASCULAR HYPORESPONSIVENESS TO ANGIOTENSIN-II IN RATS WITH CCL4-INDUCED CIRRHOSIS IS ASSOCIATED WITH COMPLEXATION OF THE ANGIOTENSIN-II TYPE1 RECEPTOR WITH THE RECEPTOR-DESENSITIZING PROTEIN, BETA-ARRESTIN 2

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Background: In cirrhosis, portal hypertension is triggered by vasodilation due to impaired vasoconstrictor-responsiveness of
extrahepatic vessels. Angiotensin-II (ang-II) induced vasoconstriction is mediated by activation of the angiotensin-II type 1 receptor (AT1R) and its associated heterotrimeric G-proteins. Activation of G-protein coupled receptors (GPCR) causes their phosphorylation by GPCR-kinases, which enables binding of β-arrestin2 to the receptor. As a result of this, receptors are desensitized and G-protein mediated signaling (e.g. contraction cascades) is blunted. We have previously shown that vascular hyporeactivity to ang-II in rats with secondary biliary cirrhosis and humans with alcohol-induced cirrhosis is associated with increased binding of β-arrestin2 to the AT1R (Hepatology2007;45(2)). Here, we analyzed the interaction of the AT1R with β-arrestin2 in vessels from rats with CCl4-induced micronodular cirrhosis. Methods: Protein-expressions in aortas from rats with CCl4-induced cirrhosis and non-cirrhotic controls were investigated by Western-blot analysis. Contractility of aortic rings was determined myographically. Interaction of the AT1R with β-arrestin2 was assessed by coimmunoprecipitation. Results: Aortic rings from CCl4 rats were hypocontractile to ang-II despite inhibition of nitric oxide synthases by L-NNAME (200 mM). Expression of the AT1R, Goα/11, and the contraction-mediating effector, RhoA, were similar in aortas from both groups. Expression of β-arrestin2 was upregulated in aortas from CCl4 rats. Interaction of the AT1R with β-arrestin2 was increased in aortas from CCl4 rats. Stimulation of isolated aortas with exogenous ang-II (300 nM, 20 min) caused recruitment of β-arrestin2 in aortas from non-cirrhotic rats, but no further complexation of the AT1R with β-arrestin2 in aortas from CCl4 rats. Conclusions: Vascular hyporesponsiveness to ang-II in CCl4 rats is due to overexpression of β-arrestin2 and a subsequently enhanced complexation of the AT1R with β-arrestin2.

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PROVE2: PHASE II STUDY OF VX950 (TELAPREVIR) IN COMBINATION WITH PegINTERFERON ALFA2A WITH OR WITHOUT RIBAVIRIN IN SUBJECTS WITH CHRONIC HEPATITIS C, FIRST INTERIM ANALYSIS

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Background: PROVE2, study VX05-950-104EU is a randomized, placebo-controlled phase II study of telaprevir (TVR), in combination with pegylated interferon alfa2a (Peg-IFN) and ribavirin (RBV), in treatment naive subjects with genotype 1 chronic hepatitis C infection. Results of a planned interim, on-treatment, safety and efficacy analysis (IA) are reported. Methods: A total of 332 subjects were randomized into four groups. Group A received standard of care Peg-IFN 180 mcg/weekly + RBV 1000-1200 mg, weight-based and TVR-placebo for 48 weeks. Group B TVR 750 mg q8h together with Peg-IFN+RBV for 12 weeks, followed by Peg-IFN and RBV for a further 12 weeks. Group C, TVR 750 q8h with Peg-IFN + RBV for 12 weeks. Group D, TVR + Peg-IFN for 12 wks. The Roche Taqman assay was used to quantify plasma HCV RNA (LOD 10 IU/mL).
This first IA was performed when at least 90% of subjects completed the week 12 visit. Results: The median age was 45 years (18-65), median weight 70.9 kg (45-115), 58.7% were male, 94.1% Caucasian, 7.5% had stage F3 fibrosis, 34.1% were infected with genotype 1a, and 54.1% with 1b. The median baseline HCV RNA was 6.4 Log10IU/ml (3.3-7.7). Overall, discontinuation occurred in 6.4% of Group A, 17.1% of Groups B+C and 10.5% of Group D subjects prior to week 12. Discontinuations for AEs occurred in 2.6% of Group A, 12.5% of Groups B+C and 7.9% of Group D subjects. Pruritis, rash, asthenia, nausea, and anemia were the most common AEs associated with TVR; anemia and nausea were less frequent in Group D. The table summarizes ITT viral responses. Conclusion: TVR (telaprevir, VX950)+Peg-IFN+RBV produces a significantly greater, on-treatment, anti-viral response at weeks 4 and 12 compared to Peg-IFN+RBV in naive subjects with genotype 1 HCV. Dosing without RBV reduced the on-treatment anti-viral response; further follow-up will evaluate effects on SVR and relapse rates. Consistent with prior studies, the adverse event profile showed that skin events, nausea and anemia are the most important AE’s associated with TVR. Further results will confirm the optimal treatment duration and dosage regimen for Phase 3.

HCV RNA Un-detected

<table>
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<tr>
<th>Week 4</th>
<th>Group A (%) (n=77)</th>
<th>Group B+C (%) (n=155)</th>
<th>Group D (%) (n=76)</th>
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<tr>
<td></td>
<td>14.3</td>
<td>74.3*</td>
<td>52.6*</td>
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<td>Week 12</td>
<td>42.9</td>
<td>78.9*</td>
<td>63.2*</td>
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</tbody>
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*p<0.001 compared to Group A;
+p=0.015 compared to group A

Disclosures:
Christophe Hezode - Consultant/Adviser: Vertex
Peter Ferenci - Consultant/Adviser: Vertex
Geoffrey M. Dusheiko - Consultant/Adviser: Vertex
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81 MULTICENTER RANDOMIZED CONTROLLED TRIAL OF CARVEDILOL VERSUS VARICEAL BAND LIGATION FOR THE PREVENTION OF THE FIRST VARICEAL BLEED
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Background: Current therapy for preventing the first variceal bleed includes beta-blockers (BB) and variceal band ligation (VBL). A recent meta-analysis has shown VBL to have lower bleeding rates, with no difference in survival. BB therapy can be limited by side effects (SEs), and there are concerns regarding the safety of variceal band ligation (VBL). Carvedilol, a non-cardioselective vasodilating BB is more effective in reducing portal pressure than propranolol, but to date there have been no clinical studies assessing the efficacy of carvedilol in primary prophylaxis. Aims: To compare carvedilol versus VBL for the prevention of the first variceal bleed in a randomized controlled multicenter trial. Methods: 152 cirrhotic patients from 5 different centres with grade II or larger esophageal varices that have not bled were studied. Patients were randomized to either carvedilol at 12.5 mg per day or VBL, performed 2 weekly till eradication using a multibandr device. The primary end point was the first variceal bleed. Secondary end points were mortality, bleeding related mortality, side effects leading to treatment discontinuation and other adverse events. Intention to treat analysis was performed for all outcomes. Results: Over a 6 year period 77 patients were randomized to carvedilol and 75 to VBL. Baseline characteristics: Alcoholic liver disease, 72%; Child Pugh Score, 8.3 ± 2.6; age, 54.1 ± 10.3 years; median follow up, 15.7 months (range 0.16–79.1 months). Patients on carvedilol had significantly lower rates of the first variceal bleed (9% vs 21%; relative hazard 0.41; 95% CI 0.19 – 0.96; p=0.04)), with no significant differences in overall mortality (35% vs 37%, p=NS), bleeding related mortality (3% vs 1%, p=NS) and treatment discontinuation due to SE’s (12% vs 4%, p=0.08). 6 patients in the VBL group bled as a result of banding ulcers. Significantly more patients in the VBL arm underwent salvage TIPSS (2 vs 8; p = 0.045). All patients in the carvedilol group who discontinued therapy due to side effects were changed to VBL, and none have bled. Conclusions: This is the first study to demonstrate lower bleeding rates with BB therapy for primary prophylaxis compared with VBL. Carvedilol was well tolerated, while VBL had an unfavorable adverse events profile. Carvedilol, along with VBL should be considered for first line therapy in primary prophylaxis. VBL remains the only alternative in patients intolerant of carvedilol or those unlikely to comply with drug therapy. Careful selection of patients and attention to technique are required to reduce the risk bleeding from the banding procedure

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82 PRESERVING RENAL FUNCTION IN LIVER TRANSPLANTATION: EFFICACY AND SAFETY OF A MYCOPHENO-LATE MOFETIL (MMF)/SIROLIMUS MAINTENANCE REGIMEN FOLLOWING CALCINEURIN INHIBITOR (CNI) WITHDRAWAL: INTERIM DATA FROM THE SPARE-THE-NEPHRON (STN) TRIAL
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Purpose: Chronic renal insufficiency has become a progressively more significant problem as the long term survival of liver transplants improves. Since CNIs are associated with renal toxicity, we examined whether a CNI-free regimen, consisting of MMF and sirolimus (SRL) can better preserve renal function in recipients of liver allografts, while maintaining adequate
immunosuppression. Methods: This trial is a 2-year multicenter, prospective, randomized, controlled, open-label study with a projected enrollment of 340 patients. Four to 12 weeks post-transplantation, patients maintained on MMF and a CNI were randomized to MMF (1-1.5 g BID), together with either SRL (2-4 mg QD, trough 5-10 ng/mL) or a CNI (cyclosporine 3-5 mg/kg BID, trough 100-250 ng/mL; tacrolimus 0.1-0.15 mg/kg/day in 2 doses; trough 3-10 ng/mL). Primary endpoints included: mean percent change in calculated glomerular filtration rate (GFR; MDRD 6 equation; mL/min), percent of patients with biopsy-proven acute rejection (BPAR), graft loss, or death. Safety data included frequencies of adverse events. Results: Six-month interim data, involving 100 patients (50 per group) are discussed herein; updated data will be presented. Patient characteristics, including GFR values, were similar at baseline for both groups. In the CNI group, 2 patients received cyclosporine, and 48 received tacrolimus. At 6 months, mean increase in GFR from baseline was 29.0% (±39.36) in the MMF/SRL group and 4.5% (±24.12) in the MMF/CNI group. BPAR occurred in 6 (12%) of MMF/SRL patients and 2 (4%) of the MMF/CNI group; withdrawal rates were 28% (14/50) and 16% (8/50) respectively. There was one patient death in the MMF/SRL group. Increases from baseline in lipid/triglyceride levels were greater with MMF/SRL. Most common adverse events (MMF/SRL vs. MMF/CNI) were leukopenia (24% vs. 10%), pyrexia (24% vs. 10%), diarrhea (18% vs. 22%), and headache (18% vs. 14%); hyperkalemia occurred in 18% of MMF/CNI patients, but was not seen in any patient in the MMF/SRL group. Pharyngolaryngeal pain (12% vs. 4%); tongue and lip ulceration (2% vs. 2% each); stomatitis (6% vs. 0%); and mouth ulceration (2% vs. 0%) were reported in the MMF/SRL and MMF/CNI groups, respectively. Conclusions: Interim data suggest that MMF/SRL maintenance therapy results in improved renal function, as compared to CNI-containing regimens. More definitive conclusions about the long-term impact of maintenance with MMF/SRL will require complete follow-up of all 340 patients.

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The following people have nothing to disclose: Lewis Teperman, Anthony Sebastianian, Juan Arenas, Linda Sher, Baburao Koneru, Paul Marotta, John P. Roberts, Dharmesh Patel

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FIRST MULTICENTER EVALUATION OF THE EFFICACY OF TENOFOVIR IN NUCLEOS(T)IDE ANALOG EXPERIENCED PATIENTS WITH HBV MONONOCCLUSION
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Background: Tenofovir disoproxil fumarate (TDF), an acyclic nucleotide reverse transcriptase inhibitor is approved for the treatment of HIV infections and has shown activity in HBV/HIV co-infected patients harboring both wild type and lamivudine resistant hepatitis B virus (HBV). In this retrospective multi center analysis we studied the effectiveness of TDF monotherapy in patients with HBV monoinfection with respect to virologic parameters and resistance. Methods: 121 patients with chronic HBV infection (m/f 87/34, mean age 45 ± 101 patients during TDF treatment (mean duration 14.8 ± 10 years) HBV DNA levels decreased from a mean baseline level of 6.7 ± 1.2 [1.4-6.7] log copies/ml at week 24 and 48, respectively. HBV DNA was undetectable (<400 copies/mL) in 72% and 91% of the patients at week 24 and week 48. There was no evidence of TDF resistance development as a rebound of HBV DNA >1 log was not observed in any of the patients studied. HBeAg seroconversion was documented in 23% of the patients after a mean TDF treatment duration of 9.3 ± 2.3 months, and HBSAg loss in 4% after 13.6 ± 9.18 months. 85 patients (70%) had elevated ALT levels at baseline while 78%
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A PILOT RANDOMIZED CONTROLLED STUDY EVALUATING EFFICACY OF AUTOLOGOUS BONE MARROW MONONUCLEAR CELLS TRANSPLANTATION IN PATIENTS WITH ADVANCED CHRONIC LIVER DISEASE

André C. Lyra1, Milena B. Soares2, Luiz F. da Silva2, Eduardo L. Braga1, Sheilla A. Oliveira2, Marcos F. Fortes2, André G. Silva2, Patricia S. Brandão1, Bernd Genser1, Ricardo R. dos Santos2, Luiz G. Lyra1

1Internal Medicine - Gastrohepatology Service, Hospital Sao Rafael and Federal University of Bahia, Salvador-Bahia, Brazil; 2Fundacao Oswaldo Cruz - FioCruz, Salvador, Brazil; 2BGStats Consulting, Graz, Belgium

Studies in animal models of liver disease have shown that bone marrow cells (BMC) transplantation improves hepatic fibrosis and liver function. Other authors and we have recently demonstrated that autologous BMC therapy in patients with chronic liver disease is safe and feasible and may induce changes in liver function tests. Aim: To evaluate the efficacy of BMC transplantation in patients with advanced chronic liver disease. Methods: Thirty patients on the waiting list for liver transplantation were randomly assigned to receive BMC therapy or no treatment in a nonblinded fashion. Approximately 50 ml of bone marrow aspirate were prepared by centrifugation in a ficoll-hypaque gradient. A minimum of 100 millions autologous mononuclear-enriched BMC were infused into the hepatic artery. Baseline assessment included clinical, laboratorial and abdominal MRI evaluation. Serum albumin, bilirubin, INR, Child-Pugh and MELD score were chosen as endpoints to assess efficacy. Patients were closely assessed for clinical events and changes in liver function for 90 days. Statistical analysis was conducted by obtaining descriptive statistics, calculating mean changes from baseline and fitting a random effects model to compare the profiles of liver tests of both groups. Results: Mean age, baseline liver function tests (except for albumin), MELD and Child-Pugh score were similar in both groups. Albumin levels (g/dl) were higher in the control group (3.31 vs 2.79, p=0.053). During follow-up one patient died and one was liver transplanted in the control group while another patient died in the intervention group. No other major complications were detected in the remaining patients. Albumin levels increased in the treatment arm while they remained stable among controls up to 90 days (P=0.034; BMC group relative change from baseline (RC) = + 16%, control group RC = + 2%). Child Pugh score significantly decreased in the therapy group compared to the control group (P=0.017, BMC group RC = -8%, controls RC = + 4%). Bilirubin levels significantly increased among controls while they decreased in the treatment arm during the first 60 days after therapy and increased to baseline levels after 90 days. INR profile was not significantly different between both groups. Conclusions: Transplantation of autologous BMC into the hepatic artery appears to improve liver function of patients with advanced chronic liver disease at least for up to 90 days and might be a promising treatment option for these subjects. Larger studies with longer follow-up are necessary to validate our findings, to define the duration of this beneficial effect and to determine if there will be improvement of survival.

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THE BASOLATERAL TRANSPORTER OSTβ-OSTβ IS ESSENTIAL FOR INTESTINAL BILE ACID ABSORPTION AND HOMEOSTASIS

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Transport of bile acids from the intestinal lumen into the portal circulation is mediated by carriers expressed on the enterocyte apical and basolateral membranes. Several candidate intestinal basolateral transporters have been proposed including Mrp3 (Abcc3) and the heteromeric organic solute transporter, Ostα-Ostβ. Mouse Ostα-Ostβ exhibits many of the properties predicted for the intestinal basolateral bile acid transporter, including highest expression in terminal ileum, basolateral membrane localization, positive regulation of expression by bile acids, and transport substrate specificity that includes the major bile acid species. However, the in vivo functions of Ostα-Ostβ have not been determined. Hypothesis: Ostα-Ostβ functions in vivo as the major ileal basolateral bile acid transporter. Methods: The Ostα gene was inactivated by conventional gene targeting. Mucosal-to-serosal taurocholate transport was measured in vitro using everted gut sacs. Analysis of the in vivo phenotype included measurements of fecal bile acid excretion, bile acid pool size and composition, and gene expression. Results: The Ostα/- mice are viable and fertile, but exhibit growth retardation. Expression of the Ostβ partner protein was significantly reduced (> 95%), despite continued high-level expression of Ostβ mRNA. In everted gut sac experiments, ileal transmucosal transport of taurocholate was reduced approximately 85% in Ostα/- versus wild type or Mrp3/- mice. Since Mrp3 may serve as a secondary export system for bile acids, everted gut sacs were also analyzed from Ostα-Mrp3 double knockout mice, where trans-mucosal taurocholate transport was further reduced to background levels. As in Asbt/- mice, the bile acid pool size was significantly reduced (> 65%) in Ostα/- mice. However in contrast to Asbt/- mice, fecal bile acid excretion was not increased in Ostα/- mice, reflecting reduced hepatic synthesis. Whereas hepatic cholesterol 7α-hydroxylase (Cyp7a1) expression is induced ~5-fold in Asbt/- mice, Cyp7a1 expression was paradoxically reduced in the Ostα/- mice. The reduced hepatic Cyp7a1 expression correlated with increased ileal expression of fibroblast growth factor 15 (Fgf15) in Ostα/- mice. Conclusions: Ostα-Ostβ is the major intestinal basolateral bile acid transporter, whereas Mrp3 is a back-up transporter whose function can be discerned in Ostα/- mice. Unlike a block in intestinal apical bile acid uptake, genetic ablation of basolateral bile acid influx mediated by Ostα-Ostβ disrupts the normal homeostatic control of hepatic bile acid biosynthesis.

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CHOLANGIOCYTE CILIÀ EXPRESS THE OSMOSENSORY PROTEIN TRPV4 AND DETECT CHANGES IN LUMINAL BICARBONATE SECRETION

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We reported that cholangiocyte cilia are sensory organelles that respond to luminal fluid flow by changes in [Ca2+]i and cAMP. Since cholangiocyte cilia are also positioned to detect changes in luminal bile composition, we hypothesized that these organelles function as osmosensors via TRPV4, a Ca2+-permeable ion channel implicated in Ca2+ signal transduction of osmotic stimuli. Using freshly isolated rat cholangiocytes and normal mouse (NMC) and rat (NRC) cholangiocyte cell lines, we found by PCR and immunoblotting that TRPV4 message and protein are expressed in cholangiocytes. Transmission electron and immunofluorescent confocal microscopy showed that TRPV4 localizes mainly on cholangiocyte cilia. Functional studies showed that fura-2 preloaded wild type NMCs responded to hypotonicity (200 mOs/L) by a 2-fold increase in [Ca2+]i, while TRPV4-overexpressing NMCs showed a 6-fold increase, a response that was reduced (P<0.05) when NMCs were cotransfected with a shRNA to trpv-4 or exposed to Ca2+-free media. Using microperfused isolated bile duct units (IBDUs) preloaded with the calcium sensitive dye, fluo4, we observed that a decrease in perfusate freshness increased [Ca2+]i by 45%; treatment of IBDUs with the deciliating agent, chloral hydrate (CHy), or a TRPV4-siRNA abolished this effect. Luminal hypotonicity of IBDUs also induced an increase in luminal pH of 0.23 unit/min, consistent with an increase in bicarbonate secretion; this effect was also abolished by treatment with a TRPV4-siRNA or CHy. To address the mechanisms involved, we found that hypotonicity induced an increase in ATP release in wild type and TRPV4-overexpressing NMCs (5- and 7-fold, respectively); this effect was impaired when cells treated with CHy or cotransfected with a shRNA to trpv-4. Finally, the hypotonicity-induced increase of luminal pH in IBDUs was abolished by suramin, a purinergic receptor antagonist. In summary, TRPV4 is expressed on cholangiocyte cilia and hypotonicity induces an increase in [Ca2+]i in a TRPV4-, ciliary- and extracellular calcium-dependent manner. This osmosensation of luminal toxicity by ciliary TRPV4 involves apical ATP release and purinergic receptors and induces apical bicarbonate secretion, the main determinant of ductal bile formation. The results provide the first evidence of TRPV4 expression in primary cilia in any epithelial cell and suggest that in cholangiocytes these organelles play an important role in the regulation of ductal bile secretion via osmosensation.

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DUAL OVER-EXPRESSION OF INSULIN RECEPTOR SUBSTRATE-1 AND HEPATITIS BX GENE CAUSES PRE-MALIGNANT CHANGES IN LIVER

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Background: The cellular changes that contribute to the development of hepatocellular carcinoma (HCC) are poorly understood. Activation of the IN/IRS-1/Ras/Raf/MAPK and the Wnt Frizzled/β-catenin signaling cascades is frequent in HCC related to persistent hepatitis B viral (HBV) and hepatitis C viral (HCV) infection. The aims of this study were to recapitulate these findings in an animal model system by providing a proliferative stimulus via IRS-1 in the context of HBx protein overexpression in transgenic mice. The purpose was to determine if alteration in these genes is sufficient to produce dysplasia and cellular transformation in the normal liver. Methods: We generated transgenic mice in which the HBx (ATX; N=16), IRS-1 (N=15) or both (ATX+/-IRS-1+; N=23) genes were over-expressed under a liver specific promoter. Wild-type litter mates were included as controls (N=11). We also assessed oxidative damage as well as upregulation of signaling molecules related to these signal transduction cascades in the liver by "real-time" RT-PCR. Histologic analysis included an assessment of lobular and periporal inflammation, macro and microsteatosis, polyploidy, dysplasia and tumor formation. Results: ATX+/IRS-1+ double transgenic livers had increased frequency of hepatocellular dysplasia and several developed HCC (N = 3). IRS-1+ and ATX+/IRS-1+ livers had more extensive hepatic steatosis compared to the other groups. All three transgenic lines had significantly increased IRS-1, IGFB1, proliferating nuclear cell antigen, Wnt 1 and Wnt 3 mRNA levels, and increased immunoreactivity for 8-OHdG and HNE indicative of DNA damage and oxidative stress. The ATX+/IRS+ double transgenic mice were distinguished by having the highest level of activation of Wnt 3 and Frizzled 7 and selectively increased expression of IGFB-1 and asparaginyl-(asparaginyl)-β-hydroxylase (AAH) a downstream insulin responsive gene associated with increased cell motility and migration. Conclusions: These results suggest that over-expression of HBx or IRS-1 can contribute to hepatocyte transformation by promoting cell turnover and DNA damage but these effects are not sufficient to trigger neoplastic changes in the liver. However, chronic over-expression of HBx together with IRS-1 is sufficient to cause hepatocellular dysplasia and HCC in vivo suggesting that upregulation of both the IN/IRS-1/MAPK and Wnt/β-catenin signaling cascades are important in the transformation of normal hepatocytes to the malignant phenotype.

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88 DISTINCT EFFECTS OF RAGE & MYD88 SIGNALING IN MASSIVE HEPATECTOMY

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BACKGROUND: Our previous studies elucidated that pharmacological blockade of RAGE in a murine model of massive hepatectomy (MH) was beneficial in reducing mortality and facilitating liver regeneration. A principal site of RAGE expression in the MH liver remnant was the CD11c+ expressing dendritic cells (DC). HMBG-1 is one of RAGE ligands and triggers cellular signals through RAGE, and possibly toll like receptors (TLRs). Here, we dissected the contribution of RAGE vs Myd88 in massive liver injury by using WT, RAGE null and Myd88 null mice. METHODS: Massive liver injury (85% hepatectomy) was performed in male C57BL/6 (WT), RAGE null mice or Myd88 null mice. Liver DCs were isolated from non-parenchymal cells by density centrifugation and CD11c+ selection. RESULTS: Up-regulation of HMBG1, TLR4 and RAGE was observed in the liver remnants after MH at 6 hrs by immunoblot. Real-time PCR revealed that RAGE and TLR4 transcripts were mainly expressed in non-parenchymal cells, especially in liver DC, and to a lesser extent in kuffer cells. Liver DCs retrieved from WT mice stimulated with LPS (100 ng/ml) for 6 hrs displayed release of HMBG1 into the supernatant, but not in total cell lysate (2 fold increase vs PBS). WT DC mediated up-regulation of TNF-alpha and IL6 transcripts when exposed to LPS (100 ng/ml) for 6 hrs compared with control. 6 hours after MH, liver DCs were isolated; transcripts for TNF-alpha and IL6 were increased in liver DC, but not from DC isolated from sham mice (3.0 vs 1.0 and 3.3 vs 1.0 fold increase, respectively, p<0.05). After MH, RAGE null mice demonstrated markedly improved survival vs WT within the first 15 hrs, while Myd88 null mice were rescued with 2.5 fold increase in survival rate compared with MH during this time period. To determine the underlying mechanisms, we assessed level of activated caspase-3, c-jun, NF-kB and TUNEL positive cells in the remnants post MH. Decreased expression of activated caspase 3, nuclear NF-kB, and TUNEL positive cells were noted in RAGE null mice and Myd88 null remnants compared to WT mice at 6 hours after MH. Up-regulation of phospho-stat3 and down-regulation of c-jun was observed in RAGE null remnants compared with WT whereas, in Myd88 null mice, phospho-stat3 signaling was dramatically suppressed vs WT, but phosph-Akt was up regulated compared with WT or RAGE null mice. CONCLUSION: These findings suggest that both RAGE and Myd88 stimulate remnants cell death after MH, but that activation of distinct signaling pathways by these molecules triggers strikingly diverse effects on pro-regenerative pathways. Blockade of RAGE, but not Myd88 signaling, may exert dramatic pro-regenerative survival mechanisms early after MH.

Disclosures: The following people have nothing to disclose: Shan Zeng, Hao Dun, Nikalesh Ippagunta, Rosa H Rosaria, Ann Marie Schmidt, Jean C Emond

89 ACUTE LIVER INJURY UPREGULATES MICRONRNA-491 IN MICE, AND ITS OVERexpression IN HEP G2 CELLS CAUSES APOPTOSIS

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BACKGROUND: Exposure of hepatocytes to various insults leads to apoptosis and liver injury. microRNAs (miRNAs) have emerged as novel genetic regulators of cell functions such as proliferation, apoptosis and cancer. Hence, the aim of the study is to evaluate the role of miRNAs in promoting apoptosis during liver injury. METHODS: C57BL/6 mice were administered Jo2 antibodies (0 or 10 mg/kg/mouse), and the mice were euthanized after 48 hours. The liver tissue was isolated and total RNA was isolated using miRNA isolation reagent followed by purification of <40 nucleotide RNAs using FlashPAGE gels. These purified miRNAs were labeled with Alexafluor 555 and hybridized with Ambion’s mirVana miRNA bioarrays, and scanned using the Genepix 4000B scanner. To evaluate the role of miRNAs in inducing apoptosis, Hep G2 cells were transfected with either miRNA precursors or nonspecific miRNAs. The cells were sensitized with either TNF-alpha (1ng/ml) or Fas ligand (FasL) (1ng/ml) and apoptosis was assessed by caspase-3 activity and DAPI staining. RESULTS: Administration of Jo2 antibodies caused the enhanced expression of several microRNAs in the liver. One of the species, microRNA-491 (miR-491), was upregulated 6-fold in Jo2-treated mice. These results were confirmed by stem-loop real-time RT-PCR (SLqPCR), using the Taqman miRNA assay system. The miRNA targets for miR-491 were then determined by bioinformatics. Major targets included hsp90, alpha-fetoprotein (AFP) and IKB kinase. The functional role of these targets is suggested that miR-491 might enhance apoptosis via inhibiting their expression. Therefore, Hep G2 cells were transfected with either the precursor miR-491 or nonspecific miRNA. There was a 1700-fold increase in miR-491 levels in the transfected cells in comparison to controls as assayed by the S lagPCR system. With transfection of miR-491, there was a significant reduction (50-60% less than control) in the targets (hsp90, AFP, and IKB) as evaluated by Western blots. The miR-491 transfected Hep G2 cells showed a 2.5±0.18- or 3.0±0.26-fold increase in caspase-3 activity with TNF-alpha or FasL sensitization when compared to cells treated with TNF-alpha or FasL and nonspecific miRNA. DAPI staining also demonstrated a significant increase in apoptosis in the miR-491-transfected cells. CONCLUSION: We have found a series of miRNAs that are associated with an acute liver injury mouse model. One of these, miR-491, appears to enhance apoptosis when over-expressed in an in vitro system, apparently by inhibiting the gene expression of several cell survival proteins.

Disclosures: The following people have nothing to disclose: Sangjeong Yoon, Ping Guo Liu, Jian Wu, Mark A. Zern, Senthil K. Venugopal

90 ROLE OF HEPATIC STELLATE CELL AND ANGIOPOIETIN ON ANGIOGENESIS DURING LIVER FIBROSIS

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[Background] Hepatic stellate cells (HSCs) contribute to the development of liver fibrosis through production of extracellular matrix and secretion of cytokines, chemokines, and growth factors. Although cirrhosis is always accompanied by neovascularization, little is known about the role of HSCs in angiogenesis during liver fibrosis. Angiopoietins are potent
angioigenic factors interacting with endothelium-specific receptor tyrosine kinase (Tie2) on endothelial cells (ECs), but their role in vascular remodeling during liver fibrosis is unknown. [Purpose] The purpose of this study is to elucidate the role of HSCs and angiopoietins on angiogenesis during liver fibrosis. [Methods] Liver fibrosis was induced in BALB/c mice by bile duct ligation (BDL) or by injection of carbon tetrachloride (CCl4) twice a week. Vessel density around the portal triads was determined by CD31 immunohistochemistry. Expression of angiopoietins was measured by quantitative real-time PCR in whole liver and isolated cell fractions. A human HSC cell line immortalized by expression of human telomerase reverse transcriptase (hTERT-HSC) was utilized to evaluate transcriptional regulation of angiopoietin-1 expression. Ad S-Tie2, an adenovirus expressing a soluble form of Tie2, was used to block angiopoietin signaling in vivo. [Results] CD31 positive area was increased around the portal triads in fibrotic livers induced by BDL (0.26% to 0.60% at 2 weeks) or by CCl4 (0.26% to 0.62% at 2 weeks). Messenger RNA expression of angiopoietin-1 was upregulated after both BDL (2.8 fold) and CCl4 (3.1 fold). Kupffer cells, ECs, and HSCs express 5, 26, and 379 times as much angiopoietin-1 mRNA as hepatocytes, respectively, indicating HSCs are the main source of angiopoietin-1 in the liver. TNFα (10 ng/ml) induced a 10-fold increase in angiopoietin-1 mRNA expression in hTERT-HSCs, and the induction was blocked by a proteasome inhibitor MG-132, an IκB kinase inhibitor PS-1145, or an adenovirus expressing the IκB superrepressor, indicating induction of angiopoietin-1 expression is NF-κB dependent. The soluble form of Tie2 was detectable in the mouse serum through 10 days after Ad S-Tie2 injection. Blockade of angiopoietin signaling in mice by Ad S-Tie2 injection prior to induction of liver fibrosis inhibited the increase in vessel density (0.44% vs 0.57% CD31-positive area) and the development of liver fibrosis (0.47% vs 1.13% sirius red-positive area) at 2 weeks after CCl4. [Conclusion] HSCs produce an angiogenic cytokine angiopoietin-1 that contributes to the development of liver fibrosis through promotion of angiogenesis. Anti-angiogenic drugs may be effective in treating hepatic fibrosis.

Disclosures:
The following people have nothing to disclose: KOJIRO TAURA, David A. Brenner

91 INTERIM RESULTS OF RANDOMIZED CONTROLLED TRIAL OF ELAD™ IN ACUTE ON CHRONIC LIVER DISEASE
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This report details the first use of ELAD™ (Vital Therapies Inc, San Diego Ca, a human hepatocyte based liver assist device, in patients with acute on chronic liver disease. Methods: A randomized (2:1), open label, controlled trial of 90 patients was initiated at 2 Chinese liver disease centers. Continuous ELAD therapy was provided until recovery (43-119 hrs). Inclusion criteria were Chronic HBV or HCV with a current episode of acute decompensation. The primary endpoint was survival. 60 patients have currently been enrolled. 6 patients were not evaluable secondary to protocol violations leaving 35 treated and 19 controls to evaluate. All patients underwent one treatment of plasma exchange after randomization and obtaining baseline biochemical values. Demographic, and biochemical data is shown in the table. Results: 50 day transplant free survival was 9/19 (47%) in the controls and 30/35 (86%) in the treated group (p= 0.004). 5 patients underwent transplantation in the control group vs. 2 in the treated group. Intent to treat analysis: transplant free survival 10/20 (50%) controls vs. 32/40 treated (80%) (p=0.034). Biochemical improvement supported the increased survival in the treated group. Safety endpoints: Thrombocytopenia was the only statistically significant safety issue. Platelets dropped by an average of 28% (see table) during ELAD therapy vs. no change in controls. Platelet count recovered within 5 days of ELAD discontinuation and could be managed by platelet transfusion. Conclusions: The first clinical trial of ELAD in acute on chronic liver disease patients was able to demonstrate that ELAD is safe in this patient population. There is statistically significant transplant free survival advantage for the ELAD treated patients. ELAD appears to be effective in bridging patients with acute decompensation of chronic liver disease to recovery.

<table>
<thead>
<tr>
<th></th>
<th>FLAD</th>
<th>CONTROL</th>
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</thead>
<tbody>
<tr>
<td>AGE</td>
<td>39.6 ± 9.9</td>
<td>39.6 ± 11.6</td>
</tr>
<tr>
<td>HEP B</td>
<td>20/30 (67%)</td>
<td>13/16 (81%)</td>
</tr>
<tr>
<td>ON HBV ANTI-VIRAL @ BASELINE (%)</td>
<td>17</td>
<td>33</td>
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<tr>
<td>ON HBV ANTI-VIRAL @ POST ELAD DAY 7 (%)</td>
<td>25</td>
<td>42</td>
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<tr>
<td>ON HBV ANTI-VIRAL @ POST ELAD DAY 36 (%)</td>
<td>25</td>
<td>33</td>
</tr>
<tr>
<td>DURATION OF ELAD (HRS)</td>
<td>72 ± 14</td>
<td></td>
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<tr>
<td>MELD @ BASELINE</td>
<td>26.4 ± 3.4</td>
<td>31.0 ± 5.7</td>
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<tr>
<td>MELD @ ELAD STOP</td>
<td>29.1 ± 6.6</td>
<td>30.1 ± 5.8</td>
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<tr>
<td>MELD @ POST ELAD DAY 6**</td>
<td>14.2 ± 5.8</td>
<td>16.0 ± 7.1</td>
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<tr>
<td>PLTs @ SCREEN</td>
<td>802.5 ± 28.8</td>
<td>100.8 ± 48.5</td>
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<tr>
<td>PLTS @ ELAD STOP***</td>
<td>31.7 ± 24.9</td>
<td>95.7 ± 66.7</td>
</tr>
</tbody>
</table>

** VALUE TAKEN AT 72 HRS (MEAN DURATION OF ELAD TREATMENT)

** P <0.001 vs MELD at baseline and ELAD Stop

** P < 0.002

Disclosures:
Dar He - Employee: Other
John D. Brotherton - Employee: Other
Kameron Maxwell - Employee: Other
Michael Millis - Consultant/Adviser: Other

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92 OLDER AGE IS NOT ASSOCIATED WITH WORSE OUTCOMES IN ACUTE LIVER FAILURE
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Background: Age > 40 years is among the poor prognostic factors in King’s College Hospital criteria for acute liver failure (ALF). Older age is considered a relative contraindication for liver transplantation in ALF. We aimed to evaluate the impact of older age, defined as age ≥ 60 years, on outcomes in patients with ALF. Methods: One thousand one hundred and twenty-six patients with acute-on-chronic liver failure were enrolled in the study and were divided into two age groups, < 60 years and ≥ 60 years. Results: There was no significant difference in the causes of ALF between the age groups (p=0.78). Older age was not associated with poor outcomes in patients with ALF. There was no significant difference in the MELD score between the age groups (p=0.27) and the survival rate was not different between the age groups (p=0.21). Conclusions: Older age is not associated with worse outcomes in acute liver failure.
L-ORNITHINE PHENYLACETATE ATTENUATES INCREASE IN ARTERIAL AMMONIA AND INTRACRANIAL PRESSURE IN A DEVASCULARISED PIG MODEL OF ACUTE LIVER FAILURE: A NOVEL AMMONIA-LOWERING STRATEGY FOR HEPATIC ENCEPHALOPATHY

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Ammonia is central in the pathogenesis of HE and its arterial levels predict brainstem herniation which accounts for mortality in about 30% of patients with ALF. Treatment options for hyperammonemia and raised intracranial pressure (ICP) in ALF remain unmet clinical needs. Ammonia levels in liver failure are regulated by the critical interplay of ammonia, glutamate and glutamine metabolism involving the muscle, gut and kidneys. Based on this knowledge, we have formulated L-Ornithine Phenylacetate (OP), a novel concept to reduce plasma ammonia. L-Ornithine stimulates ammonia removal through glutamine synthetase (glutamine production) in skeletal muscle and phenylacetate excretes the ornithine-related glutamine as phenylacetamide in the urine. To evaluate the therapeutic efficacy of OP, pigs with ALF (induced by liver devascularization) were treated with L-Ornithine 0.05g/kg/hr (i.v) and phenylbutyrate (pro-drug) 0.07g/kg/hr (i.g) and compared to pigs with ALF (vehicle treated) and shams. ICP and cardiovascular hemodynamics were continuously monitored for the duration of the experiment (8 hours) following liver devascularization. Arterial blood and urine was sampled every 2 hours to measure ammonia and phenylacetylglutamine respectively. A lumbar puncture cerebrospinal fluid (CSF) sample was taken at the end of the experiment to measure ammonia. Over 8 hours, arterial concentrations of ammonia did not change significantly in the sham group (50.7±8.7 µmol/L), but in the ALF group it increased to 589.6±56.7 µmol/L (p<0.001 compared to sham). This increase was attenuated in the OP-treated group (365.2±60.4 µmol/L, p<0.01 compared to ALF). Furthermore, ICP (expressed as percent baseline) did not change in the sham group (18.2±14.9%, but increased significantly in the ALF group (98.5±9.1%, p<0.01 compared to sham). In the ALF+OP-treated group, ICP was normalized (25.5±17.3%, p<0.05 compared to ALF). This was accompanied by a reduction in CSF ammonia in the ALF+OP treated pigs compared to ALF pigs (158.50±29.01 µmol/L vs 233.50±39.51 µmol/L respectively). In accordance with the hypothesis, treatment with OP resulted in increased urine phenylacetylglutamine levels in ALF+OP treated pigs compared to ALF pigs (9.0±4.5 µmol/L vs 0.05±0.04 µmol/L). In conclusion, OP is a novel approach to treat hyperammonemia and HE, which successfully attenuated an increase in arterial and CSF concentrations of ammonia and, normalized ICP in this large-animal model of ALF. These positive results demonstrating its efficacy in pigs with ALF provides the rationale for further development of this drug and a clinical trial of OP in ALF.

Disclosures:
The following people have nothing to disclose: Lars M. Ytrebø, Rune Gangsøy Kristiansen, Ole Martin Fuskævåg, Hanne Mæhre, Arthur Revhaug, Rajiv Jalan, Christopher Rose

STARVATION-INDUCED HEPATOCYTE AUTOPHagy: AN ORIGINAL MECHANISM OF ACUTE LIVER CELL DAMAGE IN PATIENTS WITH ANOREXIA NERVAso

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BACKGROUND/AIMS: In patients with anorexia nervosa (AN), increased serum transaminases level is a common finding, suggesting that extreme malnutrition, by itself, may cause some degree of liver cell damage. Yet, the mechanisms are unclear. Autophagy has been documented in patients with cardiomyopathy. This pathway can induce cell death under certain conditions. The aims of this study were to clarify the mechanisms of liver injury during AN and to determine if hepatocyte autophagy could play a role. PATIENTS/METHODS: The study is based on a series of 11 patients (9 females, 2 males) with AN referred to our institution for acute liver insufficiency between 1995 and 2007. Mean age was 24 years (range 18-47). Mean BMI was 11.4 (range 9.6-13). All the other causes of acute liver injury were ruled out. In particular, there was no paracetamol overdose. RESULTS: At admission, prothrombin
USE OF NUCLEOSIDE ANALOGUES IN HBV RELATED ACUTE LIVER FAILURE

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Background: Acute liver failure (ALF) due to hepatitis B virus [HBV] infection has a high mortality rate (~25% survival without transplant [OLT]). Recently, nucleoside analogues [NA] have been suggested to improve outcomes in severe acute hepatitis B in uncontrolled studies [Tillman HL et al. J Viral Hepat 2006;13:256]. We sought to determine whether use of NAs favorably influenced outcomes specifically in HBV ALF. Methods: Detailed clinical information had been collected prospectively on more than 1,133 patients with classically defined ALF [encephalopathy and INR > 1.5] from the ALF Study Group registry. Over the past 10 years, the ALF Study Group has enrolled 76 patients with HBV ALF. We obtained additional retrospective data from the sites on NA use for 57/76 patients: 32 had received a NA [29 lamivudine, 1 adefovir/lamivudine and 2 on entecavir]. Results: Median duration of NA use was 9 days (range 1 to 36). NA use increased only slightly over the decade, from 13/27 patients in the first 5 years of the study to 19/30 in the next 4.5 years. The groups were comparable in the following clinical and lab parameters: gender, INR, coma grade at entry and percent receiving OLT. However, the group receiving NA were older (51 vs. 38 yrs, p=0.03), and had higher bilirubin levels (23.4 mg/dl vs. 15.2, p=0.01), but lower ALT (1,234 IU/L vs. 2,416, p=0.06) and AST levels (676 vs. 1,347, p=0.03). Overall survival was 40/57 (70.1%), including 22/23 patients receiving a graft; 18/57 (31.5%) survived spontaneously (without a graft). Overall survival was 20/32 (62.5%) for the NA (14 were transplanted) and 20/25 (80%) for the no NA group (9 were transplanted), p=0.15. Spontaneous survival was 7/18 (39%) for the NA group and 11/18 (61%) for the no NA group, p=0.07. Conclusions: NA use for patients with ALF due to HBV did not appear to improve survival in this retrospective, non-randomized study. Selection bias, differences in treatment duration or other factors might be at play. Empiric use of nucleoside analogues in established HBV ALF does not appear to improve outcomes and cannot be recommended, given the trend toward worse overall and spontaneous survival in the NA group. A prospective study that evaluates NA vs placebo use for severe HBV infection and/or HBV ALF seems indicated. Supported in part by James and Alinda Wikert Fund of the Southwestern Medical Foundation and by U-01 56389 from NIDDK to WML for the ALFSG. We acknowledge the many contributions of the investigators, coordinators and patients of the Acute Liver Failure Study Group 1998-2007.

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96 EVALUATION OF A SCORING SYSTEM FOR ASSESSING PROGNOSIS IN PEDIATRIC ACUTE LIVER FAILURE

Brandy Lu, Jane Gralla, Edwin Liu, Emily Dobyns, Michael Narkewicz, Ronald Sokol; Pediatrics, The University of Colorado School of Medicine and the Children's Hospital, Denver, CO

Pediatric acute liver failure (PALF) is defined as sudden severe liver dysfunction and coagulopathy with or without encephalopathy in a child without known liver disease. PALF leads to death or liver transplantation [LT] in up to 50% of cases. A scoring system for predicting death or LT in PALF [Liver Injury Units [LIU] score] was previously developed by our group [J Hepatol. 2006, 44, 134-141]. Total bilirubin, prothrombin time [PT], international normalized ratio [INR], and ammonia were identified through multi-variate analysis as significant predictors. The following formula predicted outcome: LIU = 3.507 x peak total bilirubin (mg/dl) + 45.51 x peak INR + 0.254 x peak NH3 (μmol/l). The aims of this study were to test the predictive value of the LIU score using peak values and LIU score at PALF presentation in a subsequent test group of patients. Methods: Charts were reviewed and data obtained from all 53 children with PALF admitted to The Children's Hospital of Denver from 2002 – 2006. PALF was defined using standard criteria [J Pediatr. 2006, 148, 652-8]. Outcome was defined at 4 weeks as alive without LT, death, or LT. Survival curves were constructed based on the quartile of the LIU score. Individual receiver operating characteristics [ROC] curves were generated to predict death or transplant at 4 weeks following presentation. Results: Etiology of PALF was indeterminate [14], sepsis/ischemia-reperfusion [14], viral [8], metabolic [5], acetaminophen [4], hematology-oncology [4], and other [4]. 13% underwent LT, 15% died without LT, with an overall transplant-free survival of 72% at 4 weeks. The proportion of patients alive without LT was plotted in a survival analysis stratified by previously defined quartiles for the LIU score [0-209; 210-296; 297-369; > 369]. Survival without LT at 4 weeks for each quartile using the peak LIU score was 92%, 78%, 60%, and 25% respectively (p=0.0001). The Peak LIU ROC had a C index of 0.96, with a sensitivity of 96% and specificity of 88%.
86.3. The Presentation LIU score was also predictive of outcome based on survival curves (p = 0.005) with a C-index of 79.3. Conclusions: Both the Peak and Presentation LIU scores were predictive of survival without liver transplantation in this test group of PALF patients. The LIU score may be useful for establishing transplant priority in PALF and as a research tool for stratifying PALF patients into risk groups. Further prospective analysis is underway.

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97 A DEDICATED FIBROSCAN® PROBE TO EVALUATE LIVER FIBROSIS IN CHILDREN: FEASIBILITY AND PERFORMANCE FOR THE DIAGNOSIS OF CIRRHOSIS
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Liver stiffness measurement (LSM) has been shown to be correlated with liver fibrosis in adult with chronic liver disease. A preliminary study using the standard FibroScan® probe showed promising results after data were reprocessed [1] and led to the development of a probe dedicated to children. The aim of this prospective study was to evaluate the performance of this FibroScan® probe dedicated to children. From December 2005 to October 2006, all consecutive children with chronic liver diseases were enrolled after informed consent was obtained from their parents. Clinical, biological, US examination and eventually liver biopsy (LB) were performed. Liver stiffness was assessed using the METAVIR scoring system by two pathologists. Liver stiffness measurement was performed using FibroScan® (EchoSens, Paris, France) equipped with a probe dedicated to children. Examination was considered as reliable if at least 5 valid measurements were obtained. 93 children were included and LSM was unreliable in 3 patients. In the 90 children remaining (46 boys), age was 88 ± 64 [1-206] months and BMI 16.6 ± 10.4-24.2 kg/m². Etiologies were 22 biliary atresia, 7 HCV, 6 HBV, 10 autoimmune hepatitis and 45 miscellaneous. LSM was significantly (p < 0.05) correlated with platelets (-0.46), prothrombin time (-0.30), INR (0.33), albumin (-0.47), bilirubin (0.50), AST (0.44), ALT (0.34), GGT (0.37), alkaline phosphatases (0.22) and hyalurionate (0.38). US signs of portal hypertension were significantly associated with higher LSM (19.5 ± 18.1 versus 6.6 ± 3.8 kPa). Out of 56 LB, 11 were unreliable for fibrosis staging (METAIVR). Between LSM and METAIVIR fibrosis stage, Kendall correlation coefficient was 0.42 (p < 0.001). Area under the ROC curve was 0.88 (0.65-0.96) for the diagnosis of cirrhosis. Reliable liver stiffness measurement was feasible using a dedicated probe in children from 18 years down to 1 month of age. Moreover, LSM was strongly correlated with liver fibrosis assessed by histology, biological parameters and the presence of portal hypertension. This new probe needs to be rapidly available for further studies in large cohorts of children with and without cirrhosis. [1] de Ledinghen et al, JPGH (in press)

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98 OVERWEIGHT IS ASSOCIATED WITH DIMINISHED ANTIVIRAL RESPONSE IN HCV-INFECTED CHILDREN
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Background and Aims: Evidence demonstrates that obesity is associated with poor response to interferon therapy among hepatitis C (HCV)-infected adults. However, this evidence is confounded by the use of a single adult dose rather than weight-based dosing of interferon whereby overweight patients most often received lower doses relative to their body mass. Pediatric cohorts offer advantages over adult populations, including lower degrees of fibrosis, minimal comorbidities, and, in general, shorter duration of infection. The aim of this investigation was to determine the effect of overweight on response to antiviral therapy for HCV in the young. Methods: Patients younger than 20 years of age (median= 13.1, range= 5.9-19.2), who received therapy for HCV infection at Children’s Hospital Boston (CHB) between 1998 and 2007 (n=45) were included. Demographic characteristics, medical histories, HCV genotype, available liver histology, anthropometric measurements before and after treatment, and response to therapy (defined as HCV RNA levels below the limit of detection at the end of therapy) were analyzed. Treatment included at least 12 weeks of therapy with standard interferon with and without ribavirin; dosages of all medications were determined based on subjects' body weight. Subjects' BMI percentiles and subsequent z scores were calculated from CDC smoothed percentile curves of the NHANES data. Results: There were 21 males and 24 females in the study. The vast majority of patients were non-Hispanic Caucasian; there was one African American and 3 Hispanics. Most patients had mild to no fibrosis at the onset of treatment. Thirty-five had genotype 1, six had genotype 3a, three had genotype 2a, and one had genotype 4. A multiple logistic regression model controlling for change in BMI z-score demonstrated a significant association between baseline BMI z-score and response to antiviral therapy (OR=0.50; 95% CI 0.26-0.94; p=0.03). In the same model, overweight patients (BMI>85 percentile for age and gender) at onset of therapy had 85% lower odds of response to antiviral therapy (OR=0.15; 95% CI 0.03-0.69; p=0.02). Conclusion: Overweight is independently associated with poor response to antiviral therapy against HCV among children and adolescents. The use of uniform weight-based dosing and choice of a pediatric cohort minimize possible confounders. These results strongly suggest that increased BMI has an intrinsically adverse effect on treatment outcome independent of possible underdosing of interferon and/or ribavirin.

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The following people have nothing to disclose: Aymin Delgado-Borrego, Marielle Christofe, David Healey, David A. Ludwig, Maureen M. Jonas, Raymond T. Chung
HIGH THROUGHPUT SEQUENCE ANALYSIS IDENTIFIES INDIVIDUAL AND COMBINED GENETIC DEFECTS IN CHILDREN WITH SYNDROMES OF INTRAHEPATIC CHOLESTASIS

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The discovery of the genetic defects in children with intrahepatic cholestasis allows a specific diagnosis to be made despite the large overlap in clinical phenotypes. Because of the lack of predominant mutations in target genes, we developed a sequencing chip (the JaundiceChip) that reads all exons and intron boundaries of five genes known to cause syndromes of intrahepatic cholestasis: SERPA1A (for Alpha-1-Antitrypsin), A1AT, JAG1 (Alagille syndrome, AGS), ATPB81 (FIC1 deficiency), ABCB11 (BSEP deficiency), and ABCB4 (MDR3 deficiency) simultaneously. In this study, we aimed to: 1) determine the ability of the chip to detect previously reported mutations, 2) search for mutations in children with chronic cholestasis of unknown etiology, and 3) screen for the coexistence of defects in more than one gene.

Methods and Results: We tested the chip using peripheral blood DNA from 48 subjects that either had a well defined clinical/biochemical diagnosis (A1AT=10, AGS=10, MDR3=1) or carried candidate mutations previously identified by capillary sequencing (AGS=5, FIC1=11, BSEP=11). The chip accurately identified at least one candidate mutation in 38 of 48 patients (79.2%). These mutations correlated with the previously assigned clinical or molecular diagnosis in more than 90% of patients for individual diseases, except for AGS in whom mutations were detected in 7 of 15 (46.7%) subjects. Because of the known high frequency of insertion/deletion mutations in AGS, we developed a complementary capillary sequencing approach and identified potentially disease-causing mutations in 7 of the remaining 8 subjects. We then applied the JaundiceChip to screen for candidate mutations in 24 subjects with intrahepatic cholestasis of unknown etiology and without features of AGS. We found mutations in 50% of the subjects, but the mutation frequency increased to 60% in subjects with low serum GGT, all of them in the ATPB81 and ABCB11 genes. Sequence analysis of the entire cohort revealed that the previously reported ATPB81 mutations N45T and E429A were present in 21% of subjects with BSEP deficiency (harboring ABCB11 mutations) and K203R and F305I in 20% of subjects with PiZ allele. In conclusion, validation studies showed that the JaundiceChip detects candidate mutations in 79.2% of children with intrahepatic cholestasis and requires complementary capillary sequencing to detect insertions or deletions in JAG1. When used to test subjects with known and undefined forms of intrahepatic cholestasis, the JaundiceChip identifies candidate mutations in at least half of the subjects and the coexistence of previously reported mutations in more than one gene in a subgroup of subjects.

COMPARATIVE PROTEOMIC ANALYSIS OF ENTERAL NUTRITION-ASSOCIATED LIVER DISEASE (PNALD) IN INFANTS WITH INTESTINAL FAILURE

Ronald J. Sokol, Kerri Murray, Jenna Boyd, Heather Thompson, Ross Shepherd, Frederick M. Karrer, Cara Mack, Mark Duncan

Cholestatic liver disease is a serious complication of parenteral nutrition (PN) therapy in infants with intestinal failure and is the primary indication for liver-intestinal transplants in children. Because current treatments are ineffective, novel strategies are needed to discover mechanisms involved in this serious liver injury. The aim of this study was to apply comparative proteomics to determine differences in protein levels in the liver of infants with PNALD. Methods: A portion of surgical liver biopsies, obtained for clinical purposes, was flash-frozen in liquid nitrogen from 7 infants with PNALD (mean age 88 days, 5 male) and 7 with BA (BA control group) undergoing portoenterostomy (mean age 59 days, 2 male). Underlying intestinal diseases in PN group were NEC, gastrochisis, intestinal volvulus and atresias. Mean serum total bilirubin (PN:7.4, BA:7.0 mg/dl), conjugated bilirubin (6.0, 5.3), ALT (305, 129 IU/L) and alkaline phosphatase (707, 647 IU/L) did not differ between the two groups. Liver was homogenized, proteins precipitated with methanol/chloroform, and difference gel electrophoresis (DIGE) was used to compare protein levels in PNALD and BA samples (n=7 separate gels). Differentially abundant spots were excised, digested with trypsin, analyzed by MALDI-TOF MS and identified based on their peptide mass fingerprints. Results: Over 2000 protein spots were detected on each gel and 96 of the differentially abundant spots were identified by mass spectrometry. Stringent criteria were employed to establish a subset of 4 proteins of interest that were significantly overabundant in all PNALD samples compared to BA samples: peroxiredoxin-2, protein S100-A8, glutathione S-transferase P, and Mn-superoxide dismutase (mitochondrial SOD). Notably, each of these are involved in redox regulation and cellular protection against oxidative stress, a proposed mechanism involved in PNALD (Am J Physiol 1996;270:G691). Of note, lipid peroxide products (lipofovin) in Kupffer cells are characteristic of PNALD liver histology. Two overabundant proteins in BA were peroxiredoxin-2 and protein S100-A8; the latter is expressed by macrophages which have been shown to be increased in the portal tracts in BA. These findings were validated with immunoblotting. Conclusion: Comparative proteomics identified several over abundant proteins in PNALD to be key redox regulators, supporting the proposal that oxidative stress is a major pathway of tissue injury in PNALD. These findings raise the possibility that novel therapeutic approaches for this disease could be targeted at this pathway.

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HCV-SPECIFIC PRODUCTION OF IL-10 AND IFN-GAMMA-INDUCIBLE PROTEIN-10 (IP-10) LEVELS PREDICT TREATMENT RESPONSE TO PEGYLATED INTERFERON AND RIBAVIRIN IN CHILDREN WITH CHRONIC HEPATITIS C INFECTION

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Background: Chronic hepatitis C virus (HCV) infection in children manifests as slowly progressing mild disease in the first two decades with potentially severe acceleration in adulthood. In adults antiviral therapy with pegylated interferon (IFN) and ribavirin prevents disease progression, while successful control of HCV infection is associated with strong and broad HCV-specific immune reactivity of Th helper 1 (Th1) type lymphocytes. Pre-treatment plasma levels of the chemokine IFN-gamma-inducible protein-10 (IP-10) were suggested to predict response to therapy. Limited information is available on HCV-specific immune reactivity in childhood. Aim: To investigate whether baseline HCV-specific Th1/Th2 immune responses and plasma levels of IP-10 predict outcome of therapy with pegylated IFN and ribavirin in children with chronic hepatitis C. Patients: Eighteen children (9 males, median age 13.5 years) with perinatally acquired chronic hepatitis C were treated with pegylated IFN and ribavirin. Patients were divided into two groups according to treatment response: 14 responders (R) and 4 non-responders (NR). Five HBV-infected children (4 males, median age 14.2 years) served as controls. Methods: Th1/Th2 lymphocyte HCV-specific responses were evaluated by IFN-gamma and interleukin-10 (IL-10) Elispots in peripheral blood mononuclear cells (PBMC) after stimulation with genotype specific HCV antigens (core, NS3, NS4 and NS5). The non-specific stimuli phytohemagglutinin, bacterial lipopolysacharide and tetanus toxoid antigen served as positive controls. Plasma IP-10 concentrations were measured by ELISA. Results: Baseline HCV-specific IFN-gamma production was similar in R and NR. In contrast, core- and NS3-specific IL-10 production was higher in NR than in R (core: 76.3 ± 23.9 vs. 12.5 ± 6.2 specific spot forming cells (SPF) per million of PBMC (spSFC/10^6 PBMC), p=0.008 and NS3: 81.3 ± 16.8 vs. 27.5 ± 10.1 spSFC/10^6 PBMC, p=0.03). Production of IFN-gamma and IL-10 was similar in R and NR after stimulation with non-specific stimuli. Low HCV-specific IFN-gamma and IL-10 production was detected in HBV infected children (IFN-gamma HCV core: 7.5 ± 3.4 spSFC/10^6 PBMC and IL-10 HCV core: 7.9 ± 3.7 spSFC/10^6 PBMC). Plasma levels of IP-10 tended to be higher in NR (57.0 ± 12.8 vs. 31.4 ± 9.7 pg/ml, p=0.15). Conclusions: High levels of pre-treatment IP-10 and HCV specific production of IL-10 are associated with failure to respond to antiviral treatment in children with chronic HCV infection.

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DISCRIMINATING FEATURES OF BILARY ATRESIA - A PROSPECTIVE MULTI-CENTERED ANALYSIS

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Introduction: Neonatal cholestasis presents an important diagnostic challenge. Efficiently differentiating biliary atresia (BA) from other causes of cholestasis is important in order to undertake surgical intervention early when it is more effective. Identifying presenting features that effectively differentiate BA from other forms of neonatal cholestasis would contribute to an efficient diagnostic algorithm. We performed the first prospective multi-center analysis to assess the reliability of patterns of presenting features in making a diagnosis of BA. Methods: Between October 2003 and April 2007, 222 children with neonatal cholestasis were enrolled in a prospective multi-center database as part of the Biliary Atresia Research Consortium (BARC). Presenting clinical features, physical findings and laboratory data were collected prospectively and recorded prior to the ultimate assignment of a clinical diagnosis. Specific features analyzed from the time of the first evaluation at the BARC center included: age, sex, weight, height, head circumference, midarm circumference, triceps skinfold thickness, palpable liver (including cm below the costal margin), palpable spleen, acholic stools, facial features, total bilirubin, conjugated bilirubin, ALT, AST, alkaline phosphatase, GGT albumin, platelet count, and cholesterol. Findings were compared between subjects ultimately diagnosed with BA relative to all others (non-BA). Results: 106 subjects were diagnosed with BA (116 non-BA). Of the findings described above the ones that were significantly different between BA and non-BA (expressed as BA vs non-BA, mean +/- SD) were: female sex (49 vs 35%, p=0.04), measurement of liver edge below costal margin (3.4 +/- 1.7 vs. 2.6 +/- 2.0 cm, p=0.0002), normal facial features (94% vs 82%, p=0.009), palpable spleen (56% vs 38%, p=0.012), acholic stools (80% vs 31%, p<0.0001), and GGT (655 +/- 476 vs 349 +/- 376, p<0.0001). By multivariate analysis (expressed as odds ratio; 95% CI) acholic stools (8.9; 3.7 – 21.7, p<0.0001), female gender (2.3; 1.04 – 5.2, p=0.04), and GGT (1.19; 1.07 – 1.32, p=0.0015) were independent predictors of BA. Conclusion: It is difficult to distinguish BA from other cholestatic liver diseases at the time of presentation on the basis of physical findings and currently utilized routine laboratory parameters. The combination of acholic stools and elevated GGT may be the most informative findings. Supported by grants from the NIDDK, NIH

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CASPASE INHIBITION SWITCHED TNF-ALPHA-INDUCED APOPTOSIS TO AUTOPHAGIC CELL DEATH IN HEPATOCYTES

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Cell death, in particular, apoptosis, plays an important role in many liver diseases such as eatotoxicity and alcohol-induced liver injury. We have previously demonstrated that tumor necrosis factor-alpha (TNF) plus a general transcription inhibitor actinomycin D (ActD) induced apoptosis in primary cultured mouse hepatocytes. This apoptosis could be completely suppressed by a general caspase inhibitor, ZVAD-fmk (Ding et al Hepatology 2004;40:403-413). However, we found that in the presence of ZVAD-fmk, TNF/ActD-treated hepatocytes still died in a necrosis-like cell death with abundant cellular vacuoles, as examined by the propidium iodide (PI) staining and phase-contrast microscopy. In contrast, ZVAD-fmk alone did not induce cell death in the cultured hepatocytes. Using adenov-GFP-LC3 infected hepatocytes, we found that TNF/ActD/ZVAD-fmk treatment significantly increased the percentage of cells with punctuated GFP-LC3, a hallmark of autophagy, suggesting the activation of autophagy in TNF/ActD/ZVAD-fmk-treated hepatocytes. To further confirm the induction of autophagy by TNF/ActD/ZVAD-fmk treatment, we have performed electron microscopy (EM) studies. Results from the EM studies revealed that TNF/ActD/ZVAD-fmk-treated hepatocytes accumulated large amounts of autophagosomes (AV), while few were found in control cells, further supporting the induction of autophagy by TNF/ActD/ZVAD-fmk treatment. Interestingly, in the presence of two autophagy inhibitors, 3-methyadenine (3-MA) and LY294002, TNF/ActD/ZVAD-fmk-induced cell death was significantly suppressed. Moreover, necrostatin, a novel small molecule which has been shown to be a specific necrosis inhibitor, did not affect TNF/ActD/ZVAD-fmk-induced cell death. Taken together, these findings suggest that autophagic cell death could be induced by TNF/ActD/ZVAD-fmk in hepatocytes and imply that simply suppress caspase activity is not sufficient to fully protect hepatocytes from TNF-mediated cell death, even apoptosis is blocked.

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IMPAIRED AUTOPHAGY IS THE MECHANISM OF MITOCHONDRIAL DYSFUNCTION IN ISCHEMIC RAT HEPATOCYTES

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BACKGROUND: Onset of the mitochondrial permeability transition (MPT) after ischemia/reperfusion (I/R) is a key mitochondrial dysfunction causing hepatocyte death. Autophagy is a vital endogenous process that selectively removes abnormal or damaged cellular constituents and organelles such as dysfunctional mitochondria. Defects in autophagy cause accumulation of dysfunctional mitochondria, leading to cell death. However, the role and regulation of autophagy in ischemic hepatocytes is unknown. The AIM of this study was to investigate the role of mitochondrial autophagy in I/R-induced injury to hepatocytes. METHODS: Cultured rat hepatocytes were incubated in anoxic Krebs-Ringer-HEPES (KRH) buffer at pH 6.2 for 4 h to simulate tissue ischemia. To simulate reperfusion, cells were reoxygenated at pH 7.4 for 2 h. Expressions of Akt7 and Beclin-1, proteins that regulate autophagy, were analyzed by Western blot during I/R. Some hepatocytes were incubated with 50 μM of N-Acetyl-Leu-Leu-Met-CHO to block calpains, or transfected with adenoviruses encoding GFP (control), Akt7 and Beclin-1 to augment autophagy. To induce nutrient depletion, a condition that stimulates autophagy, hepatocytes were incubated in KRH for 3 h prior to onset of ischemia. Necrotic cell death was evaluated by propidium iodide (PI) fluorometry. For confocal imaging, hepatocytes were co-loaded with calcine, tetramethylrhodamine methylster and PI to visualize onset of the MPT, mitochondrial depolarization and necrosis, respectively. To further examine the role of autophagy, hepatocytes were isolated from GFP-LC3 mouse or rat hepatocytes were transfected with adenovirus expressing GFP-LC3 and subjected to I/R. Confocal images were collected to analyze green fluorescent autophagosomes (GFP-LC3) during and after ischemia.

RESULTS: Western blot analysis showed that 4 h of ischemia decreased Akt7 and Beclin-1 to 8.1% and 48.9% of normal values, respectively. After 20 min of reperfusion, expressions of Akt7 and Beclin-1 further decreased to only 0.9% and 8.9% of initial values, respectively. In control hepatocytes, reperfusion caused MPT-dependent necrotic cell death. However, calpain inhibition, adenoviral overexpression and nutrient depletion all substantially suppressed I/R-induced loss of autophagy proteins, and furthermore prevented onset of the MPT and cell death after reperfusion. Confocal imaging of GFP-LC3 confirmed I/R-induced depletion of autophagosomes, which was reversed by adenoviral overexpression and nutrient depletion.

CONCLUSION: Degradation of Akt7 and Beclin-1 by calpains impairs mitochondrial autophagy, which in turn leads to MPT-dependent hepatocyte death after I/R.

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CASPASE 8-INDUCED ENDOSONAL ACIDIFICATION IS AN UPSTREAM EVENT IN CD95-DEPENDENT HEPATOCYTE APOPTOSIS

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Introduction: In hepatocytes, almost all CD95 receptor is located inside the cell. CD95 ligand (CD95L) was recently shown to trigger a NADPH oxidase-derived generation of reactive oxygen species (ROS) through a ceramide-dependent pathway which finally leads to CD95 trafficking to the plasma membrane (Reinehr et al., J Biol Chem 2005). Methods: In primary rat hepatocytes and hepatocytes from p47phox knock-out mice ceramide formation was detected by HPTLC and ROS generation by DCFDA-fluorescence. Apparent vesicular pH was determined using endocytosed FITC-dextran and [3Cl-]cyt by MQAE-fluorescence. Acidic sphingomyelinase (ASM) protein knock-down was achieved using antisense oligonucleotides. Activation of the CD95 system was measured by CD95 immunoprecipitation and subsequent detection for EGFR-FADD- and caspase 8-association and CD95-yr phosphorylation by WBlot. CD95 trafficking to the plasma membrane was visualized by immunocytchemistry under permeabilized vs.
non-permeabilized conditions. Results: CD95L lowered within seconds the apparent vesicular pH from 6.0 to 5.7 in a FITC-dextran-accessible and ASM-containing endosomal compartment, which activates ASM-driven ceramide formation. Simultaneously, an increase of [Cl\textsuperscript{-}]-cyt was observed, which is known to activate vH\textsuperscript{+}-ATPase. Inhibition of CD95L-induced endosomal acidification by either bafilomycin or DIDS largely abolished the CD95L-induced ceramide-formation as well as downstream processes, such as p47phox-Ser phosphorylation, ROS generation, activation of the CD95 system and subsequent apoptosis. These responses were also abolished after protein knock-down of ASM in rat hepatocytes or in cells from the p47phox knock-out mouse. In addition, inhibition of caspase 8 using a caspase 8- or a pan-caspase-inhibitor abolished both, signaling events occurring almost instantaneously after CD95L-addition including increase in [Cl\textsuperscript{-}]-cyt, endosomal acidification, ceramide- and ROS-generation, as well as the subsequent activation of the CD95 system by EGFR-catalyzed CD95-Tyr phosphorylation, translocation of the intracellular located main amount of CD95 to the plasma membrane and DISC-formation. Conclusions: These data suggest that in hepatocytes some CD95 “sentinel” receptors, which are undetectable by conventional immunostaining, are present at the plasma membrane under control conditions and which after CD95L-addition, induce an instantaneous and caspase 8-dependent increase in [Cl\textsuperscript{-}]-cyt, endosomal acidification and ROS generation. This further leads to a self-amplification of the CD95 death receptor-pathway by trafficking of the main amount and intracellular localized CD95 receptors to the plasma membrane.

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106 INTERNALIZATION OF DEATH RECEPTOR 5 IS REQUI-SITE FOR LYOSOMAL PERMEABILIZATION BY TRAIL

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Tumor necrosis factor-related apoptosis inducing ligand (TRAIL) selectively triggers cell death in a wide variety of cancer cells through two death receptors, death receptor 4 (DR4) and death receptor 5 (DR5). We have recently reported that TRAIL cytotoxicity is mediated by lysosomal permeabilization (Guicciardi et al Am J Physiol, in press). However, the mechanisms responsible for TRAIL-induced lysosomal permeabilization remain unclear. Our AIM was to determine which death receptor, DR5 and/or DR4 mediates TRAIL-induced lysosomal permeabilization, and to ascertain if receptor internalization is necessary for this process. METHODS: Apoptosis was induced by treating HUH-7 cells, a human hepatocellular carcinoma cell line, with TRAIL. DR4 and DR5 were knocked down by siRNA technology. Internalization of TRAIL:TRAIL receptor complex was analyzed by confocal microscopy using Flag-tagged TRAIL. Clathrin-dependent endocytosis was inhibited by infecting cells with an adenovirus expressing the K44A dominant negative dynamin mutant. Lysosomal permeabilization was evaluated by examining the subcellular localization of cathepsin B, a lysosomal protease, by immunofluorescence. Apoptosis was assessed by characteristic nuclear staining with DAPI and caspase 3/7 activity assay. RESULTS: HUH-7 cells express both TRAIL receptors. Confirmation of selective DR4 or DR5 knockdown by specific siRNA was confirmed by immunoblot analysis. DR5 siRNA, but not DR4 siRNA significantly attenuated TRAIL-mediated apoptosis (p<0.05). DR5 siRNA also reduced cathepsin B release from lysosomes (p<0.0001), indicating that DR5 specifically mediates lysosomal release of this protease. Flag-tagged TRAIL was internalized into cytosol within 60 minutes after administration of TRAIL. TRAIL-mediated apoptosis was significantly reduced in K44A dynamin adenovirus-infected cells as assessed by both morphologic changes and caspase 3/7 activity (p<0.05). In addition, K44A dynamin adenovirus infection also reduced lysosomal permeabilization (p<0.0001). CONCLUSIONS: Our results indicate that DR5 mediates TRAIL-triggered lysosomal permeabilization. Moreover, internalization of the TRAIL:DR5 receptor complex is requisite for lysosomal permeabilization. We speculate that internalization of the receptor-ligand complex targets apoptosis effectors to lysosomes triggering this death pathway.

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107 GLUTATHIONE DEPLETION DOWN-REGULATES TUMOR NECROSIS FACTOR (TNF) α-INDUCED NF-κB ACTIVITY VIA IκB KINASE (IKK)-INDEPENDENT AND -DEPENDENT MECHANISMS

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Background and Aim: Depletion of reduced glutathione (GSH) by diethyl meleate (DEM) was shown previously to inhibit expression of NF-κB target genes induced by TNF and sensitize primary cultured mouse hepatocytes to TNF-mediated apoptotic killing. The present studies were designed to elucidate the mechanism for the inhibitory effect of GSH depletion on this pathway and to compare the effects of moderate versus severe GSH depletion. Methods: Hepatocytes were isolated from male C57BL/6 mice, 7-9 week of age, plated at 1.2x10\textsuperscript{6} cell/60-mm dish. Total glutathione levels were determined by recycling assay. Western blotting (WB) analysis and quantitative real-time PCR were used to assess protein and mRNA levels. Electrophoretic mobility shift assay and chromatin immunoprecipitation assay were used to examine NF-κB DNA binding activity in vitro and in intact cells, respectively. Results: We observed that moderately depleting cell of GSH (~50%) by 0.1 mM DEM cotreatment (1) represses expression of NF-κB responsive gene in a xB-site dependent manner using reporter gene assays, (2) inhibits TNF-induced expression of endogenous NF-κB-target genes, including IκBα, A20, cIAP1, iNOS, and FUIPL, at both mRNA and protein levels, and (3) sensitizes PMH to TNF-induced apoptosis. Similar results were obtained when moderate GSH depletion was achieved by inhibition of GSH synthesis with buthionine-sulfoximine. However, TNF-induced IκK activation, IκBα degradation, NF-κB nuclear translocation, NF-κB DNA binding in vitro or p65-DNA binding in vivo were not altered, indicating the down-regulation is independent of the IKK-IκK-pathway and suggesting repression of NF-κB transcriptional activity. On the other hand, severely depleting cell GSH (~80%) by 0.5 mM DEM results in partial blockage of TNF-induced nuclear translocation of NF-κB. Interestingly, a decrease of polyubiquitinated RIP1 in TNF-engaged TNFRI signaling complex was found under this condition, which preceded the decrease in activating phosphorylation of IKK and subsequent decrease in IκK activity, IκBα phosphorylation and degradation. Importantly, pretreatment with antioxidant trolox protects against the inhibitory effect of profound GSH depletion on IκK activation and NF-κB nuclear translocation but fails to restore expression of NF-κB target genes.
unmasking the repression of nuclear NF-κB activity. Conclusions: Down-regulation of TNF-induced NF-κB transactivation by moderate GSH depletion (~50%) is IKK-independent and acts on NF-κB transcriptional activity in nucleus, whereas with profound GSH depletion (~80%) the inhibition extends to IKK-pathway activation in a reactive oxygen species dependent manner.

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108 ACTIVATION OF FOCAL ADHESION KINASE AND JNK CONTRIBUTES TO THE EXTRACELLULAR MATRIX MEDIATED DIFFERENTIAL SENSITIVITY TO BILE ACID INDUCED APOPTOSIS IN RAT HEPATOCYTES

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The extracellular matrix modules cell survival. Hepatocytes plated on polylysine (PL) or laminin (LM) undergo more bile acid induced apoptosis than cells plated on type I collagen (CL). Bile acids activate the pro-apoptotic kinase, JNK. Focal adhesion kinase (FAK) which is phosphorylated by integrin engagement modulates extracellular matrix mediated survival in non-hepatic cells. Aim: The goal was to study the role of JNK and FAK in the extracellular matrix mediated differential susceptibility of hepatocytes to bile acid apoptosis. Methods: Rat hepatocytes were plated on CL and LM, which engage integrins, or on PL which promotes nonspecific binding. Kinase activation by glycochenodeoxycholate (GCDC, 50 μM) was determined by immunoblotting for the phosphorylated form of JNK-T183/Y185, and Fak-Y397. Apoptosis was determined morphologically by Hoechst staining of cells treated with GCDC (50 μM) or deoxycholate (100 μM) in the presence or absence of cytochalasin B, 1 μM (a concentration that inhibits FAK but not actin polymerization) or the JNK inhibitor, SP-600125, 5 μM. Results: Compared to hepatocytes on CL, cells on LM and PL had more apoptosis in response to GCDC (1.6 and 1.4 fold, respectively) and DCA (1.3 and 1.9 fold, respectively). In hepatocytes on CL, GCDC increased phosphorylation of JNK p56 and p46, 1.8 and 1.7 fold respectively at 60’, while hepatocytes plated on PL or LM had 58 and 14 fold increases in JNK p56 phosphorylation and 13 and 27 fold increases in JNK p46 phosphorylation, respectively. SP-600125 prevented LM and PL potentiation of DCA induced apoptosis suggesting that JNK activation was pro-apoptotic. An anti-apoptotic role for FAK is supported by the following observations: 1) basal FAK phosphorylation decreased 30 to 40% in hepatocytes on LM and PL compared to hepatocytes on CL; 2) cytochalasin treatment which decreased FAK phosphorylation in hepatocytes on CL increased GCDC induced apoptosis 60 +/- 6.7 % resulting in an amount similar to that seen in cells on PL and LM; 3) GCDC treatment decreased FAK phosphorylation in hepatocytes plated on CL (by contrast 50 μM of the nontoxic bile acid tauroursodeoxycholate had no effect on FAK); and 4) pre-treatment with 20 μM of 4-[4-chlorophenylthio]-2’-O-methyladenosine-3’-5’-cyclic monophosphate, a selective activator of cAMP-GEF’s, restored normal FAK phosphorylation in GCDC treated cells and resulted in cytochalasin sensitive attenuation of GCDC apoptosis. Conclusions: JNK activation augments apoptosis in hepatocytes plated on PL and LM. Decreased FAKA297 phosphorylation as seen in cells treated with bile acids or attached to PL and LM, promotes hepatocyte apoptosis.

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109 IN VITRO AND IN VIVO INHIBITION OF HBV REPLICATION BY PEPTIDE APTAMERS VIA DISRUPTION OF THE HBX-PROTEASOME INTERACTION

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The X protein (HBX) of the hepatitis B virus (HBV) is important for productive infection in vivo. Although this viral protein is not essential for viral replication in vitro, HBV negative for HBX replicates less efficiently in transfected cells. Our previous studies have suggested that the interaction between HBX and the proteasome complex may underlie the pleiotropic functions of HBX (Hu et al. JVI 2006; 8: 1405-1413, Zhang et al. JCI 2001; 108: 1523-1531, Zhang et al. JBC 2000; 257: 15157-15165, Zhang et al. JVI 2004; 78: 4566-4572). We have further demonstrated that inhibition of cellular proteasome activities enhances hepadnavirus replication in an HBX-dependent manner. In this study, we aim to develop potential anti-HBV agents by targeting the functions of HBX using a novel screening process. A modified yeast two-hybrid disruptor system was developed to screen a randomly generated library of peptide aptamers displayed on the bacterial protein Thioredoxin A (TrxA). By screening for a disruption of the interaction between HBX and the proteasome subunit PSMA7, 367 yeast transformants were isolated from 1.5 x 10^7 independent yeast colonies of the peptide aptamer library. On secondary screen against nonspecific interacting pairs, 23 colonies were confirmed to show specific disruption of the HBX-PSMA7 interaction. The peptide aptamers from these colonies were isolated, sequenced, and cloned into a plasmid expression construct for transfection into HepG2 cells for functional studies. Transcriptional activation assays by HBX demonstrated that most of these peptide aptamers interfered with HBX transactivation by decreasing the reporter activities to 20-80% of the control level. When co-transfected with an HBV replication competent construct, most of the peptide aptamers which inhibited HBX transactivation also suppressed HBV viral replication by 50-80%. Very preliminary in vivo murine studies via intravenous injection of one of the peptides suggest a beneficial effect on inhibition of HBV DNA in HBV transgenic mice. Further studies are required to confirm this observation. Our results demonstrate that selection of random peptide aptamers based on disruption of the HBX-proteasome interaction using a modified yeast two-hybrid system may identify peptide aptamers as potential therapeutic drugs for HBV infection. Also, this system may provide a molecular and structural basis for novel antiviral drug development.

Disclosures: The following people have nothing to disclose: Uwais Zaid, Zhensheng Zhang, T. Jake Liang

110 INDUCTION OF ANTIGEN-SPECIFIC HUMORAL AND CELLULAR IMMUNE RESPONSES BY ANTIGEN-PULSED HUMAN BLOOD DENDRITIC CELLS IN HEPATITIS B VACCINE NONRESPONDERS AND PATIENTS WITH CHRONIC HEPATITIS B

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Purpose: Dendritic cells (DCs) loaded with HBV-related antigens induce HBV-specific immunity in HBV-transgenic mice. However,
the utility of antigen-pulsed DCs have not been assessed in human in the context of HBV infection. This study was conducted (1) to prepare hepatitis B surface antigen (HBsAg)-pulsed human blood DCs, (2) to evaluate their safety and (3) to assess their prophylactic and therapeutic efficacies against HBV. Methods: Six hepatitis B vaccine nonresponders, who never developed antibody to HBsAg (anti-HBs) due to immunization with commercial vaccines for 6-9 times and 5 patients with chronic hepatitis B were enrolled in this study. Immature blood DCs were prepared by culturing peripheral blood mononuclear cells with human grade granulocyte-macrophage colony stimulating factor and interleukin-4 for 7 days. HBsAg-pulsed DCs were prepared by culturing immature DCs with HBsAg (Hepavax II, Banyu Pharmaceutical Co, Tokyo, Japan) for 8 hours. Five million HBsAg-pulsed DCs were administered to each individual and parameters of generalized inflammation, liver functions, kidney functions, and autoantibodies were checked at day 0, 1, 3, 7, 14, 28, 90, and 180 and after 2 years. HBsAg-specific T cell responses and anti-HBs were assessed in all subjects before and after administration of HBsAg-pulsed DCs. Results: HBsAg-pulsed human blood DCs expressed DC-related markers such as HLA DR, CD86 and CD40. In vitro, HBsAg-pulsed DCs induced proliferation of HBsAg-specific T cells and anti-HBs from HBsAg-specific B cells. Hepatitis B vaccine nonresponders or patients with chronic hepatitis B did not develop any adverse effects due to administration of HBsAg-pulsed DCs. All vaccine nonresponders and patients with chronic hepatitis B did not show anti-HBs or HBsAg-specific T cells before administration of HBsAg-pulsed DCs. Anti-HBs and HBsAg-specific T cells were detected in the peripheral blood of all vaccine nonresponders within 28 days after single administration of HBsAg-pulsed DCs. HBsAg-pulsed DCs were safe for all patients with chronic hepatitis B. Three and 2 of these patients exhibited HBsAg-specific T cells and anti-HBs in the peripheral blood, respectively. Conclusions: This study has opened a new field of immune intervention strategy against HBV infection in human in which human consumable HBsAg-pulsed DCs have been prepared in vitro and their safety and efficacy have been confirmed in vivo. The principle of this study may be used for development of cell-based prophylactic and therapeutic vaccines against hepatitis C virus and other viruses when those can not be achieved by traditional approaches.

Disclosures: The following people have nothing to disclose: Sk. Md. Fazle Akbar, Osamu Yoshida, Shinya Furukawa, Yoichi Ilias, Norio Horiike, Morikazu Onji

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POWEROUL INHIBITION OF HEPATITIS B VIRUS REPLICATION IN COMBINATION WITH LAMIVUDINE IN VITRO

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Background and aims. CpG oligodeoxynucleotides (CpG ODN) are synthetic agonists of Toll-like receptor 9 (TLR9) and potent inducers of innate and acquired immunity. Antiviral mechanisms of CpG ODN include induction of antiviral and pro-inflammatory cytokines (INF-α, IL-6), activation of natural killer cells (INF-γ), thereby promoting strong TH1, CD8 T cell and humoral responses. Current therapy of chronic hepatitis B infection, either interferon alpha or nucleoside analogs, fail to control hepatitis B virus (HBV) replication in most patients. This limitation is mainly due to the slow kinetic of viral clearance, HBV genetic variability and weak or absent anti-viral cellular immunity. This provides the rationale for the development of immunotherapeutic approaches combining nucleoside analogs and TLR9 agonists to accelerate viral clearance and restore anti-HBV specific immune responses. Our aim was therefore to evaluate the antiviral effect of CpG-induced cytokines combined with lamivudine on HBV replication in vitro. Methods. HepaRG cells were infected with purified HBV virions or transduced with HBV recombinant baculovirus. Peripheral blood mononuclear cells from naive individuals were stimulated with CpG ODN and cytokine secretion (INF-α, INF-γ, IL-6) determined by Elisa. HBV-infected or -transduced HepaRG were treated with CpG-induced cytokines in combination with lamivudine. Antiviral effects were evaluated 48h post-treatment by quantification of antigen secretion (HBsAg, HBeAg) and HBV DNA intermediates of replication by Southern blot. Results. CpG-induced cytokines inhibited HBsAg and HBeAg production from infected HepaRG cells. The antiviral effect was demonstrated by up to 90% inhibition of intracellular HBV DNA intermediates in HBV transduced-cells. More importantly, the combination of CpG-induced cytokines with lamivudine reduced by one hundred fold the lamivudine IC50. Overall, combination was more effective than CpG-induced cytokines or lamivudine treatment alone. Conclusions. CpG-induced cytokines with lamivudine represent a powerful combination to inhibit HBV replication in vitro, most likely by inhibiting different steps of viral replication. The use of TLR9 agonists combined with nucleoside analogs should be evaluated in vivo in order to assess restoration and duration of anti-HBV specific immune responses. The development of such novel immunotherapeutic combinations provides promising approaches to decrease the risk of liver disease progression in chronic hepatitis B patients.

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LONG TERM INHIBITION OF HEPATITIS B VIRUS REPLICATION USING THE NON VIRAL EPISOMAL VECTOR PEPI-1

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Introduction: Aim In the past, it has been shown that RNA interference (RNAi) has the potential to suppress hepatitis B virus (HBV) replication. For further gene therapy an optimal vector system free of common side effects as for example induction of mutagenesis has still to be found. The aim of this study was to test the non viral episomal vector system pEPI-1 as a tool for inhibition of HBV replication. Methods Recombinant vectors pEPI/HBV1 and pEPI/NonSense were constructed and transfected into HepG2.2.15 cells. After selection of transfected clones, cells were grown for 3 months in the absence of selection pressure and harvested at 2 and 12 weeks. The episomal status of the plasmid was confirmed by Southern analysis. The level of HBsAg mRNA was measured by RTPCR and the concentration of HBsAg in the supernatant was determined using ELISA. Results Transfection of HepG2.2.15 cells with pEPI/HBV1 resulted in more than 90% suppression of HBsAg mRNA 2 weeks after transfection compared with the control plasmid pEPI/NonSense. This effect was even more pronounced after 12 weeks, when almost no HBsAg mRNA could be detected. Likewise, the secretion of HBsAg was inhibited by more than 95%. Also the number of DNA copies within the culture medium was significantly decreased. Conclusion The non viral episomal vector system pEPI-1 allows efficient long term
suppression of HBV replication in the absence of selection pressure and without the risk for insertional mutagenesis or expression of additional viral proteins.

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113 ADENO-ASSOCIATED VIRUS REP78 PROTEIN INHIBITS HUMAN HEPATITIS B VIRUS REPLICATION IN HUMAN CELLS BY BINDING TO X PROMOTER AND C PROMOTER OF HBV
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Background and aims: REP78, the large rep gene product of adeno-associated virus (AAV), is a highly multifunctional protein which is required for AAV DNA replication and the trans-regulation of AAV gene expression. Apart from these essential functions prerequisite for the life cycle of AAV, REP78 is able to inhibit the replication and gene expression of some DNA virus by binding to the promoter, such as HPV and HIV. The aim of this study was to determine whether REP78 can bind to the promoter of HBV DNA and then inhibit the replication of HBV DNA. Methods and Results: Here, we demonstrated that REP78, as a chimeric with the malarious binding protein, binds to both the full length of HBV X promoter and the full length of HBV C promoter, as analyzed by electrophoretic mobility shift assay. To map the key region of binding, sequentially smaller substrates from the full length of promoter were synthesized and tested for recognition by REP78. It is demonstrated that the binding between REP78 and the full length of HBV X promoter was specific, but not with REP78 and the smaller substrates from the full length. It was therefore possible that multiple binding sites were needed or the secondary structure of the full length of HBV X promoter was needed for specifically binding with REP78. The smaller substrates from the full length, B1, the active region of HBV C promoter can bind to REP78 specifically. Furthermore, investigating the biological implications of these interactions, REP78 inhibited both the HBV X promoter and HBV C promoter activity in vitro transcription assays in Hela nuclear extracts, and the inhibition of X promoter was stronger than that of C promoter. In addition, to investigate whether the binding between REP78 and HBV DNA promoters could inhibit the replication of HBV DNA, we constructed the plasmid containing the AAV2 rep78 gene and transfected the plasmid to the HepG2.2.15 cell line, the concentration of HBV cccDNA was decreased indicating that the replication of HBV was inhibited. Conclusions: The ability of REP78 to interact with both the HBV X promoter and HBV C promoter and down-regulate the corresponding transcription and replication suggests the mechanism by which AAV may modulate the HBV life cycle.

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The following people have nothing to disclose: Tianhui Liu, Hong You, Min Cong

114 CONSTRUCTION OF THE SHRNA PLASMID OF HUMAN FGL2 PROTHROMBINASE GENE AND ITS EFFECT ON HFG2 EXPRESSION IN VITRO
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Background and aims: Our previous studies have shown an anti-sense plasmid to mouse fgl2 prothrombinase (mfgl2) gene significantly reduced mfgl2 gene expression in vivo, markedly alleviated inflammatory infiltration, fibrin deposition and hepatocyte necrosis, prolonged the survival time period and elevated the survival rate in Balb/cj mice with murine hepatitis virus type 3 (MHV-3) induced fulminant hepatitis. This study was designed to explore the opportunity of RNA interference technique in the inhibitory application of human fgl2 prothrombinase (hfgl2) expression, which has been reported to be involved in a variety of disease development including fulminant viral hepatitis, acute rejection of allogeneic/xenogeneic transplantation and fetal loss syndrome. Methods: A plasmid named p-hfgl2shRNA complementary to the sequence of hfgl2 was constructed, meanwhile irrelevant shRNA plasmid with a random combination of the hfgl2shRNA sequence was used as control. A plasmid named pEGFP-hfgl2 expressing hfgl2-EGFP fusion protein was also constructed for the screening of the effect of p-hfgl2shRNA on the hfgl2 expression. By cotransfection of p-hfgl2shRNA and pEGFP-hfgl2 or pcDNA3.1-hfgl2 expression construct into CHO cells, the inhibition of hfgl2 expression by hfgl2shRNA was analyzed by direct observation through fluorescent microscopy, FACS, real time PCR and immunohistochemistry staining. Results: The experiments showed the significant inhibitory effect of p-hfgl2shRNA on hfgl2 expression at 48h post-transfection by observation of green fluorescent cells, FACS, real time PCR and immunohistochemistry staining as well as CHO cell lines with the inhibitory efficiency reached as high as 85.5%. Conclusions: The study demonstrated that the construct of p-hfgl2shRNA successfully interfered hfgl2 expression in vitro and this provides the foundation for further investigation of this construct’s application in vivo and further more as a therapeutic strategy for a targeting intervention in the disease control to which the gene fgl2 contributed. This work was supported by the NSFC30571643, 30672380 and National Key Basic Research Program of China(2005CB522901).

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115 CHARACTERIZATION OF HEPATITIS C RNA CONTAINING PARTICLES FROM HUMAN LIVER BY DENSITY AND MORPHOLOGY
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Background & Aims: Hepatitis C RNA containing particles found in vivo are heterogeneous in size and density but detailed characterization of these particles has been restricted by the low titre of HCV in human serum. Our group has identified a liver explant from a patient in which the virus titre was unusually high, allowing detection of HCV structural proteins and full length HCV RNA genome by Western and Northern blotting. Methods: Native HCV RNA containing particles were characterized by density on iodixanol gradients and by size using gel filtration. The morphology of particles was evaluated by thin section electron microscopy from gradient fractions treated with glutaraldehyde. Triglyceride, cholesterol and phospholipid in gradient fractions were quantitated by enzymatic assays. Endogenous liver proteins and viral proteins were characterized by mass spectrometry and Western blotting. Results: Both positive and negative strand HCV RNA in liver macerate
was found in particles with density ≤1.08 g/ml. These low density fractions also contained the highest concentration of cholesterol and phospholipid in the gradient, suggesting co-distribution of viral RNA with cholesterol rich membranes. After further fractionation by size on Superose columns calibrated with purified human lipoproteins, HCV structural proteins core, E1, E2 and non-structural proteins NS3, NS4A and NS5A were found in the same peak as endoplasmic reticulum proteins and very low density lipoprotein. After fractionation with Toyopearl, a gel filtration medium capable of separating larger particles, 78% of HCV RNA eluted with mean diameter 110 nm. These particles had low positive/negative strand ratio, consistent with virus replication complexes. Particles with a high positive/negative strand ratio, comprising 8% of total viral RNA, eluted with mean diameter 55 nm, consistent with virus particles. Electron microscopy of low density fractions revealed clusters comprising up to 8 particles surrounded by a double membrane of circa 110 nm diameter, some of which had internal structures in the form of a pentagon or hexagon. These particles had the the appearance of flavivirus replication complexes. In the control, consisting of low density iodixanol fractions from an HCV negative liver, clusters with up to 4 lipid particles with diameters below 80 nm were observed. However, these particles did not contain internal structures and were less frequently observed. Conclusions: HCV particles and HCV replication complexes from human liver have equally low density, but different diameters. Structures resembling flavivirus replication complexes in liver were found in clusters of vesicles.

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The following people have nothing to disclose: Soren U. Nielsen, Margaret Bassendine, Barnabas J. King, Dermot Neely, Geoffrey L. Toms

116 DETERMINANTS FOR MEMBRANE ASSOCIATION OF THE HEPATITIS C VIRUS NS3-4A COMPLEX

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Hepatitis C virus (HCV) replicates its genome in a membrane-associated replication complex. NS3-4A, a multifunctional protein harboring serine protease and RNA helicase activities, is an essential component of this complex and a prime target for antiviral intervention. We previously demonstrated that NS3 is targeted to membranes via interaction with its cofactor NS4A. In extension of this work, we now show that the N-terminal 21 amino acids of NS4A form a transmembrane alpha-helix required for integral membrane association of the NS3-4A complex. Selected amino acid substitutions in the NS4A transmembrane segment abrogated HCV RNA replication without interfering with membrane association of NS3-4A, suggesting that this segment, similar to the membrane segments of NS5A and NS5B, has additional functions and may be involved in intramembrane protein-protein interactions essential for the assembly of a functional replication complex. Surprisingly, unprocessed NS3-NS4A was found to peripherally associate with membranes. Analyses of green fluorescent protein (GFP) fusion proteins and NS3-4A single chain constructs revealed that an amphipathic alpha-helix at the N-terminus of NS3 was responsible for this peripheral membrane association. In conclusion, membrane association of HCV NS3-4A is conferred by two determinants, an amphipathic alpha-helix in NS3 and the N-terminal transmembrane segment of NS4A. These results allow us to propose a mechanistic model for the membrane association process of NS3-4A and the final topology of NS3-4A on the membrane. This model has important implications for the functional architecture of the HCV replication complex, proteolytic targeting of host factors, and drug design.

Disclosures:
The following people have nothing to disclose: Jan Martin Berke, Volker Brass, Roland Montserret, Hubert E. Blum, Francois Penin, Darius Moradpour

117 PRODUCTION OF INFECTIOUS HEPATITIS C VIRUS IN PRIMARY CULTURE OF HUMAN ADULT HEPATOCYTES

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Background and Aim. Hepatitis C research has been heavily handicapped by the lack of relevant cell culture systems supporting the complete life cycle of hepatitis C virus (HCV). Primary cultures of human adult hepatocytes provide a relevant model for HCV natural host cells but do not support production of progeny virions upon infection with sera from HCV-infected patients. Productive infections with the HCV strain JFH1 have been achieved recently in subpopulations of the Huh-7 cell line, but these transformed cells are only distantly related to differentiated hepatocytes. These considerations prompted us to investigate whether JFH1 can be used to achieve productive infection in normal human adult hepatocytes maintained in primary culture during two weeks. Methods. Human hepatocytes isolated from liver resection by collagenase perfusion were seeded at high density on collagen-coated plates, and then infected with JFH1 produced from Huh-7.5.1 cells. Expression of liver phenotypic markers was checked by RT-qPCR or flow cytometry analysis. Replication of HCV genome was evaluated by strand-specific RT-qPCR. To assess production of progeny virions into the culture supernatants, HCV core antigen was measured with an ELISA assay, HCV RNA was quantified with a standardized viral load assay used for routine clinical follow-up of patients, and the infectious titer was evaluated by focus formation upon inoculation into naive Huh-7.5.1 cells. Results. Hepatocytes expressed albumin, cytochrome P450 isofoms CYP2E1 and CYP3A4, and CD81 over the time of the experiments. HCV genome replicated efficiently, as shown by the co-distribution of viral RNA with cholesterol rich membranes. In the control, consisting of low density iodixanol fractions from an HCV negative liver, clusters with up to 4 lipid particles with diameters below 80 nm were observed. However, these particles did not contain internal structures and were less frequently observed. Conclusions: HCV particles and HCV replication complexes from human liver have equally low density, but different diameters. Structures resembling flavivirus replication complexes in liver were found in clusters of vesicles.

Disclosures:
The following people have nothing to disclose: Jan Martin Berke, Volker Brass, Roland Montserret, Hubert E. Blum, Francois Penin, Darius Moradpour

117 PRODUCTION OF INFECTIOUS HEPATITIS C VIRUS IN PRIMARY CULTURE OF HUMAN ADULT HEPATOCYTES

Philippe Podevin1,2, Sylvie Lagaye1, Arnaud Carpentier3, Lynda Aoudjehane1, Matthieu Carrière3, Sakina Zaidi1, Jean-François Méritel1,4, Marlène Deux5, Olivier Scatton2, Takaji Wakita6, François-Loïc Cosset5, Filoména Conti2, Arielle R. Rosenberg1,4, Yvon Calmus3,1 Equipe Virus de l’Hépatite C, Institut Cochin, Paris, France; 2Pôle médico-chirurgical d’hépato-gastro-entérologie, Groupe Hospitalier Cochin, Paris, France; 3UPRES 1833, Université Paris Descartes, Paris, France; 4Service de Virologie, Groupe Hospitalier Cochin, Paris, France; 5U 758, Inserm, Lyon, France; 6Department of Virology II, National Institute of Infectious Diseases, Tokyo, Japan

Background and Aim. Hepatitis C research has been heavily handicapped by the lack of relevant cell culture systems supporting the complete life cycle of hepatitis C virus (HCV). Primary cultures of human adult hepatocytes provide a relevant model for HCV natural host cells but do not support production of progeny virions upon infection with sera from HCV-infected patients. Productive infections with the HCV strain JFH1 have been achieved recently in subpopulations of the Huh-7 cell line, but these transformed cells are only distantly related to differentiated hepatocytes. These considerations prompted us to investigate whether JFH1 can be used to achieve productive infection in normal human adult hepatocytes maintained in primary culture during two weeks. Methods. Human hepatocytes isolated from liver resection by collagenase perfusion were seeded at high density on collagen-coated plates, and then infected with JFH1 produced from Huh-7.5.1 cells. Expression of liver phenotypic markers was checked by RT-qPCR or flow cytometry analysis. Replication of HCV genome was evaluated by strand-specific RT-qPCR. To assess production of progeny virions into the culture supernatants, HCV core antigen was measured with an ELISA assay, HCV RNA was quantified with a standardized viral load assay used for routine clinical follow-up of patients, and the infectious titer was evaluated by focus formation upon inoculation into naive Huh-7.5.1 cells. Results. Hepatocytes expressed albumin, cytochrome P450 isofoms CYP2E1 and CYP3A4, and CD81 over the time of the experiments. HCV genome replicated efficiently, as shown by the co-distribution of viral RNA with cholesterol rich membranes. In the control, consisting of low density iodixanol fractions from an HCV negative liver, clusters with up to 4 lipid particles with diameters below 80 nm were observed. However, these particles did not contain internal structures and were less frequently observed. Conclusions: HCV particles and HCV replication complexes from human liver have equally low density, but different diameters. Structures resembling flavivirus replication complexes in liver were found in clusters of vesicles.
Disclosures:
The following people have nothing to disclose: Philippe Podevin, Sylvie Lagaye, Arnaud Carpentier, Lynda Aoudjehane, Matthieu Carrière, Sakina Zaidi, Jean-François Mératet, Marlene Dreux, Olivier Scatto, Takaji Wakita, François-Laï Cossel, Filéména Conk, Arielle R. Rosenberg, Yvan Calmus

118 ANALYSIS OF NS5A REGION IMPORTANT FOR HEPATITIS C VIRUS PARTICLE PRODUCTION
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Background/Aim: NS5A is essential for HCV genome replication and is considered to be an RNA-binding protein. Its C terminal region is poorly conserved among different genotypes and tolerates deletions as well as insertion of large heterologous sequences such as green fluorescent protein (GFP). Although insertion of GFP into NS5A allows the visualization of replication and is applicable to a study of viral replication dynamics in living cells as reported previously, transfection of Huh-7 cells with the genotype 2a isolate JFH-1 genome that carries GFP-inserted NS5A gene results in a marked decrease in the virus level in culture medium compared to the wild type (Schaller et al, J Virol 2007;81:4591-4603). The aim of this study is to elucidate the involvement of NS5A in virus assembly and maturation. Methods: Effects of NS5A mutations on HCV production and RNA replication were analyzed in the context of JFH-1 genome as well as subgenomic luciferase-reporter replication. The binding between NS5A and HCV structural proteins was examined by co-immunoprecipitation assay. Results: From site directed mutagenesis analyses, we identified serine residues in the NS5A region important for the generation of HCV particles; substitutions of some serine residues to alanine residues in the NS5A region did not affect the viral RNA replication but markedly reduced the amount of extracellular core protein (nearly 20-fold decrease compared to the wild type). Co-immunoprecipitation analyses revealed that HCV core interact with wild-type NS5A but does not interact with NS5A mutants. Conclusions: The results suggest that the determinants of RNA replication and virus assembly can be dissected at a molecular level and that the interaction of NS5A with HCV core can be important for viral particle production because there was a correlation between the ability of NS5A to interact with HCV core and to participate in the virus production. This study may provide a valuable insight into the elucidation of the mechanism of HCV virion assembly and maturation. Disclosures: The following people have nothing to disclose: Takahiro Masaki, Takaji Wakita

119 INHIBITION OF HCV 5’NCR AND CORE EXPRESSION USING SMALL HAIRPIN RNA MEDIATED THROUGH AN NEW GENE CARRIER HPHA
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Objective: To develop a suitable delivery system of siRNA as a therapeutic agent of hepatitis C virus (HCV) infection, a new noviral transgene carrier —Recombinant archaeal histone from the hyperthermophile Pyrococcus horikoshii OT3 (HPHa) for transfection of short hairpin RNA (shRNA) plasmid DNA into HL-7702 cells was studied. Methods: HCV-specific siRNA expression plasmid targeted HCV 5’NCR and C region (Psilencircle C) were constructed by SilenCircleTM RNAi Transcription Kit. Each of the above plasmids and plasmid pCMV/TP-NRCA-luc containing HCV 5’NCR and partial core region as well as luciferase sequences were transfected into HL-7702 cells by LipofectamineTM2000 and HPHA respectively. The inhibiting effect of shRNA was determined by luciferase activity assay and Western blot at 72 hours after coinflection. Gel electrophoresis mobility shift assay and MIT methods were used to demonstrate the affinity of HPHA to DNA and the toxicity of HPHA to cells. Results: Gel electrophoresis mobility shift assay demonstrated that the purified HPHA had high affinity to DNA and that the HPHA could increase plasmid electrophoresis mobilities at a proper HPHA/DNA mass ratio (3:1). Compared with LipofectamineTM 2000, HPHA didn’t have any affect on growth of the cells in a low concentration (20µg/ml), however, LipofectamineTM 2000 could cause cell viability coming down obviously. Cell death appeared at concentrations of HPHA more than 90µg/ml. The toxicity of 900µg/ml HPHA to HL-7702 cells was similar with that of 40µg/ml LipofectamineTM 2000. The maximum amount of HPHA in this study was only 9µg per well for 24-well plate in transfection indicating that HPHA was non-toxic to cells. Transfer of HCV-specific shRNA expression plasmids by HPHA could inhibit the expression of HCV 5’NCR and core protein showing a similar effect with LipofectamineTM2000. Psilencircle A, B or D transfected by HPHA could inhibit the expression of HCV 5’NCR and core protein in HL-7702 cells with inhibiting rates of 58%, 62%, 54%, respectively. The transfection efficiency of HPHA in HL-7702 cells with 10% fetal calf serum (FCS) was similar with that without FCS. Conclusion: HCV-specific shRNA mediated by HPHA can obviously inhibit the expression of HCV 5’NCR and core protein. HPHA exhibit effects of transfection in safety and high affinity to DNA even in media with 10% FCS, it is therefore suggest that HPHA probably plays an important role in RNAi-based gene therapy in vivo.

Disclosures: The following people have nothing to disclose: Yanhua Ding, Junqi Niu, Runping Gao, Di Wu

120 INTERACTION OF APOLIPROTEIN E WITH STEM LOOP E1 OF THE 3’ END OF HEPATITIS C VIRUS MINUS STRAND RNA
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Formation of the hepatitis C virus (HCV) replication complex represents a key step in the viral life cycle. We have previously demonstrated that the last 220 nucleotides of the HCV 3’ minus strand fold into five stable stem-loops forming domain I (Schuster et al. 2002, J. Virol, 76:8058-68). Comparison of the replication initiation sites on minus (−) and plus (+) strands revealed common structural features including a marked sequence homology between stem loop E1 (SLE1) of (−) strand and the (+) strand stem loop II. Aiming to investigate the function of SLE1 for the viral life cycle and virus-host interactions, we screened cellular proteins interacting with SLE1 using a yeast three-hybrid assay with SLE1 as bait and a human liver cDNA library as prey. A reverse mutant of the homologous sequence was used as a negative control to confirm specificity of the interaction. The yeast three-hybrid screen identified apolipoprotein E (Apo E) as a hepatocyte protein interacting specifically with SLE1 of HCV (+) strand. To investigate the functional re-
of this interaction, we silenced Apo E expression by siRNAs in Huh7.5 cells transfected with HCV JFH-1 full-length RNA. Silencing of Apo E expression resulted in a marked inhibition of infectious particle production without affecting the level of viral replication. These results indicate that SLE1 may recruit ApoE to the viral replication complex and that this recruitment may play a role for completion of the viral life cycle resulting in production of infectious particles.

Disclosures:
The following people have nothing to disclose: Wagané J. Benga, Maria Dimitrova, Sophie Krieger, Thomas F. Baumert, Catherine Schuster

121 THE CC CHEMOKINE RANTES (CCL5) IS AN IMPORTANT MEDIATOR OF LIVER FIBROSIS IN MICE AND HUMANS
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BACKGROUND: Chemokines are inflammatory mediators during acute and chronic liver injury. The chemokine RANTES (CCL5) directs lymphocytes to inflamed liver through interaction with the chemokine receptors CCR1 and CCR5. Notably, RANTES has also been shown to directly activate hepatic stellate cells in vitro. This functional duality defines RANTES as an interesting target for antifibrotic therapies. Therefore, the aim of the study was to analyze the role of RANTES in murine and human liver fibrogenesis. METHODS: We performed a genetic analysis with two haplotype tagging RANTES SNPs from the HapMap project in 260 individuals with chronic hepatitis C (HCV). Furthermore, the intrahepatic mRNA expression of RANTES was analysed by quantitative RTPCR in 54 human liver samples from HCV patients and in mice treated with carbon tetrachloride (CCl4). We also challenged RANTES-/- mice and wild-type littermates with CCl4 for 6 weeks to induce chronic liver damage. Fibrosis was analyzed by staging of histology after Sirius red staining and hydroxyproline measurement in liver samples. Additionally, the intrahepatic mRNA expression of fibrosis related genes (Collagen Iα1, TIMP-1, MMP-9) and interleukin-10 (IL-10) was determined by quantitative RTPCR. RESULTS: The genetic analysis revealed an association of RANTES haplotypes with the degree of liver fibrosis due to HCV (P = 0.01 by permutation testing). In human HCV infected liver, RANTES mRNA expression was positively correlated with higher stages of liver fibrosis. Similarly, RANTES mRNA expression was 4-fold increased in C57BL/6 mice treated with CCl4 for 6 weeks (P = 0.01). Genetic deletion of RANTES in fibrosis prone mice led to a significantly reduced degree of fibrosis as assessed by liver histology and hydroxyproline content of liver samples (P = 0.01). RANTES-/- mice had significantly reduced mRNA expression of Collagen Iα1 (P = 0.02), but elevated mRNA expression of MMP-9 (P = 0.05) compared to wild-type littermates. Inhibition of fibrogenesis was furthermore associated with an increased mRNA expression of IL-10 in RANTES-/- mice (P = 0.003). CONCLUSIONS: The results identify the chemokine RANTES as an important mediator of liver fibrogenesis in mice and humans. The attenuation of fibrosis in RANTES-/- mice sets the stage for the evaluation of RANTES antagonistic strategies for the treatment of chronic liver diseases.

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The following people have nothing to disclose: Anna Rueland, Mirko Moreno Zaldivar, David Scholten, Nikolaus Gassler, Christian Weber, Christian Trautwein, Hermann E. Wasmuth

122 FUNCTIONAL CONSEQUENCES OF SINGLE NUCLEOTIDE POLYMORPHISMS OF TOLL-LIKE RECEPTOR 4 ON HEPATIC STELLATE CELLS
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Background & Aims: A gene-centric functional genome scan in patients with chronic HCV yielded a 7 gene variant Cirrhosis Risk Score (CRS) signature that can predict the risk of developing cirrhosis (Hepatology, 2007, E-pub April 26). Among the variant genotypes is the CC genotype (Thr399) in Toll-like receptor 4 (TLR4) that is associated with higher risk of fibrosis. In addition, both the Asp299Gly and the Thr399Ile SNPs of the human TLR4 gene lead to missense mutations in the extracellular domain of TLR4 that confer lipopolysacharide (LPS) hypo-responsiveness. Because activated hepatic stellate cells (HSCs) express inflammatory cytokines in response to LPS via TLR4, we examined the impact of these SNPs on HSC biology. Methods & Results: cDNAs expressing either human wild type TLR4 (WT; Thr399), Asp299Gly, Thr399Ile, or dual SNPs (Asp299Gly & Thr399Ile) were transduced by lentivirus into either a human HSC line (Hx2), or into immortalized mouse HSC lines that were either TLR4 WT, TLR4-/-, or MyD88-/- (an adaptor protein of TLR4). LPS stimulation up-regulated inflammatory & chemotactic cytokine mRNAs (i.e., MCP-1, IL-1β, TNF-α) and MCP-1 secretion in both Hx2 (which express WT TLR4) and WT mouse HSCs; this was associated with enhanced activation of a nuclear factor kappa-B (NF-kB)-responsive reporter, and phosphorylation of ERK. LPS responsiveness was abrogated by siRNA-mediated TLR4 knockdown in LX-2 cells, and was absent in TLR4-/- and MyD88-/- mouse HSCs. Reconstitution of human TLR4 expression restored LPS responsiveness and NF-kB reporter activity in TLR4-/- but not MyD88-/- HSCs. However, TLR4-/ HSCs transduced with either TLR4 Asp299Gly and/or Thr399Ile cDNAs were hypo-responsive to LPS compared to cells transduced with WT TLR4 cDNA. In addition, as reported by others (Seki et al, Hepatology 4:225A), LPS stimulated a TLR4- and MyD88-dependent down-regulation of BAMBI (an inhibitory TGF-beta pseudoreceptor) in mouse HSCs. Spontaneous apoptosis was greatly increased in TLR4-/- and MyD88-/- mouse HSCs. Conclusion: TLR4-MyD88 signaling mediates an LPS-stimulated inflammatory phenotype of activated HSCs and contributes to cell survival. Importantly, these data provide a mechanistic link that explains how specific TLR4 SNPs may confer risk of fibrosis progression in patients with chronic HCV, and further validate our functional SNP genome scan approach to identifying fibrosis risk genes.

Disclosures:
The following people have nothing to disclose: Jinsheng Guo, Johnny C. Loke, Feng Zheng, Feng Hong, Steven Yea, Hongjin Huang, Scott L. Friedman
HEPATOCYTE GROWTH FACTOR SUPPRESSES COLLAGEN GENE TRANSCRIPTION VIA NUCLEAR EXPORT OF SMAD3 WITH GALACTIN-7

Yutaka Inagaki, Miwa Kushida, Goshi Shiota, Ichiro Kuwabara, Johbu Itoh, Yun Yu Hong, Sachie Nakao, Reiichi Higashiyama, Tadashi Moro, Isao Okazaki, Toshiyuki Mikami, Toru Kimura, Kiyoshi Higashi

Background/Aim: Hepatocyte growth factor (HGF) and transforming growth factor-β (TGF-β) regulate a number of cellular functions, and often act antagonistically against each other. For example, TGF-β is a potent factor accelerating liver fibrosis, whereas HGF treatment prevents its progression. In the present study, we examined the molecular mechanisms by which HGF counter-represses TGF-β-stimulated gene transcription of type I collagen, the major extracellular matrix component in fibrotic liver. Methods: Effects of HGF on α2(I) collagen gene (COL1A2) transcription were examined by using transgenic mice harboring a COL1A2 promoter sequence and cultured hepatic stellate cells (HSC) transfected with COL1A2 promoter/luciferase reporter constructs. Subcellular localization of Smad3 was analyzed by an immunofluorescence study, and a mass spectrometric analysis was employed to identify immunoprecipitated proteins with anti-phosphoSmad3 antibodies. Results: Overexpression of HGF significantly suppressed COL1A2 promoter activation in liver tissue induced by carbon tetrachloride administration, and inhibited COL1A2 transcription in cultured HSC. A mass spectrometric analysis identified galectin-7 as one of the proteins interacting with phosphorylated Smad3. HGF enhanced the interaction between nuclear phosphorylated Smad3 and galectin-7, and subsequently accelerated their nuclear export independently of mitogen-activated protein kinase activation. Transfection of cells with galectin-7 small interfering RNA inhibited nuclear export of Smad3 following HGF treatment, and abolished the effect of HGF suppressing COL1A2 transcription. On the other hand, overexpression of galectin-7 significantly suppressed both basal and TGF-β-stimulated COL1A2 expression. Conclusion: These results provide a new insight into the function of galectin-7 as a transcriptional regulator via its interaction with nuclear Smad3 and a molecular basis on the anti-fibrotic effects of HGF.

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ACIDIC SPHINGOMYELINASE-MEDIATED CATHEPSINS REGULATION MODULATES THE FIBROGENIC POTENTIAL OF HEPATIC STELLATE CELLS

Anna Moles, Jose C. Fernandez-Checa, Montserrat Mari; Liver Unit, Hospital Clinic, Barcelona, Spain

Hepatic stellate cells (HSC) are the primary cell type responsible for the excess extracellular matrix production during fibrogenesis. During chronic liver injury HSC undergo a phenotypic transformation or activation promoting the fibrogenic response of these cells. Sphingolipids in general and ceramide, in particular, serve as second messengers for transducing signals to the cell interior and trigger specific cellular responses. Activation of acidic sphingomyelinase (ASMase), an endosomal/lysosomal enzyme that generates ceramide upon sphingomyelin hydrolysis, is involved in apoptotic hepatocellular death by different stimuli such as stress and death-ligands. However, the role of ASMase in HCS biology and fibrogenesis has not been previously examined and hence, the aim of this study was to analyze if ASMase modulates HSC activation in vitro and their fibrogenic potential. Methods: HSC isolated from wild type and ASMase−/− mice (C57BL/6) and LX2, a human derived HSC, were cultured in plastic (6-10 days). For in vivo fibrosis induction, CCI4 was administered at a dose of 0.5µl/gr body weight for 4-6 weeks. Collagen deposition was analyzed by Sirius red staining. HSC activation was determined by real-time PCR of α-SMA and TGF-β. α-SMA and cathepsins B and D protein expression were determined by western blot. Cathepsin B activity was determined fluorimetrically. Results: HSC from ASMase−/− mice displayed an increase in α-SMA mRNA (aprox. 7-fold), COL1A1 (2.5-fold) compared to wild type HSC after 2 days in culture. TGF-β expression was also increased (5-fold) in ASMase−/− after 6 days in culture compared to HCS isolated from wild-type mice. Moreover, an increase in cathepsin B (catB) and D, endosomal/lysosomal cystein-proteases involved in tumour growth and apoptosis, was observed during HSC activation. Of note, ASMase−/− HSC exhibited enhanced catB expression and activity vs wild-type HSC that correlated with a stimulated proliferation rate. A cathepsin B inhibitor (CA-074 Me, 10 µM) or transfection with catB siRNA decreased the levels of α-SMA and TGF-β mRNA in both ASMase−/+ and ASMase−/− HSC activated in vitro, and also in the LX2 cells. Moreover, after induction of fibrosis in vivo, ASMase−/− livers displayed increased Sirius red-stained areas, compared to
ASMsase+/+, with increased presence of catb that co-localized with α-SMA positive cells. Conclusion: These findings underscore a functional relation between ASMsase and cathepsins. The modulation of ASMsase and/or catB activity regulates the fibrogenic potential of HSC and can be putative targets for therapy in the treatment of liver fibrogenesis.

Disclosures:
The following people have nothing to disclose: Anna Moles, Jose C. Fernandez-Checa, Montserrat Mari

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GP120 INDUCES DIRECTIONAL MIGRATION OF HUMAN HEPATIC STELLATE CELLS: A LINK BETWEEN HIV INFECTION AND LIVER FIBROGENESIS
Raffaele Bruno1, Sara Galastri2, Fabio Marra2; 1University of Pavia, Pavia, Italy; 2University of Florence, Florence, Italy

With the advent of antiretroviral therapy, increased liver-related mortality has been observed in HCV/HIV coinfected individuals. These patients have a faster progression of fibrosis, but the underlying molecular mechanisms are only partially understood. Hepatic stellate cells (HSC) are the key cell type involved in the progression of liver fibrosis. Exposure of HSC to HIV proteins results in induction of pro-fibrogenic actions, but no information is currently available on the possible role of HIV proteins. gp120 is a HIV envelope protein that regulates biological functions in different cell types. Aim of the present study was to establish whether HIV-gp120 has the ability to impact the biology of HSC. HSC were isolated from normal human liver tissue and culture-activated on plastic dishes. Cells were used between the 4th and the 9th passage, after complete transition to a myofibroblast-like phenotype. Different biologic action of HSC were evaluated after exposure to increasing concentrations of HIV-gp120 (10-1000 ng/ml). HSC migration was evaluated using modified Boyden chambers. Secretion of cytokines and type I procollagen was measured by ELISA. The HIV-gp120 has been shown to bind surface molecules belonging to the chemokine receptor superfamily. HSC have been previously shown to express CCR5, which binds M-tropic gp120. When exposed to two different types of M-tropic gp120 (CN54 or SF162), a significant increase in HSC chemotaxis was observed, as evaluated in modified Boyden chambers (see Table). Incubation of HSC in the presence of HIV-gp120 for 24-48 hours also resulted in increased secretion and gene expression of the pro-inflammatory chemokine, MCP-1, in the conditioned medium. In addition, collagen secretion in culture supernatant was modestly increased after a 48-hour incubation. These effects were accompanied by a time-dependent increase in phosphorylation of p38MAPK, that transduces pro-inflammatory stimuli in HSC and by activation of Akt, implicated in cell migration. Conclusions: This study show that HIV-gp120 modulates different aspects of HSC biology, including stimulation of chemotaxis, and increased expression of collagen and pro-inflammatory chemokines. These results suggest a direct role of HIV proteins in the process of liver fibrogenesis.

Effects of recombinant gp120 or PDGF (positive control) on migration of HSC in Boyden chambers.

<table>
<thead>
<tr>
<th>Mean±SD of 3 independent experiments</th>
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<tbody>
<tr>
<td>Gp120 (ng/ml)</td>
</tr>
<tr>
<td>-fold over control</td>
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<tr>
<td>P vs. control</td>
</tr>
</tbody>
</table>

Disclosures:
The following people have nothing to disclose: Raffaele Bruno, Sara Galastri, Fabio Marra

126
APOLIPOPROTEIN AI REGULATORY PROTEIN 1 (ARP-1) COORDINATES PROFIBROGENIC RESPONSES DURING LIVER FIBROSIS
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Background: Liver fibrosis represents a general wound healing response elicited by different etiologic causes. The cells mainly in charge of this process are liver myofibroblasts, mesenchymal cells responsible for the synthesis of the extracellular matrix proteins. The main source of fibrogenic myofibroblasts are Hepatic Stellate Cells (HSC). During the last few years it has been given much value to the role that nuclear receptors play in impairing the HSC trans-differentiation process: indeed it’s well known that PPARg maintains HSC in the quiescent state, and inhibit collagen gene transcription. However, the detailed molecular mechanisms of this process are still unclear. ARP-1 is an orphan receptor capable of homo and heterodimerization with other nuclear receptors and is expressed during embryogenesis mainly in the mesenchimal, vascular and cardiac tissues. The aim of this study is to evaluate the role of ARP-1 nuclear receptor on liver fibrosis. Methods: ARP-1 expression has been investigated on CCl4 treated C57/Bl6 mice by immunofluorescence experiments. Murine and human HSC were isolated following standard procedures. To evaluate the role of ARP-1 in HSC activation and liver fibrosis, cultured HSC where transfected with an ARP-1 expression plasmid with specific reporter plasmids for ARP-1 and PPARg. Cell migration and proliferation as well as MMP2 activity and collagen gene expression assays, were performed. Proteomics profile of ARP-1 overexpressing or silenced HSC was performed with Fluorescence 2-D Difference Gel Electrophoresis (DIGE). The transcriptional effect of ARP-1 on collagen 1a2 (Col1A2) expression was evaluated on ARP-1 expressing-HSC transfected with various fragments or the full Col1A2 promoter; CHIP and EMSA experiments were also performed. Results: ARP-1 expression is increased during HSC activation both in vivo and in vitro. Increased expression is correlated with a specific inhibition of PPARg expression and transcriptional activity. In HSC cell culture, ARP-1 induces both MMP2 and Col1A2 expression and significantly increases cell invasiveness; these effects are reverted by dominant negative ARP-1 and by specific siRNA. Moreover ARP-1 increases the transcriptional activity of Col1A2 promoter. To further investigate this effect, we performed experiments with fragments of various length of Col1A2 promoter as well as CHIP and EMSA assays, identifying three regions containing putative ARP-1 responsive elements. Conclusions: ARP-1 expression is increased during HSC activation and regulates HSC invasiveness and collagen synthesis. These data indicate that ARP-1 is an important regulator of liver fibrogenesis.

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The following people have nothing to disclose: Elisabetta Ceni, Simone Polvani, Tommaso Mello, Laura Cioni, Francesca Buccoliero, Barbara Ottanelli, Francesca Lisi, Stefano Milani, Andrea Galli
LB1
PROLONGED ANTIVIRAL THERAPY WITH PEGINTERFERON TO PREVENT COMPLICATIONS OF ADVANCED LIVER DISEASE ASSOCIATED WITH HEPATITIS C: RESULTS OF THE HEPATITIS C ANTIVIRAL LONG-TERM TREATMENT AGAINST CIRRHOSIS (HALT-C) TRIAL
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Background: Chronic hepatitis C may lead to progressive liver disease with cirrhosis, liver failure, hepatocellular carcinoma (HCC) and death. Not all pts treated with peginterferon and ribavirin achieve a sustained virologic response (SVR); whether long-term antiviral therapy can prevent progressive liver disease in those who do not achieve SVR remains uncertain. Aims: To determine if long-term maintenance therapy with peginterferon prevents progressive liver disease resulting from hepatitis C.
Methods: We conducted a randomized controlled trial of peginterferon alpha-2a (90 mcg per week) for 3.5 years vs. no treatment in pts with chronic hepatitis C and advanced fibrosis who were nonresponders to prior therapy with peginterferon and ribavirin. Subjects eligible for enrollment had chronic hepatitis C with an Ishak fibrosis score of ≥3 on liver biopsy, a Child-Turcotte-Pugh (CTP) score of ≥6, no history of ascites, encephalopathy or bleeding varices, and no other identifiable cause of liver disease. Participants were stratified according to their stage of fibrosis – Ishak stage 3 or 4 (622 with fibrosis) vs. 5 or 6 (428 with cirrhosis). Pts were seen at 3 month intervals, underwent liver biopsy at 1.5 and 3.5 years after randomization, and were monitored for the following outcomes: death, HCC, hepatic decompensation (variceal hemorrhage, ascites, spontaneous bacterial peritonitis, encephalopathy or a CTP score of ≥7), and, for those with pre-cirrhotic fibrosis at baseline, an increase in fibrosis score of ≥2 points. Results: 1050 pts were randomized (517 treatment, 533 control). By the end of the study, 34.1% of the treatment and 33.8% of the control group had experienced an outcome (hazard ratio=1.01; 95% CI=0.81-1.26; p=0.91). Although mean serum ALT and HCV RNA levels decreased significantly with treatment (both p<0.0001), as did necroinflammatory changes on liver biopsy (p<0.0001), no significant difference was observed in rates of any of the primary outcomes between groups (Table). The rate of serious adverse events was similar in both groups (284 events among 173 treated and 283 events among 155 control pts). Among treated pts, 17% stopped peginterferon by year 1.5 and 30% by year 3.5. Summary and Conclusions: Long-term therapy with peginterferon did not reduce the rate of disease progression. These findings do not support maintenance therapy with peginterferon in patients with chronic hepatitis C and advanced hepatic fibrosis who are nonresponders to a course of peginterferon/ribavirin therapy.

<table>
<thead>
<tr>
<th>Treatment (n=517)</th>
<th>Death (%)</th>
<th>Decompensation (%)</th>
<th>HCC (%)</th>
<th>Increase in fibrosis (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n=533)</td>
<td>6.6</td>
<td>14.3</td>
<td>2.8</td>
<td>28.2</td>
</tr>
</tbody>
</table>

LB2
Patrick Marcellin1, Maria Buti2, Zahary Kravstov3, George Germaniadis4, Kelly D. Kaita5, Iskren Kotzev6, Peter Buggisch7, Frank Weiler8, Huy N. Trinh9, Jeff Sorbel10, Jane Anderson10, Elsa Mondou10, Franck Rousseau10, 1Hospital Beaujon, Clichy, France; 2Hebron Hospital, Barcelona, Spain; 3University Hospital “St Ivan Rilsky”, Sofia, Bulgaria; 4General Hospital of Thessaloniki, Thessaloniki, Greece; 5Joh Bjelke Research Centre, University of Manitoba, Winnipeg, MB, Canada; 6University Hospital “Sveta Marina”, Varna, Bulgaria; 7Medizinische Universitätsklinik Eppendorf, Hamburg, Germany; 8Wairake Hospital, Hamilton, New Zealand; 9San Jose Gastroenterology, San Jose, CA; 10Gilead Sciences, Durham, NC
Background: Tenofovir Disoproxil (TDF) is a nucleotide analogue approved for the treatment of HIV-1 with activity against hepatitis B virus (HBV). The primary objective of this Phase III, 5 year study was to compare the safety and efficacy of 300 mg TDF versus 10 mg ADV at 48 weeks in subjects with HBeAg-CHB. Methods: This was a double-blind, active-controlled, multi-national study of mono-infected subjects with HBeAg-CHB who were randomized in a 2:1 ratio to TDF:ADV. Entry criteria included subjects 18-69 years of age with compensated liver disease, ALT < ULN, HBV DNA > 10^5 copies/ml and a Knodell necroinflammatory score ≤3. Biopsies were performed pre-treatment and Week 48. HBV DNA was measured using the Roche COBAS TaqMan HBV assay (LLQ=169 c/mL). The pre-
mary efficacy endpoint at Week 48 was the proportion of randomized and treated subjects with complete response [i.e., HBV DNA < 400 c/mL and histologic improvement (≥2-point reduction in the Knodell necroinflammatory score without worsening in fibrosis)]. Results: 375 subjects (250 TDF:125 ADV) were randomized and treated. Treatment groups were well balanced at baseline with an overall mean age of 44 years, 77% male, 65% white, 25% Asian, 62% from Europe and 18% lamivudine or emtricitabine experienced. At baseline, mean HBV DNA was 6.9 log_{10} c/mL, 64% had ALT>2xULN and 64% were Genotype D. Mean Knodell necroinflammatory and fibrosis scores were 7.8 and 2.3, respectively with 19% cirrhosis. Evaluation of resistance surveillance data are ongoing. Safety and tolerability were comparable between TDF and ADV treatment groups. Renal safety was excellent; no TDF treated subject had a confirmed 0.5 mg/dL creatinine increase or creatinine clearance value <50 mL. The majority of patients in both treatment groups (TDF:ADV) completed primary endpoint assessments (96%,93%). Primary and secondary efficacy endpoints are summarized below. Conclusion: TDF, at a dose of 300 mg QD, was well tolerated and demonstrated superior efficacy to ADV through 48 weeks as illustrated by the higher percentage of HBeAg- subjects achieving the primary efficacy endpoint.

<table>
<thead>
<tr>
<th>Efficacy Endpoints</th>
<th>TDF 300 mg N=250</th>
<th>ADV 10 MG N=125</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary Composite Endpoint</td>
<td>71%</td>
<td>49%</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Histologic Response</td>
<td>72%</td>
<td>69%</td>
<td>NS</td>
</tr>
<tr>
<td>&lt;HBV DNA&lt;100 c/mL (IL2)</td>
<td>91%</td>
<td>56%</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>&lt;HBV DNA &lt;300 c/mL</td>
<td>92%</td>
<td>59%</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Normal ALT</td>
<td>77%</td>
<td>78%</td>
<td>NS</td>
</tr>
</tbody>
</table>

Disclosures:
Patrick Marcellin - Consultant/Adviser: Gilead; Grant/Research Support: Gilead; Speaker's Bureau: Gilead
Maria Buti - Consultant/Adviser: Gilead; Speaker: Gilead
George Germanidis - Grant/Research Support: Gilead
Jeff Sorbel - Employee: Gilead
Jane Anderson - Employee: Gilead
Elsa Mondou - Employee: Gilead
Franck Rousseau - Employee: Gilead
The following people have nothing to disclose: Zahary Krastev, Kelly D. Kaita, Iskren Katzev, Peter Buggisch, Frank Weiller, Huy N. Trinh

**LB3**

**HIV ENTRY AND REPLIATION IN STELLATE CELLS PROMOTES CELLULAR ACTIVATION AND FIBROGENESIS: IMPLICATIONS FOR HEPATIC FIBROSIS IN HIV/HCV CO-INFECTION**

Ana C. Tuyama, Feng Hong, Alison D. Schecter, Arevik Mosaion, Benjamin K. Chen, Ping Chen, Mary E. Klotman, Meena B. Bansal; Medicine, Mount Sinai School of Medicine, New York, NY

**Introduction:** Patients co-infected with HIV/HCV develop more rapid fibrosis than patients mono-infected with HCV. Fibrosis progression correlates with HIV viremia suggesting a direct role of HIV in liver fibrogenesis. CCR5 and CXCR4 are the 2 major co-receptors required for HIV entry into cells. CCR5 has been reported on hepatic stellate cells (HSCs) and we have recently demonstrated the expression of CXCR4 on HSCs (Hong,#1400; AASLD 2007). Gp120 is the envelope protein for HIV and can activate cells independent of direct infection. The aim(s) of this study are to 1) Examine whether HIV enters HSCs and actively replicates 2) Characterize the impact of HIV gp120 on HSC biology. First the capacity of HIV IIIB (CXCR4-tropic or X4) and HIV-Bal (CCR5-tropic or R5) to infect HSCs was assessed by EUSA for supernatant p24, a marker for virus being released from the cells. LX2 cells, a human HSC line, were infected and washed to remove unbound virus. Significant concentrations of p24 (>2ng/ml) were detected on all days examined (up to 7 days). Since X4 viruses predominate later in the course of HIV disease coincident with chronic liver disease in patients with HCV/HIV, we examined whether HIV IIIB infects primary human HSCs (passage #3-4) and replicates using p24 assay and qRT-PCR for unspliced(US) and multiply-spliced(MS) HIV-1 RNA. The detection of p24 (>8ng/ml) associated with intracellular US and MS HIV suggests active replication (confirmed by sequencing of MS HIV-1). The ability of HIV to infect HSCs was confirmed by challenging the cells with a recombinant HIV expressing GFP in place of the early gene nef. The finding of GFP-positive cells indicates HIV entry and early gene expression. As HIV infection may be either CD4-dependent or -independent, CD4 expression by HSCs was documented by immunofluorescence. CD4-blocking experiments revealed that HIV IIIB entry into HSCs was CD4-independent.

**Conclusions:** HIV enters and actively replicates within HSCs independent of CD4. Both viral entry as well as exposure of cells to viral envelope glycoproteins can promote activation and collagen induction in HSCs. These results suggest that direct infection or Env-mediated activation of HSCs may contribute to rapid development of fibrosis in patients co-infected with HIV/HCV.

**Disclosures:** The following people have nothing to disclose: Ana C. Tuyama, Feng Hong, Alison D. Schecter, Arevik Mosaion, Benjamin K. Chen, Ping Chen, Mary E. Klotman, Meena B. Bansal

**LB4**

**PEGYLATED INTERFERON ALFA-2A (40KD) PLUS RIBAVIRIN (RBV) IN PRIOR NON-RESPONDERS TO PEGYLATED INTERFERON ALFA-2B (12KD)/RBV: FINAL EFFICACY AND SAFETY OUTCOMES OF THE REPEAT STUDY**

Donald M. Jensen1, Bradley Freilich2, Pietro Andreone3, Adrian DiBisceglie4, Carlos E. Brandão-Mello5, K Rajender Reddy6, Antonio Craxi7, Antonio Oliveira Martin8, Gerlinde Teuber9, Diethelm Messinger10, Greg Hooper11, Matei Popescu12, Patrick Marcellin13; 1Director, Center for Liver Diseases, University of Chicago Hospitals, Chicago, IL; 2Liver and Pancreas Institute of Kansas City, Kansas City, MO; 3Department of Internal Medicine and Gastroenterology, University of Bologna, Bologna, Italy; 4Division of Gastroenterology and Hepatology, Saint Louis University School of Medicine, St. Louis, MO; 5Hospital Universitário Gaffee e Guinle, Rio de Janeiro, Brazil; 6Division of Gastroenterology, University of Pennsylvania, Philadelphia, PA; 7GI & Liver Unit, University of Palermo, Palermo, Italy; 8Hospital La Paz, Madrid, Spain; 9Department of Internal Medicine, Johann Wolfgang Goethe University Medical Centre, Frankfurt, Germany; 10IST GmbH, Mannheim, Germany; 11Roche, Welwyn, United Kingdom; 12Roche, Basel, Switzerland; 13Centre de Recherches Claude Bernard sur les Hépatites Virales, Hôpital Beaujon, Clichy, France

**Introduction:** Treatment options are limited for patients (pts) who are previous non-responders to Peg-IFN/RBV. Intensified treatment with higher fixed-dose induction of Peg-IFN and/or longer treatment duration may increase SVR rates in these pts. REPEAT compared both strategies in prior non-responders to...
≥12 wks of Peg-IFN α-2b (12KD, PegIntron®/RBV. Methods: Eligible pts were randomized [2:1:1:2] to 4 regimens, Arms A and B Peg-IFN α-2a (40KD; PEGASYS®) 360 μg/wk for 12 wks then 180 μg/wk for a further 60 or 36 wks, respectively; Arms C and D Peg-IFN α-2a (40KD) 180 μg/wk for 72 or 48 wks, respectively. All pts received RBV (COPEGUS®, 1000/1200 mg/day). The primary endpoint was SVR (HCV RNA <50 IU/mL 24 weeks post-treatment). Results: A total of 942 pts were randomized and dosed. Key baseline (BL) characteristics were similar across arms. For the protocol-defined primary analysis the SVR rate was higher for the 72-wk induction arm (Arm A: 16%) compared to the 48-wk non-induction arm (Arm D: 9%) [p=0.006, OR 2.00 (95% CI 1.21-3.31)]. Furthermore, SVR was higher for pooled 72-wk arms vs pooled 48-wk arms [p=0.0006, OR 2.22 (95% CI 1.40-3.52)]. Tolerability of induction and non-induction regimens of Peg-IFN α-2a were similar and overall the rate/type of AEs/SAEs were similar across arms. Discontinuation rates due to safety were lower for the 48-wks arms. Conclusion: In these difficult to cure pts with prior documented non-response to Peg-IFN α-2b/RBV, re-treatment with fixed-dose induction and longer duration with Peg-IFN α-2a (40KD)/RBV provided the highest SVR rates and the lowest relapse rates. Re-treatment with 72-wks of Peg-IFN α-2a provided higher SVR rates than 48-wks, irrespective of induction.

<table>
<thead>
<tr>
<th>Peg-IFN α-2a (40KD) plus RBV 1000/1200 mg/day</th>
<th>A (n=317)</th>
<th>B (n=156)</th>
<th>C (n=156)</th>
<th>D (n=313)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Males (%)</td>
<td>64%</td>
<td>60%</td>
<td>69%</td>
<td>68%</td>
</tr>
<tr>
<td>Mean ± SD Age (yrs)</td>
<td>48.1 ± 8.7</td>
<td>48.1 ± 9.9</td>
<td>49.1 ± 8.5</td>
<td>48.5 ± 9.0</td>
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<tr>
<td>Cataract (%)</td>
<td>88%</td>
<td>90%</td>
<td>88%</td>
<td>90%</td>
</tr>
<tr>
<td>Mean ± SD Weight (kg)</td>
<td>81.5 ± 18.2</td>
<td>81.1 ± 16.8</td>
<td>81.2 ± 16.0</td>
<td>80.9 ± 16.9</td>
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<tr>
<td>Genotype (%)</td>
<td>91%</td>
<td>91%</td>
<td>91%</td>
<td>91%</td>
</tr>
<tr>
<td>Mean ± SD BL, HCV RNA (×10⁶ IU/mL)</td>
<td>5.4 ± 6.4</td>
<td>5.3 ± 7.1</td>
<td>4.9 ± 5.1</td>
<td>4.9 ± 6.0</td>
</tr>
<tr>
<td>Cirrhosis (%)</td>
<td>25%</td>
<td>29%</td>
<td>30%</td>
<td>28%</td>
</tr>
<tr>
<td>EoT response rate, n (%)</td>
<td>90 (31%)</td>
<td>52 (33%)</td>
<td>48 (31%)</td>
<td>88 (28%)</td>
</tr>
<tr>
<td>SVR rate, n/N (%) (Overall BL, VL, s800 000 BL, VL) ≥800 000</td>
<td>52/917 (15%)</td>
<td>22/264 (9%)</td>
<td>11/116 (7%)</td>
<td>22/156 (14%)</td>
</tr>
<tr>
<td>Relapse Rate, n/N (%)</td>
<td>40/92 (55%)</td>
<td>38/89 (43%)</td>
<td>30/47 (64%)</td>
<td>55/81 (68%)</td>
</tr>
<tr>
<td>Pre-existing no for safety, n (%)</td>
<td>37 (12%)</td>
<td>7 (7%)</td>
<td>18 (12%)</td>
<td>29 (15%)</td>
</tr>
<tr>
<td>SCAH, n (%)</td>
<td>33 (13%)</td>
<td>32 (12%)</td>
<td>31 (13%)</td>
<td>26 (13%)</td>
</tr>
<tr>
<td>Peg-IFN α-2a dose modifications, n (%)</td>
<td>72 (23%)</td>
<td>41 (26%)</td>
<td>36 (23%)</td>
<td>57 (18%)</td>
</tr>
</tbody>
</table>

*N=Pts with negative Eot HCV RNA and ≥1 post-Eot HCV RNA value


**LB5**

**TOTAL TUMOR VOLUME PREDICTS RISK OF RECURRENCE FOLLOWING LIVER TRANSPLANTATION IN PATIENTS WITH HEPATOCELLULAR CARCINOMA**

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Background: Criteria for selection of candidates for liver transplantation in the presence of hepatocellular carcinoma (HCC) should accurately predict post-transplant recurrence, while not excluding excessive numbers of patients from candidacy. Existing criteria are challenged by the limited accuracy of radiological assessment. Methods: Total Tumor Volume (TTV) was calculated by adding the volume of each individual tumor. A preliminary analysis was carried out on HCC patient data from the Alberta Liver Transplant Program (52 patients) and then validated on the populations of the Universities of Toronto and of Colorado programs (154 and 82 patients). Results: A TTV cutoff of 115 cm³ was chosen based on the risk of recurrence with use of a ROC curve. Radiology correlated more closely to pathology with TTV than with Milan and UCSF criteria (91 vs 69 and 75% of patients, p < 0.0001). While more patients met qualifying criteria for transplant with TTV, no deterioration of outcome were demonstrated on analysis of patients within TTV ≤ 115 cm³, in comparison to those meeting Milan or UCSF classifications at all institutions. Patients with TTV >115 cm³, experienced more recurrences and lower tumor-free survival in the Alberta and Colorado series (p < 0.005). Conclusions: Using Total Tumor Volume with a cut-off of 115 cm³ for candidate selection, the accuracy of pretransplant radiological assessment is enhanced, with post-transplant outcomes not different from those achieved with Milan and UCSF classifications despite a more inclusive patient population.

Disclosures: The following people have nothing to disclose: Christian Toso, James F. Tratter, Alice Wei, David Bigam, Shimul Shah, Joshua Lancaster, David Grant, Paul Greig, James Shapiro, Norman Kneteman

**127**

**HEREDITABILITY OF NONALCOHOLIC FATTY LIVER DISEASE**

Jeffrey B. Schwimmer1, Manuel Celedón1, Rany Salem2, Nicholas J. Schork2, Takeshi Yokoo3, Alyssa Chavez2, Michael S. Middleton2, Claude B. Sirlin3, 1Pediatrics, University of California, San Diego, San Diego, CA; 2Psychiatry, University of California, San Diego, San Diego, CA; 3Radiology, University of California, San Diego, San Diego, CA

Introduction: Nonalcoholic fatty liver disease (NAFLD) is the most common chronic liver disease in adults and children in the United States. Ethnic variability in the risk for NAFLD argues for a genetic component in the development of fatty liver. However, the impact of genetic factors in NAFLD is largely unknown. Therefore, we sought to determine the hereditability of NAFLD.

Methods: In order to test the hypothesis that NAFLD is highly heritable, children with biopsy-proven NAFLD (N=30) were recruited to serve as index cases. These probands had a mean age of 13 years with a mean BMI of 30 Kg/m². Family members age 8 and older were studied in a fasting visit. Anthropometric and laboratory studies were performed. Liver fat was quantified using in- and out-of-phase gradient echo magnetic resonance images (MRSI) obtained at 1.3T. Fatty liver was
defined as a fractional fat content ≥ 5%. Etiologies for fatty liver other than NAFLD were excluded (alcohol, Hepatitis C virus, and medications). Narrow sense heritability estimates for fatty liver were calculated using sequential oligogenic linkage analysis routines (SOLAR). Results: Family members (N = 93) of children with NAFLD had a mean age of 32 years (range 8-69) and mean BMI of 29 Kg/m2. Alanine aminotransferase (ALT) was abnormal in 40% of children and 25% of adults. Overall, fatty liver was present in 45% of siblings and 64% of adult family members. At least 1 case of NAFLD, beyond the index child, was identified in 84% of families. In 2 cases, previously unrecognized cirrhosis was detected by MRI. The unadjusted heritability of NAFLD was 56% (P=0.01). Adjusted for age, gender, and BMI, the heritability of NAFLD was 68% (P=0.01). Conclusion: Family members of children with NAFLD should be considered at high risk for NAFLD. The current data suggest that familial factors are the major determinant of whether an individual has NAFLD. Further family studies are needed to unravel the complex issues of the interaction of genes and environment in the development of NAFLD.

Disclosures:
The following people have nothing to disclose: Jeffrey B. Schwimmer, Manuel Celedan, Rany Salem, Nicholas J. Schork, Takeshi Yokao, Alyssa Chavez, Michael S. Middleton, Claude B. Sirlin

128 FRUCTOSE INDUCED HYPERURICEMIA AS A CAUSAL MECHANISM FOR NONALCOHOLIC FATTY LIVER DISEASE

Manal F. Abdelmalek, Ayako Suzuki, Cynthia Guy, Richard J. Johnson, Anna Mae Diehl, for the NASH Clinical Research Group, Division of Gastroenterology, Duke University, Durham, NC; Division of Nephrology, University of Florida, Gainesville, FL; NIDDK, National Institutes of Health, Baltimore, MD

The rise in obesity coincides with an increase in total fructose (FRU) intake. Excess FRU intake leads to hepatic increases in pyruvate and lactate production and a shift in balance from oxidation to esterification of fatty acids. Acute FRU loading of the liver causes sequestration of inorganic phosphate in FRU-1-phosphate and diminished ATP synthesis. Hepatic ATP depletion increases uric acid formation. FRU-induced hyperuricemia results in endothelial dysfunction and insulin resistance (IR) and may be a novel mechanism underlying NAFLD. Methods: We studied 341 adults enrolled in the NASH Clinical Research Network for whom dietary data using the Block Food Questionnaire was collected within 6 months of a liver biopsy. Total FRU consumption was estimated based on reporting (frequency x amount) of kool-aid, fruit juices, and non-dietary soda intake and expressed as servings per wk and classified into none, occasional (<7 servings/wk) and daily (≥7 servings/wk). Multiple linear and logistic regression analyses were used to analyze the association between FRU intake, hyperuricemia [serum uric acid (SUA) >5.5 mg/dl], HOMA-IR and the histologic features of NAFLD. Results: Of the 341 pts, 40% were male and 80% were Caucasian. The mean ± SD of age, BMI and caloric intake was 47 ± 12 yrs, 34 ± 6.3 and 1896 ± 957 kcal /day respectively. Overall, mean SUA was 6.2 ± 1.5 mg/dl, triglycerides (TG) was 196 ± 42 mg/dl, and total, HDL and LDL cholesterol was 196 ± 42 mg/dl, 44 ± 11 mg/dl and 120 ± 35 mg/dl respectively. Daily FRU intake was dose-dependently associated with hyperuricemia compared to non-consumers of FRU (p<0.002), even after controlling for total calorie intake and BMI: OR [95% CI] vs non-consumer was 2.4[1.1, 5.6], p=0.036. Elevated SUA was associated with high TG (p=0.0175), total cholesterol (p=0.008), and a trend towards low HDL cholesterol (p=0.06) which did not alter after controlling for HOMA-IR. On the other hand, even after controlling for other confounders, increased FRU intake was significantly associated with lower steatosis score but higher ballooning, inflammation, and fibrosis score (p<0.05 for each). However, SUA did not correlate with NAFLD Activity Score or fibrosis stage. Conclusions: Increased FRU intake is significantly associated with SUA. Elevated SUA is associated with altered lipid profiles independent of the severity of IR but not with histologic liver damage. However, increased FRU intake correlated negatively with steatosis and positively with ballooning and fibrosis stage. Whether increased FRU intake promotes NAFLD progression via the esterification of fatty acids and hepatic ATP depletion warrants further investigation.

Disclosures:
The following people have nothing to disclose: Manal F. Abdelmalek, Ayako Suzuki, Cynthia Guy, Richard J. Johnson, Anna Mae Diehl, for the NASH Clinical Research Group

129 DECREASED GM1 EXPRESSION IN MEMBRANE LIPID RAFT IN CD4 AND CD8 CELLS OF PATIENTS WITH NASH: A POTENTIAL BIOMARKER AND THERAPEUTIC TARGET

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The plasma membrane of lymphocytes contains sphingolipids and cholesterol, which cluster together in distinct domains called rafts. Lipid rafts have been shown to play a role in T cell activation. Altered lipid raft content may be associated with hyperactivation of immune cells associated with uncontrolled inflammation. GM1 a membrane-bound ganglioside is a main marker of lipid rafts. Current diagnosis of NASH includes serum liver enzymes and imaging studies, but the gold standard for diagnosis requires a liver biopsy. Aim: To compare the lymphocyte lipid raft GM1 content in human peripheral blood lymphocytes (PBLs) from patients with NASH and healthy controls. Methods: PBLs were harvested from five subjects with biopsy-proven NASH, and compared with three healthy controls. Cholera-toxin conjugated to FITC was used to stain GM1. Cells were sorted to CD4, CD8, and NKT lymphocyte subsets using FACS analysis. Plasma membrane lipid rafts were isolated using Triton X-100 insolubility assay. The lipid raft microdomains extracted from the plasma membrane, were identified by dot blot analysis for GM1. Disease activity was assessed via grading of liver biopsies for inflammation, steatosis and fibrosis and measurement of serum ALT levels. Results: Lipid rafts from CD4, CD8 but not NKT cells from patients with NASH, expressed significantly lower GM1 levels compared to healthy subjects (19.6%, 14.2%, 1.5%, 2.8%, 2.7% vs. 31.9%, 35.7%, 26.5% for CD4+FITC in NASH vs. healthy controls, respectively, and 6%, 7.1%, 2.2%, 2.3%, 3% vs. 33.3%, 38.3%, 9.78% for CD8+FITC, in NASH vs. healthy controls, respectively). Lipid raft microdomains from patients with NASH exhibited different GM1 partitioning patterns. GM1 was significantly expressed in the raft non-cytosolic fractions (numbers 1-5) in NASH patients, vs. higher expression in the cytosolic fractions (numbers 9-12) in healthy controls. In all NASH patients, alterations in CD4 and CD8 lymphocyte lipid rafts were associated with an increased inflammatory score, but not
with the degree of fibrosis or steatosis on liver biopsies and serum ALT levels. Conclusions: Patients with NASH exhibited altered lymphocyte lipid raft structure and lower GM1 expression. Alteration of GM1 expression in PBLs may serve as a non-invasive biomarker for disease activity and as a therapeutic target for these patients.

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EFFECT OF BARIATRIC SURGERY ON NONALCOHOLIC FATTY LIVER DISEASE (NAFLD): A META-ANALYSIS
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Background: NAFLD is widely prevalent in patients with morbid obesity. Effects of marked weight loss induced by bariatric surgery on histological features of NAFLD are unclear. Aim: To perform a systematic review and meta-analysis of the effect of weight loss after bariatric surgery on histological features of NAFLD. Methods: Electronic database (MEDLINE, EMBASE, Cochrane controlled trials register) were searched (1990 to 2007) for the studies that compared the histological features of NAFLD before and after bariatric surgery. A total of 42 studies were identified and 16 studies with adequate histological follow up were included for data extraction. Results: Total 762 patients, (81% F 19% M). Average time from surgery to second biopsy was 8 to 41 months. The most common weight loss surgery was RYGBP (8 out of 16) studies. The histological changes of NAFLD were seen in 558/762(72.33%). Overall in 456/568 an (80.28%) patient the changes of steatosis/steatohepatitis improved or resolved significantly and complete resolution of histological changes was achieved in 296/568(52.1%). In 17/568(2.9%) the inflammation progressed after surgery. A total of 268 biopsies showed evidence of fibrosis and among those 112/268(41.79%) showed improvement, 68/268(25.37%) remained the same and 44/268(16.41%) worsened. A pooled analysis of proportions using Random effects model (DerSimonian-Laird) of patients with complete resolution was 58.76% (95% CI = 42.68% to 73.94%). The studies were heterogeneous (I2=93%). Conclusions: Steatosis and necroinflammatory changes improve or completely resolve in majority of patients after bariatric surgery. There appears to be very low risk of progression of fibrosis or steatohepatitis.

Disclosures:
The following people have nothing to disclose: Rajasekhara r. Mummadi, Krishna S. Kasturi, Gagan Sood

131
DIFFERENTIAL HEPATIC EXPRESSION OF LUMICAN AND FATTY ACID BINDING PROTEIN-1 IN HISTOLOGICALLY PROGRESSIVE NAFLD – NOVEL POTENTIAL INSIGHTS INTO HISTOLOGICAL SPECTRUM OF DISEASE
Michael R. Charlton, Allard Jan Kalsbeek, Kimberly Viker, Anuradha Krishnan, Bart J. Veldt, Geoffrey Thompson, Florencia G. Que, Michael Sarr; Liver Transplant, Mayo Clinic, Rochester, MN
Background: The basis of hepatocellular injury and progressive fibrosis in a subset of patients with NAFLD is poorly understood. Recent advances in proteomic methodology have facilitated the detection of low abundance proteins in analyses of the hepatic proteome. Aims: To develop new insight into the pathogenesis of NAFLD by identifying hepatic proteins that are differentially abundant across the histological spectrum of NAFLD. Methods: The hepatic proteome was measured in liver samples from four groups of obese (BMI>30kg/m2), BMI and gender matched patients: 1) obese normal group (normal liver histology, n=10), 2) Simple steatosis (SS, n=10), 3) NASH-mild (steatosis, inflammation grade 1 and fibrosis stage 0-1, 4) NASH-progressive (as for NASH-mild but with fibrosis stage 2-4). iTRAQ LC/MS/MS analysis of peptides was performed on an API Qstar XL quadrupole time of flight mass spectrometer using Analyst QS software. Linear trends tests were performed and used to screen for differential abundance. Results: A total of 1362 hepatic proteins were identified. Nine known proteins were consistently differentially abundant between study groups. Seven proteins (albumin, hemoglobin beta, hemoglobin delta, dihydroxyrimidinase, enolase, metal transport protein ATX1 and HSP gp96) are likely to have been differentially abundant on a predictable basis. The other differentially expressed proteins were: 1) Lumican, a regulator collagen fibril assembly and activator of TGF-beta and smooth muscle actin. Lumican was overexpressed in a dose-dependent manner in NASH-mild vs. SS (124%, p<0.001), NASH-progressive vs. NASH-mild (156%, p<0.001) and NASH-progressive vs. obese normal (178%, p<0.001). 2) Fatty acid binding protein-1 (FABP-1) was appropriately relatively overexpressed in SS when compared
A randomized, double blind, placebo controlled trial of one year of pioglitazone in non-diabetic subjects with nonalcoholic steatohepatitis

Guruprasad P. Aithal¹, James A. Thomas², Philip Kaye¹,², Adam Lawson¹, Stephen D. Ryder¹, Andrew S. Austin⁴, Jan G. Freeman⁵, Linda Morgan⁵, Webber Jonathan⁵; ¹Wolfson Digestive Diseases Centre, University Hospital, Nottingham, United Kingdom; ²Histopathology, University Hospital, Nottingham, United Kingdom; ³Clinical Chemistry, Nottingham University, Nottingham, United Kingdom; ⁴Gastroenterology, Derby Hospital, Derby, United Kingdom; ⁵Diabetes Centre, Selly Oak Hospital, Birmingham, United Kingdom

Background: Nonalcoholic steatohepatitis (NASH) is a leading cause of chronic liver disease for which there is currently limited therapy. Thiazolidinediones have demonstrated some promise in treating NASH in diabetics. Their insulin sensitizing, anti-inflammatory and anti-fibrotic actions support their use in non-diabetic patients with NASH. Aim: To evaluate pioglitazone in the treatment of non-diabetic patients with NASH. Methods: We randomized 74 non-diabetic patients (45 male; median age 54 yr) with histologically proven NASH to 12 months of standard diet, exercise and either placebo or pioglitazone (30 mg/day). Of these, 61 patients (30 placebo, 31 pioglitazone) had liver biopsies both at the start and end of the study. Histological change was scored by a single pathologist who was blinded to all the information including the timing of the biopsy. Results: In the placebo group, diastolic BP (p<0.001) and ALT (p=0.016) decreased, while glucose (p=0.002), HbA1c (p=0.045) and serum markers of liver fibrosis (hyaluronic acid (p=0.012), tissue inhibitor of metalloproteinase (TIMP)-1 (p=0.008), aminoterminal propeptide of type III collagen (p=0.015)) increased over the study period. In the pioglitazone group, diastolic BP (p=0.02), transaminases (ALT & GGT p=0.001) and ferritin (p=0.016) decreased, while subjects gained weight (p=0.004) over the study period. Comparing the pioglitazone group against placebo, there was an increase in weight (+2.77 v -0.55kg, p=0.042) and reduction in glucose (p=0.021), HbA1c (p=0.006), insulin C peptide (p=0.015), ALT (p=0.009), GGT (p=0.002), ferritin (p=0.012), and TIMP-1 (p=0.015) associated with pioglitazone therapy. The histological changes during the study are summarised in the table. Conclusions: Pioglitazone therapy over a 12 month period in non-diabetic subjects with NASH resulted in improvements in metabolic and histological parameters, most notably liver injury and fibrosis. Larger extended trials are justified to assess the long-term efficacy of pioglitazone in this patient group.

Histological changes during study

<table>
<thead>
<tr>
<th>Histological feature (score)</th>
<th>Pioglitazone (start v end)</th>
<th>Placebo (start v end)</th>
<th>Pioglitazone (end) v placebo (end)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Steatosis</td>
<td>↓ (p&lt;0.001)</td>
<td>↓ (p&lt;0.025)</td>
<td>↓(p=0.193)</td>
</tr>
<tr>
<td>Lobular inflammation</td>
<td>↓ (p=0.013)</td>
<td>↑ (p=0.285)</td>
<td>↑(p=0.250)</td>
</tr>
<tr>
<td>Hepatocellular injury</td>
<td>↓ (p=0.09)</td>
<td>↑ (p=0.04)</td>
<td>↑(p=0.005)</td>
</tr>
<tr>
<td>Mallory bodies</td>
<td>↑ (p=0.005)</td>
<td>↑ (p=0.257)</td>
<td>↑(p=0.004)</td>
</tr>
<tr>
<td>Fibrosis</td>
<td>↑ (p=0.006)</td>
<td>↑ (p=0.805)</td>
<td>↑(p=0.85)</td>
</tr>
</tbody>
</table>

↓ indicates decreased score; ↑ indicates increased score.

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detectable genotype 1 HCV RNA, and anti-HCV-seropositives with detectable genotype non-1 HCV RNA was 25.7% (7.3-90.9), 10.7 (3.3-34.6) and 11.3 (3.3-39.7), respectively, for those with ALT levels at entry >45 IU/L. The corresponding figures were 5.2 (2.2-12.0), 9.8 (5.3-18.2), and 7.5 (3.2-17.5) for those with ALT levels at entry <45 IU/L. **Conclusion:** HCV infection, elevated serum ALT levels, and cigarette smoking are important HCC risk predictors for HBsAg-seronegatives in Taiwan.

Disclosures:
The following people have nothing to disclose: Mei-Hsuan Lee, Hwai-I Yang, Chun-Jen Liu, San-Lin You, Pei-Jer Chen, Chien-Jen Chen

## 134 LOW UPTAKE OF TREATMENT FOR HEPATITIS C VIRUS (HCV) INFECTION IN A LARGE COMMUNITY-BASED COHORT OF ILICIT DRUG USERS IN VANCAYVER

Jason Grebely, Jesse D. Raffa, Calvin Lai, Mel Krajden, Benedikt Fischer, Thomas Kerr, Mark W. Tyndall

**Purpose:** To estimate HCV treatment uptake in a large community-based inner city cohort in Vancouver and compare this to the incidence of HCV infection over the period January 2000 to December 2004. Methods: CHASE is a cohort study of inner city residents recruited from January 2003 to June 2004. HIV and HCV status were determined through linkage with provincial databases. Treatment information was derived from the British Columbia Ministry of Health Pharmacare database (January 2000 to December 2004), which contains information on all HCV treatment prescriptions in the province. The incidence of HCV infection and the rate of HCV treatment uptake were calculated, expressed in terms of person-years of observation and compared over the follow-up period. Results: As of December 2004, among 3,553 subjects enrolled into the cohort, HCV antibody testing was performed in 2,117 and the HCV seroprevalence was 64.3% (n=1,361). In total, between January 2000 and December 2004, 15 HCV antibody positive subjects initiated treatment for HCV (1.1%), accounting for a total of 5420 person years of follow-up, yielding an overall treatment uptake of 0.3 cases per 100 person years (95% CI, 0.1-0.4). Overall, only 3 of 15 (0.2%) HCV antibody positive subjects achieved an SVR. During the same period, 91 HCV seroconversions were observed among a total of 1285 person years of follow-up, yielding an overall incidence of 7.1 cases per 100 person years (95% CI, 5.6-8.5). The incidence of HCV infection among recent injection drug users was 23.1 cases per 100 person years (95% CI, 17.5-28.5). Conclusions: We have documented extremely low rates of HCV treatment initiation and limited effectiveness of antiviral treatment for HCV, despite a high prevalence and incidence of infection in a large community-based cohort of inner city residents in Vancouver. Overall, the incidence of HCV was 24 times the rate of treatment uptake. Many studies have demonstrated that the treatment of this group is safe and effective, but the reality is that very few people in marginalized communities are accessing treatment for HCV. Given that the majority of new and existing cases of HCV occur in this group, efforts are urgently needed to expand programs for testing and treatment of HCV infection in illicit drug users.

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Eric Rosenthal,1,10 Dominique Salmon,2 Charlotte Lewden,3 Fabrice Bonnet,4,10 Thierry May,5 Philippe Marlès,4,10 Muriel Français,3 Christine Burty,6, Eric Jouglar,9 Dominique Castagliola,7,8 Geneviève Chêne,1 Patrice Cacoub,5,10 Bonnet4,10, Thierry May,5, Philippe Morlat,4,10 Muriel François,3

**Objective:** To determine mortality due to end-stage liver disease (ESLD) and the profile of patients who died from ESLD in a nationwide population of HIV-infected patients and the evolution over a 10-years period. Design and methods: All departments of internal medicine and infectious diseases from the GERMIVIC Study Group prospectively recorded all deaths in HIV-infected patients during 2005. Fifty six departments, following around 35,000 HIV-infected patients, participated in the study. Results were compared with those of previous surveys conducted using similar methodology in 1995, 1997, 2001 and 2003. Results: Among 364 deaths documented during 2005, 142 (39.0%) were related to AIDS, 64 (17.6%) to ESLD, 55 (15.1%) to cancers neither related to HIV nor hepatitis viruses, 20 (5.5%) to cardiovascular diseases and 83 (22.8%) to other causes. Mortality due to ESLD represented 28.8% of non AIDS-related deaths. Patients dying from ESLD had chronic hepatitis due to virus C in 79.6% of cases and 46.6% of these patients had high alcohol consumption (> 30g/day) (table 1). In the Germivic survey, the proportion of ESLD-deaths has increased: 5% in 1995, 6.6% in 1997, 14.3% in 2001, 12.6% in 2003 and 17.6% in 2005, p<0.01. The proportion of hepatocellular carcinoma as a cause of death increased over this 10-years period (4.7% in 1995 vs 31.2% in 2005, p<0.01). Treatment of hepatitis C in patients who died from ESLD was more frequent in 2005 (37.5%) than in 1995 (19.0%). p<0.01. Conclusions: In HIV/HCV coinfected patients, the proportion of HCV/ESLD is still increasing and it constitutes a leading cause of mortality in this population.

<table>
<thead>
<tr>
<th>Year</th>
<th>1995 n=21</th>
<th>1997 n=36</th>
<th>2001 n=38</th>
<th>2003 n=27</th>
<th>2005 n=64</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex, male, no (%)</td>
<td>15 (71.4)</td>
<td>30 (83.3)</td>
<td>30 (78.9)</td>
<td>22 (81)</td>
<td>53 (83)</td>
</tr>
<tr>
<td>Mean age, y (range)</td>
<td>41 (26-66)</td>
<td>42 (37-62)</td>
<td>42 (32-69)</td>
<td>42 (36-54)</td>
<td>45 (39-68)</td>
</tr>
<tr>
<td>Injection drug use, no (%)</td>
<td>6 (28.5)</td>
<td>14 (38)</td>
<td>29 (76.3)</td>
<td>26 (96)</td>
<td>39 (60.9)</td>
</tr>
<tr>
<td>Alcohol consumption &gt; Xg/day, no (%)</td>
<td>6 (28.5)</td>
<td>16 (44.4)</td>
<td>19 (50)</td>
<td>16 (59.3)</td>
<td>28 (46.6)</td>
</tr>
<tr>
<td>HBsAg positive, no (%)</td>
<td>8 (38.1)</td>
<td>15 (41.7)</td>
<td>8 (21.1)</td>
<td>2 (7.4)</td>
<td>17 (26.5)</td>
</tr>
<tr>
<td>HCC, no (%)</td>
<td>1 (4.7)</td>
<td>4 (11.1)</td>
<td>9 (25)</td>
<td>4 (14.8)</td>
<td>20 (31.2)</td>
</tr>
<tr>
<td>Anti-HCV treatment, no (%)</td>
<td>4 (19)</td>
<td>3 (8.3)</td>
<td>10 (26.3)</td>
<td>12 (44.1)</td>
<td>24 (37.5)</td>
</tr>
<tr>
<td>CD4 count (cell/μl), median (IQR)</td>
<td>27 (25-72)</td>
<td>31 (21-114)</td>
<td>46 (30-172)</td>
<td>58 (66-350)</td>
<td>110 (105-355)</td>
</tr>
<tr>
<td>HAART, no (%)</td>
<td>0</td>
<td>15 (41.6)</td>
<td>28 (73.6)</td>
<td>23 (85)</td>
<td>58 (90.6)</td>
</tr>
</tbody>
</table>

ESLD, end-stage liver disease; HCV, hepatitis C virus; HCC, hepatocellular carcinoma; HAART, highly active antiretroviral therapy

Disclosures:
The following people have nothing to disclose: Eric Rosenthal, Dominique Salmon, Charlotte Lewden, Fabrice Bonnet, Thierry May, Philippe Morlat, Muriel Francais, Christine Burtu, Eric Jaugla, Dominique Castagliola, Genevieve Chêne, Patrice Cacoub

136 STRONG ASSOCIATION BETWEEN TATTOOS AND HEPATITIS C VIRUS INFECTION: A MULTICENTER STUDY OF 3,871 PATIENTS

Sameer Dhalia1, Craig T. Tenner2, Ayse Aytaman3, Nilesh B. Shukla4, Gerald Villanueva5, Grace Punla6, Carlie Patterson7, JoAnn Comas8, Edmund J. Bini9

Methods: Patients with chronic HCV infection (HCV RNA positive) and controls (HCV antibody negative) completed a detailed questionnaire at the time of their scheduled visit to the outpatient primary care or GI clinic at 3 study sites. Data collected included patient demographics and information on HCV risk factors. Results: A total of 3,871 patients were enrolled, including 1,930 with chronic HCV infection and 1,941 HCV negative controls. There were no differences in the mean age (55.2 ± 9.0 vs. 55.6 ± 11.3 years, p = 0.34) or the proportion who were male (80.3% vs. 81.4%, p = 0.39) between HCV-infected patients and controls. However, HCV positive patients were more likely to be racial/ethnic minorities (78.5% vs. 56.5%, p < 0.001). As expected, injection drug use (63.9% vs. 17.8%, p < 0.001) and blood transfusions prior to 1992 (22.3% vs. 11.1%, p < 0.001) were more common in HCV-infected patients than in control subjects. Patients with HCV infection were significantly more likely to have had one or more tattoos (53.2% vs. 12.5%; OR = 3.81; 95% CI, 3.24 – 4.49; p < 0.001) and this remained highly significant after adjustment for age, sex, and race/ethnicity (OR = 4.57; 95% CI, 3.83 – 5.43; p < 0.001). After excluding all patients with a history of ever injecting drugs and those who have had a blood transfusion prior to 1992, a total of 1,887 subjects remained for analysis (466 HCV positive and 1,421 controls). Among these 1,887 patients without traditional risk factors for HCV infection, we found that HCV positive patients were still significantly more likely to have a history of tattoos (34.1% vs. 11.9%; OR = 3.84; 95% CI, 2.99 – 4.93; p < 0.001) and this remained highly statistically significant after adjustment for age, sex, and race/ethnicity (OR = 4.47; 95% CI, 3.42 – 5.83; p < 0.001). Conclusions: Tattoos are strongly associated with HCV infection, even among those without traditional HCV risk factors such as injection drug use and blood transfusions. All patients with tattoos should be offered HCV testing.

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The following people have nothing to disclose: Sameer Dhalia, Craig T. Tenner, Ayse Aytaman, Nilesh B. Shukla, Gerald Villanueva, Grace Punla, Carlie Patterson, JoAnn Comas, Edmund J. Bini

137 EVIDENCE OF INTERNATIONAL TRANSMISSION OF HCV IN PAN-EUROPEAN STUDY OF HIV-POSITIVE MEN WHO HAVE SEX WITH MEN (MSM)

Mark Danta1,2, Thijs van de Laar3, David Brown2, Oliver Pybus3, Sanjay Bhagani4, Martin Vogel5, Stefan Neifer6, Axel Baumgarten7, Helena Goltz8, Jurgen Rockstroh9, Sylvia Bruisten10, Geofrey M. Dusheiko11, 1St Vincent’s Clinical School, Sydney, NSW, Australia; 2Centre for Hepatology, University College and Royal Free Medical Schools, London, United Kingdom; 3Department of Zoology, University of Oxford, Oxford, United Kingdom; 4Department of HIV Medicine, Royal Free Hospital, London, United Kingdom; 5University of Bonn, Bonn, Germany; 6Practice Dupke, Carganico, Baumgarten, Berlin, Germany; 7Department of Infectious Diseases, Health Service, Rotterdam, Netherlands; 8Cluster of Infectious Disease, Health Service, Amsterdam, Netherlands

Since 2000, there has been a reported rise in percutaneous HCV transmission among European HIV-positive MSM related to high-risk sexual behaviours. We conducted a phylogenetic study to investigate the presence of a HCV transmission network among European MSM. Methods: HIV-positive MSM diagnosed with acute HCV (n=178) in England, Netherlands and Germany between January 2000 and December 2006 were enrolled into a molecular phylogenetic study. Part of the NS5B region of the HCV genome (436 bp) was amplified using RT-PCR and subsequently sequenced and genotyped. NS5B phylogenetic trees were constructed using MEGA 3.1 software, comparing MSM cases with unrelated NS5B sequences. Results: NS5B sequences were obtained from 154/178 (87%) of cases; UK 86/107 (80%); Netherlands 46/47 (98%); Germany 22/24 (92%). Circulating HCV strains were of subtype 1a (60%), 4d (23%), 3a (8%), 1b (6%), 2b and 2c (3%). Phylogenetic analysis revealed 10 distinct HCV clusters (containing between 3-36 individuals; bootstrap values >70%) involving 129 (84%) of the sequences. Six of the ten clusters contained sequences from more than one country; 3 clusters contained sequences from all three countries. The majority (66%) of HCV sequences were in the five largest clusters, all of which contained sequences from different countries. There was a trend to country specific segregation occurring in smaller clusters compared with country non-specific clusters (4.5 median sequences/cluster versus 13.5 median sequences/cluster, p=0.07). Conclusion: This phylogenetic analysis reveals a large HCV transmission network among HIV-positive MSM in
Europe. International mixing increases with cluster size, emphasising the rapid spread of regional outbreaks to neighbouring countries, presumably through increased travel associated with high-risk behaviours. The emergence of co-circulating HCV lineages supports transmission related to behavioural change among MSM rather than intrinsic viral change. This has important implications for public health agencies mitigating HCV transmission.

Disclosures:
The following people have nothing to disclose: Mark Danta, Thijs van de Laar, David Brown, Oliver Pybus, Sanjay Bhagani, Martin Vogel, Stefan Neller, Axel Baumgarten, Helena Gout, Juergen Rockstoh, Sylvia Bruisten, Geoffrey M. Dusheiko

### Table 1. Clinical Outcomes

<table>
<thead>
<tr>
<th></th>
<th>HIV-HCV</th>
<th>Deaths</th>
<th>Overall</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>48 (27%)</td>
</tr>
<tr>
<td>HIV-Associated</td>
<td>7 (4%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>18 (10%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ESLD-Associated</td>
<td>12 (7%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Drug-related</td>
<td>0 (0%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unknown</td>
<td>11 (6%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>29 (16%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HCV</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cirrhosis</td>
<td>34 (19%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>16 (9%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ESLD-Associated</td>
<td>6 (3%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Drug-related</td>
<td>1 (1%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unknown</td>
<td>11 (6%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>31 (18%)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Disclosures:
The following people have nothing to disclose: Alexander Monto, Sue Currie, Lorna M. Dove, Daniel Tracy, Sally George, Angela Myers, James C. Ryan, Teresa L. Wright

### 139 ANGIOTENSIN-CONVERTING-ENZYME 2 IS A NEGATIVE REGULATOR OF CHRONIC LIVER INJURY

Christoph H. Oesterreicher1, Ekihiro Seki1, Samuele De Minicis1, KOJIRO TAURA1, Melissa Penz-Oesterreicher1, Johannes Kluwer2, David A. Brenner1; 1Medicine, University of California, San Diego, CA; 2Medicine, Columbia University, New York, NY

Background: The renin-angiotensin system (RAS) plays a major role in liver fibrogenesis. The formation of angiotensin (ang) II and the ang II-induced effects in hepatic stellate cells (HSCs) are fibrogenic in the liver. Recently, a homologue of ang-converting-enzyme 1, named ACE2, has been identified that appears to be a negative regulator of the RAS by degrading ang II to ang 1-7. Interestingly, ACE2 expression and activity is increased in patients with chronic HCV infection. So far the role of ACE2 in liver disease remains elusive. Aims: To investigate the role of ACE2 in acute and chronic models of liver injury in vivo. Methods: ACE2 ko mice and wild-type (wt) littermates underwent bile-duct-ligation (BDL) for up to 21 days or received 1-8 injections of CCl4 and were sacrificed and analyzed at various time points. Liver pathology was analyzed by H&E and Sirius Red staining, immunohistochemistry, α-SMA immunoblotting and real-time PCR for collagen1(I), α-SMA and TIMP-1. Murine HSCs were isolated by collagenase-pronase perfusion and Nycodenz gradient centrifugation Results: After 21 days of BDL, ACE2 ko mice displayed a 2.5 fold increase in Sirius Red positive area as well as increased α-SMA protein levels compared to wt siblings. Livers of ACE2 ko mice were characterized by inflammatory cells infiltrating and increased expression of lipid peroxidation products. Collagen1(I), α-SMA, and TIMP-1 mRNA levels were significantly higher in ACE2 ko mice compared to wt littermates. Similar results were obtained from mice receiving 8 injections of CCl4. In contrast, ACE2 ko mice undergoing BDL for 5 days or receiving a single injection of CCl4 did not differ from their wt littermates with respect to serum ALT or AST levels or mRNA expression of collagen1(I), α-SMA or TIMP-1. Real-time PCR analysis revealed that ACE2 mRNA is significantly upregulated after 3 weeks of BDL or 8 injections of CCl4 in contrast to ACE1 mRNA which is already significantly increased 5 days after BDL or 1 injection of CCl4. Interestingly, 1 year old untreated ACE2 ko mice displayed inflammatory cell infiltration and collagen deposition. Finally, HSCs expressed the ang 1-7 receptor MAS, and ang 1-7 inhibited the activation of cultured HSCs by ang II. Conclusion:
140 REDUCED HEPATIC FIBROSIS IS ASSOCIATED WITH FEWER INTRAHEPATIC B CELLS IN FIBROBLAST ACTIVATION PROTEIN AND DIPEPTIDYL PEPTIDASE IV GENE KNOCKOUT MICE

Xin M. Wang1, Shaun Cordoba1, Didier Marguet2, Wolfgang Retting3, Andreas Schnapp2, Geoff W. McCaughan1, Mark D. Gorrell1; 1Centenary Institute, Faculty of Medicine, University of Sydney, Sydney, NSW, Australia; 2Boehringer Ingelheim, Vienna, Austria; 3Inserm, Paris, France

Cell adhesion and migration are essential in pathologic processes that involve wound healing, such as chronic liver disease. Fibroblast activation protein (FAP) and dipeptidyl peptidase IV (DPIV) are type II cell surface proteins of the prolyl oligopeptidase gene family but both proteins have soluble forms in human serum. FAP has both peptidase and collagen type I specific gelatinase activities. DPIV binds to fibronectin and both proteins bind beta 1 integrin and influence cell-extracellular matrix interactions in vitro (X.M. Wang et al. Hepatology 2005;42:935). In chronic liver injury, FAP is selectively expressed by activated hepatic stellate cells (HSC) and myofibroblasts and the expression level correlates with fibrosis severity. The role of DPIV in metabolic regulation has been exploited by a new type 2 diabetes therapy that selectively inhibits the peptidase activity of DPIV. To further investigate the roles of FAP and DPIV in liver fibrosis, chronic liver injury was induced in wild type, FAP gene knockout (gko) and DPIV gko mice by carbon tetrachloride (CCL4) injection. The level of liver fibrosis was quantified by Sirius red staining and automated Axiovision v.4.3 – assisted image analysis. Inflammation was assessed using both H&E staining and CD45 immunostaining. Frozen sections were immunostained for desmin, GFAP, alpha SMA, CD4, CD8, B220 and F4/80 expression. Immunostained cells were counted in 20 adjacent fields under a 20x objective by fluorescence microscopy. Global intrahepatic gene expression at 3 weeks of CCL4 treatment was measured by DNA microarray and quantitative PCR. Statistical analyses used Student’s t test. Both FAP and DPIV gko mice developed less liver fibrosis and less inflammation compared with the wild type mice (p<0.05). DNA microarray detected 22 genes up-regulated and 172 genes down-regulated in DPIV gko fibrotic mice and 12 genes up-regulated and 37 genes down-regulated in DPIV gko fibrotic mice, compared to fibrotic wild type mice. Quantitative PCR confirmed that some cell-ECM and metabolism-associated genes were differentially expressed. Most of the down-regulated genes were immunoglobin genes. Reduced fibrosis severity was associated with reduced intrahepatic B cell densities, as has been reported by Novobrantseva et al. (J Clin Invest 2005, 115:3072). Fewer CD4+ cells were seen in DPIV gko (p<0.05) but not FAP gko livers. No differences in HSC, CD8+ or macrophage numbers were detected. The data suggest pro-fibrotic roles for FAP and DPIV in experimental chronic liver injury. Future work should investigate interactions between FAP and B cells in the development of liver fibrosis.

Disclosures:
The following people have nothing to disclose: Xin M. Wang, Shaun Cordoba, Didier Marguet, Wolfgang Retting, Andreas Schnapp, Geoff W. McCaughan, Mark D. Gorrell

141 GHRELIN ATTENUATES ACUTE AND CHRONIC HEPATIC INJURY IN RATS AND INFLUENCES FIBROSIS PROGRESSION IN PATIENTS WITH CHRONIC HEPATITIS C

Montserrat Moreno1, Javier F. Chaves2, Pau Sancho-Bru1, Fernando Ramalho3, Leandra N. Ramalho1, Josep Vidal3, Xavier Forns1, Montserrat Mari3, Maria L. Mansego2, Albert Morales3, Jordi Colmenero1, Marlene Dominguez1, Vicente Arroyo1, Juan Caballeria1, Pere Gines1, Ramón Bataller1; 1Liver Unit, IDIBAPS, CIBER, Hospital Clinic de Barcelona, Barcelona, Spain; 2Laboratorio de Estudios Genéticos, Fundación de Investigación, Hospital Clínico Universitario de València, Valencia, Spain; 3Consejo Superior de Investigaciones Científicas, Barcelona, Spain; 4Endocrinology Unit, Hospital Clinic de Barcelona, Barcelona, Spain

Background&Aims: Ghrelin is a gut-derived hormone with powerful orexigenic properties. Ghrelin is also expressed in some peripheral organs, and exerts pleiotropic effects such as protection of parenchymal cells against cell death. The aims of this study were to study whether ghrelin is expressed in the liver and regulates the hepatic response to acute and chronic injury. Methods: Ghrelin gene expression was assessed by quantitative PCR in normal livers (n=5), alcoholic hepatitis (n=22) and chronic hepatitis C (n=13). Serum ghrelin levels were analyzed by RIA in healthy subjects (n=34); alcoholic hepatitis (n=25) and chronic hepatitis C (n=73). Recombinant ghrelin or saline were administered to rats with acute liver injury (single CCl4 administration) and rats with liver fibrosis (bile duct ligation). The effects of ghrelin were also studied in primary cultures of hepatic stellate cells (HSC) and hepatocytes. Six SNPs of the ghrelin gene were analyzed in 266 patients with chronic hepatitis C infection with mild and advanced fibrosis. Results: Ghrelin gene expression was detected in both normal and diseased livers. Ghrelin serum levels were decreased in patients with chronic liver diseases and correlated with the degree of fibrosis. Recombinant ghrelin markedly attenuated the fibrogenic response of rats to chronic injury, as assessed by decreased collagen deposition (morphometric quantification of the area stained with Sirius Red), accumulation of αSMA-positive cells and expression of procollagen αI(III), TGFβ1 and TIMP-1. These effects were reproduced in cultured HSC. The anti-fibrotic effects of ghrelin in vivo and in vitro were prevented by the use of a ghrelin receptor antagonist. Ghrelin also protected rat livers from acute liver injury, as indicated by reduced serum levels of AST, necroinflammatory score, infiltration by CD43-positive inflammatory cells, hepatocellular apoptosis (TUNEL) and oxidative stress (HNE). Ghrelin also stimulated pro-survival signaling pathways in cultured hepatocytes. Finally, we studied whether ghrelin gene variations regulate fibrosis development in patients with chronic hepatitis C. Two SNPs of the ghrelin gene (9974CT and -604GA) influenced the degree of liver fibrosis in female patients with chronic hepatitis C. Conclusions: We conclude that ghrelin attenuates liver fibrosis and hepatocellular injury and it may influence the progression of liver fibrosis in patients with chronic hepatitis C. Therefore, ghrelin could be a potential therapeutic agent for patients with liver diseases.

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142
COMPETITIVE INHIBITION OF LEPTIN SIGNALING BY A MUTAGENIC PEPTIDE RESULTS IN IMPROVEMENT OF HEPATIC FIBROSIS THROUGH INHIBITION OF HEPATIC STELLATE CELL ACTIVATION
Eran Elinav1, Rafi Bruck1, Muhammed Ali1, Eli Brasowski1, Adam Phillips1, Zamir Halpern1, Arieh Gertler2; 1Tel Aviv Sourasky Medical Center, Tel Aviv, Israel; 2Faculty of Agriculture, Hebrew University, Rehovot, Israel

Background: Leptin signaling is required for hepatic stellate cell pro-fibrotic function. In leptin deficient ob/ob mice, fibrosis does not develop despite the presence of severe longstanding steatohepatitis, while administration of leptin to ob/ob mice results in development of fibrosis and exacerbation of existing fibrosis in wildtype mice. Aims: Assessment of the effect of competitive inhibition of leptin signaling on chronic liver fibrosis. Methods: A mutated leptin protein carrying four substituted amino acids at the leptin receptor binding site III was generated by site-directed mutagenesis. This mutated molecule was found to potently bind leptin receptor, but does not induce intra-cellular signaling events. The anti-leptinogenic and anti-fibrotic activity of the leptin mutant was assessed using various in-vitro systems, while leptin antagonist’s in-vivo effect on liver fibrosis was assessed using the chronic thioacetamide (TAA) chronic inflammation and fibrosis model. Results: The mutated leptin protein was found to potently inhibit leptin-induced phosphorylation of STAT3 and MAP kinase, and leptin-induced oligomerization of leptin receptors. In the chronic TAA fibrosis model, leptin administration was associated with significantly enhanced liver disease leading to a 100% 5-week mortality rate. In contrast, administration of inhibitory doses of leptin antagonist alone or in concomitantly with leptin was associated with markedly improved survival and reduced chronic liver inflammation and fibrosis. No significant changes in weight, serum cholesterol or triglycerides were noted. In-vitro addition of leptin antagonists to hepatic stellate cells was associated with reduced PDGF-induced proliferation. To prove leptin antagonist’s inhibitory effect on HSC’s profibrogenic function, HSC’s were transiently transfected with a bicistronic vector encoding alpha 1 procollagen promoter and luciferase as a reporter gene. Incubation with leptin antagonists was associated with significant reduction in luciferase luminescence, reflecting inhibition of leptin-induced activation of alpha 1 procollagen expression. Conclusion: Inhibition of leptin’s activity by a mutated peptide results in potent inhibition of chronic inflammation and fibrosis, partly through inhibitory effects on hepatic stellate cell activity. Inhibition of leptin’s activity may hold promise as a future anti-fibrotic therapeutic modality.

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143
INTEGRIN ALPHAV BETA 6 IS A UNIQUE PROGRESSION MARKER AND TARGET FOR ANTIFIBROTIC THERAPIES IN LIVER FIBROSIS
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Background: The integrin αvβ6 promotes proliferation and wound healing of specialized epithelial cells and acts as a receptor for the activation of latent TGF-β1. Since molecular targeting of this integrin could serve as a novel strategy to treat hepatic fibrosis, we studied αvβ6 expression in experimental and human liver fibrosis and its pharmacological inhibition for treatment of liver fibrosis. Materials and methods: αvβ6 expression was studied by quantitative PCR and immunohistochemistry in rats with cirrhosis due to bile duct ligation (BDL), chronic administration of thioacetamide (TAA), in Mdr2(abcb4)-/- mice that develop progressive biliary fibrosis, and in 18 explant livers of patients who underwent liver transplantation for end-stage liver disease due to HBV, HCV, PBC, PSC and alcohol. The effect of the selective αvβ6 inhibitor EMDS27040 was evaluated in Mdr2(abcb4)-/- mice with advanced hepatic fibrosis. Results: Integrin αvβ6 mRNA was upregulated 25- and 90-fold in TAA- and BDL-induced cirrhosis, respectively, and up to 85-fold in Mdr2(abcb4)-/- mice in correlation with fibrosis progression. In human end-stage liver disease αvβ6 transcripts were up to 100-fold upregulated, with no association to the underlying disease. αvβ6 protein was almost absent in normal livers and expressed de novo on (activated) bile duct epithelia and transitional hepatocytes adjacent to fibrous septa. A single dose of EMDS27040 into Mdr2(abcb4)-/- mice resulted in a transient 40% downregulation of profibrogenic transcripts (procollagen 1, MMP-2, TGFβ2) and a transient 3-fold induction of a pro-fibrolytic transcripts (MMP-8, -9, -13), paralleled by increased extracellular matrix-degrading activities in the liver 3h after injection. Conclusions: 1) Integrin αvβ6 is highly upregulated in rodent and human liver fibrosis, where it is expressed on bile duct epithelia and (transitional) hepatocytes and correlates with fibrosis progression. 2) In vivo a single dose of a small molecule αvβ6 inhibitor modifies hepatic gene expression and enzymatic activities in an antifibrogenic and profibrolytic fashion, confirming αvβ6 integrin as a unique target to monitor and treat liver fibrosis.

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REGRESSION OF FIBROSIS AMONG LONG-TERM RESPONDERS TO ANTIVIRAL TREATMENT FOR CHRONIC VIRAL HEPATITIS
Cosimo Colletta1, Carlo Sinirne2, Carlo Fabris3, Anna M. Foscolo4, Pierluigi Toniutto5, Rachele Rapetti5, Rosalba Minisini5, Lisa Sala5, Stefano Fangazio5, Mario Pirisi1; 1Division of Medicine, COQ, Omegna, Italy; 2Dpt of Clinical & Experimental Medicine, University of Eastern Piedmont, Novara, Italy; 3DPMSC, University of Udine, Udine, Italy; 4Pathology, ASL 14, Verbania, Italy

Introduction. Evidence of fibrosis regression among long-term responders to antiviral treatment for chronic viral hepatitis B and C is scanty, since, for a variety of reasons, liver biopsy is rarely performed on these patients. We aimed to estimate the degree of long-term improvement in necroinflammatory and fibrosis scores after successful antiviral treatment of chronic viral hepatitis, and to verify whether hepatic ultrasonic transient elastography might be a reliable non-invasive alternative to liver biopsy in these patients. Methods. One-hundred one patients (70 males, median age 52 years, range 34-68), who had undergone percutaneous liver biopsy prior to antiviral treatment for chronic hepatitis C (N. = 78) or B (N. = 23), and had achieved sustained viral response or persistent viral suppression, respectively, were studied. A second liver biopsy was performed a median of 76 months (range, 35 to 113) after the index biopsy; all biopsy specimens were scored according to the method of Ishak et al. Within 24 hours from this second
biopsy, liver stiffness was measured by hepatic ultrasonic transient elastography. Results. In the Table, initial and final grading and staging scores are summarized according to the viral agent involved. The grading and staging scores observed at the end of follow-up were significantly lower than to those observed prior to antiviral therapy (p ≤ 0.0001 for both). The mean reductions in the grading scores were 4.2±3.1 in hepatitis C patients vs. 4.8±2.5 in hepatitis B patients (p: NS). The mean reductions in the staging scores were 2.9±1.1 in hepatitis C patients vs. 3.0±1.0 in hepatitis B patients (p: NS). The individual final staging scores were always lower than the initial staging scores; none of the patients had a final staging score >2. Liver stiffness was 4.7±0.8 kPa in the 72 patients with final staging score = 0; 7.6±0.7 kPa in the 27 patients with final staging score = 1; and 8.9±0.1 in the two patients with final staging score = 2. Conclusions. Achievement of virologic end-points is accompanied, in the long term, by significant improvements in the necroinflammatory and fibrosis scores both in hepatitis B and C. Transient elastography may reliably substitute liver biopsy to confirm fibrosis regression in this setting.

### Main histologic features at the index and final biopsies, according to viral etiology

<table>
<thead>
<tr>
<th>Feature</th>
<th>Hepatitis C N. = 78</th>
<th>Hepatitis B N. = 35</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grading at the index biopsy median range</td>
<td>7 4-16</td>
<td>9 5-14</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Staging at the index biopsy median range</td>
<td>3 1-6</td>
<td>3 2-6</td>
<td>NS</td>
</tr>
<tr>
<td>N. with staging score ≥5 at the index biopsy</td>
<td>12 (15%)</td>
<td>6 (20%)</td>
<td>NS</td>
</tr>
<tr>
<td>N. with staging score ≥5 at the final biopsy</td>
<td>3 1-6</td>
<td>4 2-6</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Staging at the final biopsy median range</td>
<td>0 0-1</td>
<td>1 0-2</td>
<td>&lt;0.005</td>
</tr>
</tbody>
</table>

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146 CHRONIC HBV EVOLUTION IS ASSOCIATED WITH NUMERIC AND PHENOTYPIC CHANGES IN PERIPHERAL HBV-CORE EPITOPE SPECIFIC CD4+ T-CELLS: A STUDY USING A NOVEL HBV-CORE SPECIFIC HLA-DRB1*0101 TETRAMER FOR THE ANALYSIS OF ANTIVIRAL CD4+ T-CELL RESPONSES DURING ACUTE AND CHRONIC HEPATITIS B

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A strong HBV-specific CD4+ T cell response is associated with spontaneous viral clearance in acute hepatitis B but is weak or short-lived in patients developing chronic disease. So far, this CD4+ T cell response could just be investigated by functional assays which lack the possibility of further T-cell phenotyping and that are unable to identify dysfunctional cells. HBV-specific HLA-class-II tetramers now can negotiate those limitations. To establish a new HBV-core MHC-class-II tetramer we screened 33 patients with acute HBV infection by overlapping HBV-core peptides and identified a HLA-DRB1*0101 restricted HBV-core epitope. This epitope was recognized by all DRB1*0101 patients with acute HBV infection as well as by 77 % (17/22) of all patients with acute HBV. Using this epitope we assembled a new DRB1*0101 MHC-class-II tetramer. The tetramer specifically stained a corresponding CD4+ T cell clone. To proof the tetramer sensitivity dilution experiments of Tet+ clone cells into PBMC were performed and a strong correlation between added and measured frequencies was found. In six HBV-negative DR1+ individuals and nine patients with acute hepatitis B lacking DR1, no CD4+ cells were tetramer positive. In three HLA-DR1+ patients with acute hepatitis B, HBV-specific CD4+ T cells were detectable during the acute phase of disease, ranging from 900 to 1680/106 CD4+ T cells during the peak phase. The peak phase was associated with an A Th peak and viral clearance as well as with a peak level of IFN-γ secretion. The frequencies of HBV specific CD4+ T-cells decreased rapidly after viral clearance but were detectable in considerable levels over several months. In contrast, at different time points of disease 15 investigated patients with chronic hepatitis B showed demonstrate that cross-presentation by LSEC leads to CD8 T cell tolerance in vivo characterized by lack of cytokine expression and lack of in vivo cytolysis. Our findings suggest that T cell tolerance towards circulating antigens is not primarily induced by tolerogenic DC, but rather results from rapid antigen-specific hepatic retention of naive CD8+ T cells by cross-presenting scavenger LSEC. Although tolerant T cells redistributed in the entire organism, LSEC-induced tolerance remained dominant over restimulation of T cells in lymphatic tissue by dendritic cells. This identifies intra-hepatic naive T cell sequestration as the first step in peripheral CD8+ T cell tolerance towards circulating antigen. LSEC constitute the key cell population combining extraordinary scavenger activity with the capacity of cross-presentation and antigen-specific retrieval of naive CD8+ T cells from the circulation for subsequent induction of non-deleterious tolerance. These mechanisms may be amenable to therapeutic manipulation in order to influence autoimmunity or persistence of viral infection.

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145 CROSSPRESENTING LSEC PROMOTE LIVER-SPECIFIC NAIVE CD8 T CELL RETENTION AND TOLERANCE IN VIVO

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Systemically circulating antigen in the absence of inflammation does not cause immunity but tolerance. Likewise, circulating viral antigens may skew virus-specific CD8+ T cell immune responses promoting viral immune escape. Here we demonstrate in a murine system that organ-resident liver sinusoidal endothelial cells (LSEC) but not dendritic cells scavenge circulating antigens from the blood. Subsequently cross-present of circulating antigens by LSEC caused rapid liver-selective and antigen specific retention of naive CD8+ T cells as evidenced by intravital microscopy. Generation of bone-marrow chimeras, where only organ-resident LSEC but not bone-marrow derived immune cells cross-presented circulating antigens to naive CD8 T cells, clearly revealed together with the extraordinary scavenger function for circulating antigens the decisive role of LSEC in antigen-specific hepatic retention of naive CD8 T cells. Using these bone-marrow chimeras as a novel model system we
no or only very low frequencies of tetrameric positive cells. During the course of acute self limiting hepatitis B, phenotypic characterization demonstrated a rapid increase of IL7R and CD62L expression on Tet+/CD4+ T-cells. In conclusion, using a new MHC-class-II tetramer containing one immunodominant HLA-DRB1*0101 restricted epitope within HBV-core, we were able to quantify and characterize HBV specific CD4+ T cell responses patients with acute hepatitis B and chronic hepatitis B. In patients with chronic disease almost no Tet+/CD4+ T-cells were found indicating a complete loss of antiviral CD4+ T-cells rather than a loss of function of those cells. Furthermore those cells showed an impaired capacity to develop a memory phenotype. So far tools which allow to distinguish between the absence of specific cells or their non-function were not available.

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147 A NOVEL THERAPEUTIC HBV VACCINE INDUCES POTENT SURFACE- AND CORE-SPECIFIC IMMUNOGENICITY IN MICE, RHESUS MACAQUES AND HBV TRANSGENIC MICE

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HBV infects approximately 350 million people worldwide, and carriers are at increased risk for cirrhosis and hepatocellular carcinoma. Current HBV therapies do not eradicate HBV and have limited long-term efficacy. We have developed a novel immunotherapeutic vaccine for treatment of chronic HBV infection. The vaccine, composed of HBsAg (HBV surface antigen), HBeAg (HBV core antigen) and a proprietary adjuvant, was designed to induce virus-specific cellular immune responses such as are typically correlated with HBV clearance. Mice immunized with the vaccine exhibited robust cellular and humoral immune responses to both HBeAg and HBsAg. The vaccine induced HBV-specific cell-mediated immunity in both CD4 and CD8 compartments. The total CD8-restricted T cell responses, including antigen-restricted responses directed against both HBeAg and HBsAg (as high 500 SFC/106 cells) were demonstrated using IFN-γ ELISPOT assays. Additionally, CTL lytic activity as measured by Granzyme B release was observed in response to HBeAg and HBeAg peptides. The vaccine also elicited strong anti-HBsAg and anti-HBeAg immunoglobulin responses in both IgG1 and IgG2a isotypes. In rhesus macaques, immunization induced antigen-specific T cells at frequencies as high as 1000 SFC/106 PBMC. These T cells secreted IFN-γ and IL-2 in response to stimulation with either HBeAg or HBeAg peptides in ELISPOT assays. Treated rhesus also exhibited anti-HBsAg IgG levels, greater than 105 mIU/mL after two immunizations. As a model for chronic hepatitis, we are conducting studies of HBV transgenic mice to examine the effect of this vaccine on viral replication, seroconversion, and generation of HBV-specific T cell responses. Preliminary results indicate that the vaccine is effective in breaking immune tolerance, inducing HBV-specific T cell responses and high anti-HBsAg levels simultaneously with a vigorous multivalent cell-mediated T cell response. These data suggest this dual-antigen immunotherapeutic vaccine may be valuable in the treatment of chronic HBV infection. Currently, this vaccine is being tested in Phase 1 human clinical trials.

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Pascale Buchmann - Employee: Other
Eduardo Martins - Employee: Other
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Karl Melber - Employee: Other
Gary Van Nest - Employee: Other
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A COMBINATION OF BETA-GLYCOLIPIDS OVERCOMES
A CD1D DEPENDENT INHIBITION OF NKT LYMPHO-
CYTES: AN ALTERNATIVE FLOTILLIN-2 RAFT PROTEIN-
DEPENDENT REGULATORY CELL ACTIVATION PATHWAY
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CD1-restricted natural killer T (NKT) cells are regulatory lymphocytes that co-express a conserved T-cell and natural killer (NK) receptors. Lipid rafts are highly dynamic, submicroscopic assemblies enriched in sphingolipids and cholesterol. CD1d, a raft-localized receptor, is an essential restriction element in raft glycolipid-mediated activation. Lipid raft disruption inhibits NKT cell activation but does not interrupt ligand loading onto CD1d, in contrast to direct blocking of the CD1d receptor by a monoclonal antibody. Raft disrupters were shown to inhibit IL-
6/STAT3 and IFN-γ/STAT1 signaling suggesting that almost all signaling by these cytokines is initiated in raft microdomains. Purpose: To determine the signaling pathway of NKT activation using naturally occurring beta glycolipids, and its dependence on the CD1d membrane receptor. Methods: NKT hybridoma cells (DN32.D3) were stimulated in vitro using 500ng/ml of beta-glucosylceramide (GC), beta-lactosylceramide (LC), or a 1:1 combination of both (IGL), or PBS. The detergent-insoluble membrane complexes were floated on nycodenz gradient and their GM1 content was analyzed by dot blot. NKT signaling pathway was assessed using western-blot analysis of FLOTILLIN-2, LCK STAT1 and 3. NKT activation was measured using a 3[H] thymi-
dine incorporation assay. To determine the effect of CD1d on the signaling pathway, anti-CD1d monoclonal blocking anti-
bodies were used. Results: Administration of IGL led to a more prominent flotilltin-2 recruitment to the raft fractions (1-2) compared with GC, LC and PBS. No effect was noted on LCK and GM1 in all treatment groups. All glycolipids led to a significant increase in STAT1 expression in the cytosolic factions (8-12), but STAT3 expression was prominent for IGL only. Administration of IGL, resulted in stimulation of NKT cell proliferation, in contrast with an inhibition with GC or LC alone (increase by 18%, versus a decrease by 3% and 10%, respectively, p<0.05). The addition of anti CD1d antibodies resulted in increased cell proliferation for GC, LC, IGL compared with PBS (13%, 27%, and 12% respectively, p<0.05). Conclusion: Administration of the combination of beta-glycolipids, IGL over-
comes the CD1d dependent inhibition of NKT activation by nat-
urally occurring beta glycolipids. An alternative membrane raft FLOTILLIN-2 mediated pathway may be an important mechanism for the effect of these novel compounds on regulatory cells. These data suggest that IGL may serve as a novel immune mod-
ulator in NKT mediated liver disease.

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NATURAL KILLER DENDRITIC CELLS ARE A DISTINCT
CLASS OF HYBRID DENDRITIC CELLS WITHIN THE
MURINE LIVER NK1.1+ CELL POPULATION
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Introduction: Natural killer dendritic cells (NKDC, NK1.1+CD1c+CD3-) are a novel subtype of dendritic cells (DC, NK1.1+CD1c+CD3-) that share with natural killer cells (NK cells, NK1.1+CD1c+CD3) the ability to lyse tumor cells and produce copious amounts of interferon-gamma (IFN-γ). The study of NKDC has thus far been limited to the spleen and the function of NKDC in the liver is unknown. We hypothesized that liver NKDC are the dominant IFN-γ producing DC in the liver and participate in the defense against viral and bacterial hepa-
ritis. Methods: Five color flow cytometry was used to study NKDC in the livers of naive C57BL/6 mice and in mice infected intravenously with 5 x 10¹⁰ particles of unattenuated human adenovirus type 5 (Ad, viral hepatitis model) or 1 x 10⁷ CFU of Listeria monocytogenes 10403S (LM, bacterial hepatitis model). For functional assays, liver NKDC were purified with fluorescence-activated cell sorting. Results: NKDC comprised 39 ± 5% of hepatic NK1.1+CD3 cells and were equally as prevalent as classical liver DC (NK1.1+CD1c+CD3). Liver NKDC had unique cellular morphology and a resting surface phenotype resembling both activated NK cells and immature DC. Liver NKDC were potent lytic cells effecting greater lysis of YAC-1 lymphoma cell targets than liver NK cells; this activity was dependent upon the effector molecule perforin. In vitro, liver NKDC had the capacity to stimulate allogeneic T cells in a mixed leukocyte reaction when activated with the toll-like recep-
tor 9 ligand CpG. In vivo, liver NKDC were able to prime anti-
gen specific CDF T cells in a mouse footpad model. The combination of CpG and IL-18 stimulated liver NKDC to secrete large amounts of IFN-γ in vitro whereas NK cells produced only low levels (55.1 ± 8.0 vs. 1.3 ± 0.6 ng/ml). In response to hepatic infection in vivo, liver NKDC expanded (17-fold with Ad and 8-fold with LM) and upregulated surface expression of natural killer receptors Ly49D and Ly-49C/1 as well as DC cos-
timulatory molecules MHCIi, CD40, CD80, and CD86. Using intracellular cytokine analysis, we determined that liver NKDC respond to viral and bacterial hepatitis by producing IFN-γ and were the dominant producers of IFN-γ in Ad-infected animals 48 hours post infection. Similar experiments performed in IL-12-/ / and IL-18-/ / mice demonstrated that the production of IFN-γ by NKDC was dependent upon these cytokines. Conclusion: Liver NKDC are a distinct component of the hepatic immune system. These multifunctional cells are the principal IFN-γ producing DC of the liver and play an important role in the innate immune response to viral and bacterial hepatitis.

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RAS/P13-K/ Akt PROMOTES CELLULAR GROWTH BY
ENHANCING ALTERNATIVE SPlicing-MEDIATED
INACTIVATION OF THE KL6 TUMOR SUPPRESSOR IN
HUMAN HEPATOCELLULAR CARCINOMA
Steven Yea, Goutham Narla, Xia Zhao, Augusto Villanueva, John A. Martignetti, Josep M. Llovet, Scott L. Friedman; Mount Sinai School of Medicine, New York, NY

Background & Aims: Hepatocellular carcinoma is the fifth most prevalent cancer worldwide and the third most lethal. Dysregu-
lation of alternative splicing underlies a growing number of
human neoplasms, yet its contribution to HCC has not been explored. The KLF6 tumor suppressor is a zinc finger transcription factor that inhibits cellular growth by either transcriptional activation of p21, antagonism of c-jun, and/or sequestration of cyclin D1. KLF6 function is abrogated in several human cancers due to increased alternative splicing that yields a dominant negative isoform, ‘KLF6 SV1’, which antagonizes full length KLF6 (‘KLF6Full’) mediated growth suppression. The molecular basis for increased KLF6 splicing is unknown. Methods & Results: KLF6 isoform-specific qRT PCR was developed to quantify KLF6 alternative splicing in human cell lines and tissues. In 67 human HCCs, there was a significant correlation (p < 0.05) between activated Ras signaling, as measured by H-Ras mRNA overexpression, and increased KLF6 alternative splicing, as assayed by the ratio of KLF6Full to KLF6 SV1. In cultured cells, ectopic activation of Ras via PMA or plasmid transfection, increased the expression of KLF6 SV1 ~3 to 4 fold relative to KLF6Full, thereby enhancing cellular proliferation. Abrogation of oncogenic Ras-signaling by siRNA or a farnesyl-transferase inhibitor (FTS) decreased KLF6 SV1 ~50-80% and suppressed growth. Moreover, HCT116 and DLD1 colon cancer cell lines, which have one oncogenic K-Ras allele deleted by homologous recombination, express 40-60% less KLF6 SV1 than their parental lines containing two Ras alleles. Growth inhibition by FTS in transformed cell lines was rescued by forced expression of KLF6 SV1, indicating that SV1 partially mediates Ras’s growth promoting phenotype. Downregulation of the splice factor ASF/SF2 by siRNA increased KLF6 SV1 mRNA levels, suggesting a requirement for ASF/SF2 in KLF6Full production. Analysis of the two major signaling cascades downstream of Ras, MAP kinases and PI3-kinase using siRNAs and chemical inhibitors, implicates PI3-K/Akt in regulating enhanced KLF6 splicing. Finally, promoter-luciferase assays indicated that KLF6 alternative splicing occurs independently of its transcription. Conclusions: We have functionally linked oncogenic Ras signaling to increased splicing of KLF6 through signaling by PI3 kinase and Akt, mediated by the splice regulatory protein ASF/SF2. Our findings expand the role of both Ras and KLF6 in human HCC by identifying a novel mechanism of tumor suppressor inactivation through increased alternative splicing mediated by an oncogenic signaling cascade.

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The following people have nothing to disclose: Steven Yea, Goutham Narla, Xiao Zhao, Augusto Villanueva, John A. Martignetti, Josep M. Llovet, Scott L. Friedman

mimic human disease. To examine the significance of cytokine-Stat5 signaling in the prevention of liver fibrosis and disease, control mice and mice from which the Stat5 locus had been deleted in hepatocytes using Cre-mediated recombination were treated for 8 weeks with CCI4. While strong activation of Stat5 was observed in hepatocytes of control mice upon stimulation with growth hormone (GH), reduced activation was detected after CCI4 treatment, suggesting that cells lose their response to cytokines during early stages of fibrosis. Histological analyses revealed that Stat5 mutant mice displayed severe progression of liver fibrosis compared to control mice. Surprisingly, hepatic TGF-beta levels were greatly elevated in Stat5-ko mice after 8 weeks of treatment with CCI4. To define the molecular link between Stat5 silencing and TGF-beta upregulation, we examined mouse embryo fibroblasts from Stat5-null and control mice. In vitro experiments demonstrated that TGF-beta levels were highly elevated in the absence of Stat5 whereas TGF-beta mRNA levels remained unchanged. Protease inhibitor studies revealed that Stat5 deficiency enhances the stability of mature TGF-beta protein. These results indicate that deletion of Stat5 causes upregulation of TGF-beta protein through changing its stability.

Conclusions: This study provides evidence for a novel role of Stat5 in the development of liver fibrosis through changing the stability of TGF-beta protein. We propose that cytokine-Stat5 signaling confines the levels of TGF-beta, which in turn is protective for liver fibrosis.

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153 TGF-β PATHWAY MEMBERS SMAD3 AND ELF ARE KEY REGULATORS OF HEPATOCELLULAR CANCERS

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The transforming growth factor-beta (TGF-β) pathway constitutes a central signaling network that plays an essential role in cell differentiation, proliferation, motility, apoptosis and tumorigenesis. TGF-β signals are conveyed through type I/II receptors to specific intracellular mediators known as SMAD. Activation of SMAD3, a receptor activated SMAD, results in nuclear translocation and activation of target genes. We have shown that the adaptor protein ELF plays a key role in TGF-β signaling through SMAD3 and SMAD4 (Science, 2003, 299:574-577). Further analysis has revealed that over 40% of elf+- mice spontaneously develop hepatocellular carcinoma (HCC) at the age of 12-15 months. SMAD3 has long been suspected as a major component in tumor suppression; however, studying this potential role remains elusive. Aims: 1. To investigate the importance of the ELF-SMAD3 interactions in tumor suppression. 2. To determine whether elf somatic mutations occur in human HCC. Methods: 1. We generated elf+/-/smad3+-/- double heterozygous mice. 8 heterozygous mice were dissected at the age of 10 months; tumors from various tissues were collected; RNA was isolated from each tumor tissues and was used to synthesize cDNA. The cDNA obtained was used to examine p53 mutation or to perform qPCR analysis to test the alteration of the gene expression. 2. One gastric cancer (GC) and three HCC cell lines were cultured in DMEM. cDNA was prepared and used to amplify the exons of elf to examine their mutation. Results: 1. The elf+/-/smad3+-/- mice strikingly produce high frequency of tumors in organs of mostly endodermal lineage including liver,
pancreas, stomach, small intestine, lung, thymus, and kidney. 2. Of the 28 tumor tissues, only two shows p53 point mutations replacing amino acids. Statistically, this demonstrates the existence of very low frequency of p53 mutations compared to the other reported cases of GCs and HCCs. 3. All the mice tumor tissues show dramatic decrease in elf mRNA levels (200- to 2000-fold) compared to its non-tumor tissues. In contrast, p53 mRNA levels were relatively stable in all the tissue samples. 4. SNU475, an HCC cell line shows an Arg to Ile mutation at exon 15 of elf locus. Conclusion: 1. These studies suggest that the ELF/SMAD3 interaction is a primary and powerful event in a major tumor suppressor pathway for multiple cancers that includes HCC. Loss of this interaction in whole animal system leads to multiple tumors. 2. These studies also indicate that p53 mutation is not a major secondary event during the elf-mutation-induced tumorigenesis. 3. Thus, potentially TGF-β pathway can be utilized as a predictor for HCC formation and treatment prognosis.

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REGULATION OF WNT/β-CATENIN PATHWAY BY CPLA2α-MEDIATED PPARδ ACTIVATION IN HUMAN CHOLANGIOCARCINOMA CELLS

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Cytosolic phospholipase A2α (cPLA2α) is a rate-limiting key enzyme that releases arachidonic acid (AA) from membrane phospholipid for the production of prostaglandins via cyclooxygenase-2. Our previous studies have shown an important role of cPLA2α in human cholangiocarcinoma growth (Hepatology 2002; 36:363-373; J. Biol. Chem. 2004, 279:44344-44354; J. Biol. Chem. 2006, 281:24831-24846; J. Biol. Chem. 2006, 281:33982-33995). Here we describe a novel role of cPLA2α for activation of peroxisome proliferator-activated receptor-δ (PPARδ) and β-catenin in human cholangiocarcinoma cells. Overexpression of cPLA2α induced the binding of PPARδ to β-catenin and increased their association with the TCF/LEF response element. These effects were inhibited by the cPLA2α siRNA and inhibitors as well as by siRNA knockdown of PPAR. Overexpression of PPARδ or treatment with the selective PPARδ ligand, GW501516, also increased β-catenin binding to TCF/LEF response element and increased its reporter activity. Addition of AA and GW501516 to nuclear extracts induced a comparable degree of β-catenin binding to TCF/LEF response element. Furthermore, cPLA2α protein is present in the PPARδ and β-catenin binding complex, suggesting that PPARδ may directly import the cPLA2α into the nucleus. Thus the close proximity between cPLA2α and PPARδ provides a unique advantage for their efficient functional coupling in the nucleus, where AA produced by cPLA2α becomes immediately available for PPARδ binding and subsequent β-catenin activation. These findings disclose a novel connection linking the cPLA2α, PPARδ and Wnt/β-catenin signaling pathways in human cholangiocarcinoma cells and suggest that these molecules may be targeted for future chemoprevention and treatment.

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POTENTIATION OF PERIPHERAL INSULIN SENSITIVITY IN OB/OB MICE BY BETA-GLYCOLIPIDS IS MEDIATED BY IRS-1 MTOR SIGNALING PATHWAYS: A NOVEL THERAPEUTIC TARGET FOR NASH

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The serine threonine kinase mTOR is regulated by nutrients and growth factors. Activation of mTOR and its downstream target S6K1 results in down regulation of insulin signaling. It was previously shown that naturally occurring beta-glycolipids alleviate the metabolic derangement of NASH. Aim: To study whether the beneficial effect of beta-glycolipids is mediated via the mTOR/S6K1 pathway. Methods: Four groups of ob/ob mice were treated by oral administration of beta-glycosylceramide (GC), beta-lactosylceramide (LC), GC and LC together or with PBS alone. The effect of the different glycolipids on the metabolic state of the treated animals was assessed by an i.p. glucose tolerance test (GTT) and measurement of fasting serum insulin, lipids, liver enzymes and TNF-alpha after treatment for 45 days. Liver steatosis was determined by Oil-red-O staining. The effects of glycolipids on insulin signaling were studied by immunoblot analysis of IRS-1, mTOR, AKT/PkB and S6K phosphorylation in liver extracts of treated animals. Results: Treatment with beta-glycolipids decreased mTOR and S6K phosphorylation. This was associated with markedly increased total IRS1 and a decreased IRS1 serine 636/639 phosphorylation, corrected for total IRS1. Moreover, AKT/PkB phosphorylation was significantly increased indicating that liver insulin action was enhanced. These effects were associated with a marked improvement of glucose tolerance in animals treated with GC and the combination of GC and LC, but not with LC alone. The beneficial effect was manifested by decreased serum insulin levels (81.33 and 56.4 vs. 131.59 and 103.21 microgram/L for GC and the combination, compared to the LC group and PBS, respectively). Alleviation of liver injury was noted by decreased liver enzymes and by markedly reduced TNF-alpha serum levels (62 and 105 vs. 181 and 155 pg/ml in GC and the combination compared with LC and PBS groups, respectively); reduced hepatic steatosis as evidenced by Oil-red-O staining showed an improvement in the lipid profile noted by increased HDL cholesterol (3.76 of 4.5 mmol/l total cholesterol vs. 3.6 of 5 mmol/l total cholesterol, in GC vs. PBS, respectively). Conclusion: beta-glycolipid treatment inhibited the mTOR/S6K1 pathway, thereby improving insulin sensitivity. These effects were associated with significant amelioration of the metabolic abnormalities in NASH and reduced serum TNF-alpha levels. Inhibition of the mTOR pathway may serve as a novel therapeutic target for this disease.

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IMPACT OF HEPATOCYTE SPECIFIC LOSS OF C-MET SIGNALING ON LIVER REGENERATION

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c-Met is a receptor tyrosine kinase for hepatocyte growth factor (HGF). Previous work has established that HGF plays a pivotal role...
role in regulating the onset of S phase and DNA replication following partial hepatectomy. We used c-Met conditional knockout mice in which c-met gene is inactivated in postnatal hepatocytes by Alb-Cre recombinase (MetLivKO) to directly evaluate the biological outcome of a selective loss of c-Met in hepatocytes on liver regeneration. The priming events appear to be intact in MetLivKO livers. Up-regulation of stress response (e.g. MAFK, IKBZ, SOCS3) and early growth response (e.g. MYC, DUSP1 and 6) genes as judged by microarray profiling was similar in MetLivKO and Alb-Cre control regenerating livers. This was consistent with an early induction of NF-kB, STAT3, and MAPK/ERK. Nevertheless, in the absence of c-Met signaling in the hepatocytes, ERK phosphorylation rapidly declined although it remained high in Alb-Cre control livers. Also, MetLivKO mice displayed impaired liver regeneration as determined by a decrease in BrdU incorporation and a delay in timely progression into mitosis. Upstream signaling pathways involved in the block of G2/M transition included lack of EGR1 transcription factor induction, and inability to increase cdc2, aurora B and Mad2 expression followed by decreased histone 3 phosphorylation and lag in chromatin condensation. However, after a delayed passage through G2 phase, c-Met deficient cells eventually entered mitosis. In culture, EGF treatment increased proliferation of MetLivKO hepatocytes and restored expression levels of cell cycle regulators aurora B and Mad2 albeit to a lesser degree than in similarly treated Alb-Cre control hepatocytes. In conclusion, our results assign a novel function for HGF/c-Met signaling in the regulation of G2/M transition during liver regeneration and implicate EGR1 as a potential G2/M target of HGF/c-Met pathway.

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157 DIFFERENTIAL EFFECTS OF JNK1 AND JNK2 ON THE DEVELOPMENT OF STEATOHEPATITIS
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Prior studies in the murine methionine- and choline-deficient diet-induced model of steatohepatitis demonstrated that a genetic knockout of the c-Jun N-terminal kinase (JNK) isoform JNK1, but not JNK2, decreased the development of steatosis and liver injury. However, the function of JNK1/2 in an obese/insulin resistant model of steatohepatitis, and the ability of JNK inhibition to reverse established NAFLD, were not determined. The aim of this study was to examine the effect of inhibition of JNK1/2 function on the development and treatment of high fat diet (HFD)-induced steatohepatitis. Wild-type C57BL/6 (WT), jnk1−/− and jnk2−/− mice were fed a regular diet (RD) or HFD. After 16 weeks of HFD, jnk1−/− mice had significantly decreased body weights, liver weights, serum glucose and insulin levels, and HOMA as compared to WT and jnk2−/− mice. Hepatic triglyceride (TG) content was equivalent in both RD-fed WT mice and HFD-fed jnk1−/− mice (11.1 vs. 10.0 ug/mg), but increased 10-fold with HFD in WT and jnk2−/− mice (110.2 and 108.3). The histological grade of steatosis was also significantly decreased in HFD-fed jnk1−/− mice from that in WT mice (0.4 vs. 2.4). Liver injury was significantly reduced in HFD-fed jnk1−/− mice as compared to WT mice by measures of ALT (57 vs. 153 IU/ml), numbers of TUNEL positive cells (1.0 vs. 4.4 per HPF), and inflammation score (0.5 vs. 1.1). To determine whether JNK1 inhibition could reverse established steatohepatitis, WT mice were injected with control, JNK1 or JNK2 antisense oligonucleotides (ASO) after 12 weeks of HFD, a point at which stable steatohepatitis was established. JNK1 ASO partially reversed the steatohepatitis as indicated by decreases in hepatic TG (26.9 vs. 48.0), ALT (44 vs. 76) and TUNEL positivity (1.8 vs. 3.9) as compared to control ASO injected mice. JNK2 ASO injected mice had increased TG content (61.8), ALT (6,334), and TUNEL positivity (11.8) with histological evidence of lobular disarray, inflammation and apoptosis. Immunoblots revealed increased levels of proapoptotic Bim in JNK2 ASO-injected mice on HFD. Bim mediated the increase in hepatitis as HFD-fed, JNK2 ASO-injected bim−/− mice had significantly less liver injury. In conclusion, JNK1 mediates the development of HFD-induced steatohepatitis and JNK1 inhibition decreases both established steatosis and hepatitis. In contrast, JNK2 is not involved in the development of this disease and JNK2 inhibition markedly worsens existing disease through Bim up regulation. Targeting the metabolic syndrome or NAFLD with anti-JNK therapy may therefore require selective inhibition of JNK1 and not JNK2 function.

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158 A PIVOTAL ROLE FOR CD154 IN LIVER STEATOSIS
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Aim: Liver steatosis is a first pathogenic step in non-alcoholic fatty liver disease and is regulated by inflammatory signals. IL-6 is thought to play an essential role in fat processing in the liver. CD154 is a major inducer of inflammatory mediators, among which is IL-6, through triggering of its receptor, CD40. Our purpose was to investigate the contribution of CD154 in liver steatosis. Animal model: We developed a mouse model of steatosis by olive oil force-feeding. Male Balb/c CD154 KO mice were generated from male B6/C CD154 KO mice (Jackson laboratory). Results: [1] Major liver steatosis occurs in CD154 KO animals. Fat diet led to obvious steatosis in CD154 KO mice, in contrast to the results with wild type (WT) mice (n = 10 and 5 groups of mice analyzed at 3 and 10 weeks, respectively). The difference was consistent in each group studied and readily apparent on macroscopic examination of livers. CD154 KO livers showed an important centrilobular macrovesicular steatosis on Haematoxylin-Eosin stained tissue sections, lipid accumulation being confirmed by oil-red staining. There were no foci of lobular inflammation in either setting. The difference in fat processing between WT and KO animals was further shown by comparing perigonadal fat pad storage (decreased fat pad weight by an average of 3.5 fold in KO mice, n = 6), showing that CD154 KO mice failed to export lipids from their liver. [2] The expression of Interleukin-6 (IL-6) mRNA is reduced in CD154 KO mice livers. As IL-6 controls fat processing in the liver and is induced by CD154 in cells expressing CD40, we investigated IL-6 mRNA expression by quantitative PCR in livers of both groups. There was a profound reduction of IL-6 mRNA expression in CD154 KO livers (average of 6-fold [n = 10]). [3] Hepatic Stellate Cell (HSCs) activation is absent in CD154 KO mice. HSC activation was assessed by the neo-expression of α-smooth muscle actin. In WT animals, HSCs were diffusely activated; in contrast, there was a lack of
HSC activation in CD154 KO animals. Conclusions: CD154 is therefore pivotal in liver steatosis. We propose that CD154 represents a link between lipid metabolism and the inflammatory response in the liver and that IL-6 is in the pathway. Absence of CD154 is accompanied by a lack of HSC activation. We hypothesize a model in which CD154 is essential to the activation of CD40-expressing cells, notably HSCs, Kupffer and endothelial cells and subsequent increased production of IL-6. Upon activation of these cells, an IL-6-dependent loop helps processing of lipids by the hepatocytes. Absence of CD154 leads to a lower IL-6 production and defective handling of the triglycerides by the liver.

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159 TRANSCRIPTION FACTOR NRF2 REGULATES HEPATIC LIPID METABOLISM THROUGH ORPHAN NUCLEAR RECEPTOR SHP
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Background: Nuclear factor erythroid-2 related factor 2 (Nrf2) plays a pivotal role in cytoprotection against endogenous oxidative stress and exogenous xenobiotics in the liver. However, its role in lipid homeostasis has not been explored before. The aim of this study is to elucidate the potential function of Nrf2 in hepatic lipid metabolism. Methods: Nrf2 knockout mice were used as in vivo mouse models. Assays include: LC- GC (liquid and gas chromatography) analysis of serum and hepatic lipid metabolites, real-time PCR, hepatic oil red O and H&E staining, GTT and ITT, in vitro islet perfusion, lipase activities, VLDL production, TG clearance, transient transfection, gel-shift, mutagenesis, ChIP assay, adenosine transduction, RNAi interference, hepatocyte culture, Northern and Western blots. Results: Changes in serum and hepatic lipid content was not evident in 2 month old Nrf2KO mice. However, LC-GC analysis revealed markedly decreased hepatic triacylglycerol, but increased level in cholesterol ester in 6 month old Nrf2KO. In contrast, no changes of free fatty acids, lysophosphatidylcholine, phosphatidylcholine and phosphatidylethanolamine were observed between wild-type and Nrf2KO. Neutral lipid staining using oil-red O appeared to be smaller and lighter in Nrf2KO hepatocytes as compared to wild-type mice. Interestingly, the Nrf2KO exhibited significantly increased glucose tolerance and insulin sensitivity as examined by GTT and ITT, which was strongly associated with increased expression of genes in glucose metabolism and insulin sensing in the liver. Both RT-PCR and real-time PCR analysis revealed that genes in lipid synthesis were generally downregulated, whereas genes in lipid oxidation were generally upregulated. Surprisingly, challenging mice with high fat diet diminished the differences in liver lipid phenotype observed in control diet fed wild-type and Nrf2KO mice. Among nuclear receptor genes that were examined, the small heterodimer partner SHP expression was markedly decreased in Nrf2KO liver. By using transient transfection, mutagenesis, ChIP assay and gel-shift assay, we demonstrated that Nrf2 was a potent transcriptional activator of SHP gene expression. Thus the decreased hepatic lipid observed in Nrf2KO may be due to a secondary effect of decreased SHP function. Conclusions: We conclude that Nrf2 may play an important role in regulating hepatic lipid homeostasis through indirect targeting of nuclear receptor SHP and other lipid metabolic regulators. [Grant support: Liver Scholar Award from AASLD/ALF, NIH 1P20 RR021940, Junior Faculty Award from ADA, Pilot Award from KMCRI, BGIA Award from AHA]

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160 PROBIOTICS IMPROVE HIGH-FAT DIET INDUCED STEATOSIS AND INSULIN RESISTANCE THROUGH MODULATION OF HEPATIC NKT CELLS
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Background: Obesity and its associated conditions, such as fatty liver disease and insulin-resistance, have increased rapidly. Among various factors that contribute to obesity-related diseases, dietary macronutrients and intestinal bacteria play an important role. High fat diets induce both obesity and insulin-resistance in experimental animals and humans. Germ free mice colonized with gut bacteria from obese mice gain a greater amount of weight than mice colonized with gut bacteria from lean mice. Probiotics, live microbial food supplements, help maintain the balance of intestinal micro-flora. Our previous study showed that probiotic therapy improved steatohepatitis and insulin resistance in leptin deficient, ob/ob mice. In the current study, we evaluated the effect of probiotics on high fat diet induced obesity, fatty liver and insulin resistance. Methods: Wild type C57BL6 mice were fed high fat diet to induce obesity, insulin resistance and hepatic steatosis. Some high fat diet fed mice also received probiotics (VSL#3) via oral lavage. Liver histology, glucose tolerance test and hepatic NKT cell content were evaluated at different time points. In addition, hepatic cytokines, IKKbeta activity and insulin signaling were also evaluated. Results: High fat diet induced a hepatic NKT cell depletion that preceded the formation of insulin resistance and hepatic steatosis. Adoptive transfer of NKT cells also improved insulin resistance and steatosis. The effects of probiotics on high fat induced insulin resistance and steatosis were diminished in CD1d knock out mice that lack NKT cells. In addition, high fat diet also increased the expression of the proinflammatory cytokine, tumor necrosis factor-alpha, that activated IKKbeta and reduced the sensitivity of insulin signaling, which were all reversed by either probiotic treatment or adoptive transfer NKT cells. Conclusions: Probiotics improve high fat diet induced steatosis and insulin resistance. These effects of probiotic are likely due to modulation of hepatic NKT cells to inhibit inflammatory signaling. These observations support the concept that dietary factors and intestinal bacteria promote hepatic insulin resistance and NAFLD. Results from our current study may have profound therapeutic implications for the management of obesity-related fatty liver and insulin-resistance.

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RATS SELECTIVELY BRED FOR LOW AEROBIC CAPACITY DEVELOP NONALCOHOLIC FATTY LIVER DISEASE

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Non-alcoholic fatty liver disease (NAFLD) has been linked to a sedentary lifestyle and low aerobic fitness. There is a paucity of knowledge detailing the link between low aerobic fitness and susceptibility for NAFLD. We previously reported a novel model in which rats were artificially selected over several generations for high and low exercise endurance capacity (Science, 307:418-20, 2005). The selected breeding resulted in high capacity runners (HCR) and low capacity runners (LCR) with robustly different aerobic fitness levels. Because these rats intrinsically display contrasting phenotypes, we chose this model to assess the role of aerobic fitness in the development of NAFLD. Sedentary (only cage activity) normal chow-fed LCR and HCR rats were sacrificed at ~25 weeks of age (generation 17; max running distance equaled 1,514±91 vs. 200±12 meters for HCR and LCR, respectively). LCR rats displayed metabolic syndrome characteristics including elevated plasma insulin and triglycerides, heavier body weights, and larger adipocyte pad weights and cell volumes compared to the HCR rats. LCR rats also had higher liver triglycerides (6.00±0.71 vs. 4.20±0.39 nmol/g, p =0.020 value), a higher steatosis score (P=0.004), and >2-fold higher percentage of hepatocytes associated with lipid droplets (54.0±9.2 vs. 22.0±3.5%, p=0.006) than the HCR rats, demonstrating evidence of early steatosis in the LCR rats. The LCR livers also displayed a lipogenic phenotype with higher protein content of both sterol regulatory element binding protein-1c and acetyl CoA carboxylase. In addition, the LCR livers displayed reduced mitochondrial oxidative capacity as shown by lower β-hydroxyacyl-CoA dehydrogenase and citrate synthase enzyme activities. Further, analysis of hepatic 4-hydroxynonenal levels revealed a significant increase in hepatic lipid peroxidation in the LCR compared to the HCR rats (P=0.04). In conclusion, normal chow-fed LCR rats display early development of NAFLD, while HCR rats show no abnormalities. These data support a link between low aerobic fitness and NAFLD and demonstrate the usefulness of this novel model to study underlying molecular mechanisms.

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CB2 RECEPTOR ANTAGONISM REDUCES DIET-INDUCED OBESITY, INSULIN RESISTANCE AND HEPATIC STEATOSIS

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Recent data have established the crucial role of inflammation in obesity, insulin resistance and metabolic steatosis. Growing evidences indicate that the endocannabinoid system plays a major role in obesity and steatosis via CB1 receptors. However, the possible contribution of the cannabinoid receptor CB2 has not been reported. Interestingly, CB2 receptors are predominantly expressed in inflammatory cells and regulate immune and inflammatory responses. Therefore, in the present study, we investigated the role of CB2 receptors in insulin resistance and hepatic steatosis, owing to the use of CB2KO and wild type mice subjected to a high fat diet for 15 weeks. After 15 weeks of high fat diet, WT mice exhibited marked induction of CB2 receptor expression in white epididymal adipose tissue, and unchanged levels in the liver. Strikingly, body weight was significantly lower in CB2 KO mice [41.6 ±1.3g for CB2/- vs 46.7±1.2g for WT, p<0.05], despite similar food intake. As expected, WT mice developed insulin resistance as assessed by elevated serum insulin levels, increased HOMA-IR and reduced insulin tolerance tests. In contrast, these parameters were significantly improved in CB2 KO animals, indicating reduced insulin resistance. Finally, whereas WT mice developed severe fatty liver, as shown by histological analysis of liver tissue sections and increased hepatic triglycerides, hepatic CB2 KO mice exhibited minimal hepatic steatosis. We also investigated the consequences of CB2 antagonism on inflammation. As expected, obese WT mice displayed increased inflammation in the white epididymal adipose tissue and in the liver, as indicated by a strong increase in the density of macrophages and a parallel induction of TNF-a and MCP-1 mRNA expressions. In contrast, high fat diet-fed CB2/- mice showed significantly lower inductions of F4/80, TNF-a and MCP-1 mRNAs in white adipose tissue and in the liver. In conclusion, our results unravel a novel role for CB2 in the pathogenesis of obesity, insulin resistance and hepatic steatosis induced by a high fat diet, by a mechanism that probably involves a proinflammatory effect of CB2 receptors in the adipose tissue. These data identify CB2 as a potential novel therapeutic target for the treatment of liver injury associated to the metabolic syndrome.

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163 EXCELLENT LONG-TERM OUTCOME FOLLOWING DOWNSTAGING OF HEPATOCELLULAR CARCINOMA PRIOR TO LIVER TRANSPLANTATION: AN INTENTION-TO-TREAT ANALYSIS

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BACKGROUND: We previously reported encouraging results of down-staging of hepatocellular carcinoma (HCC) to meet conventional criteria for orthotopic liver transplantation (OLT) (Liver Transpl 2005; 11:1505). In this ongoing prospective study, we present longer follow-up data in a larger cohort.

METHODS: Between June 2002 and January 2007, 61 patients with tumor stage exceeding conventional T2 criteria were enrolled in a down-staging protocol. The eligibility criteria for down-staging included 1 lesion > 5 cm but within 8 cm, 2 or 3 lesions at least one > 3 cm but all within 5 cm with total tumor diameter up to 8 cm, or 4 to 5 nodules none greater than 3 cm, with total tumor diameter within 8 cm. Imaging criteria for successful down-staging included a decrease in tumor size to within T2 criteria, or complete tumor necrosis with no contrast enhancement. Patients were eligible for living-donor liver transplantation (LDLT) if the tumors were down-staged to within the proposed UCSF criteria (1 lesion up to 6.5 cm, or 2 to 3 lesions none greater than 4.5 cm with total tumor diameter within 8 cm). A minimum follow-up period of 3 months after down-staging was required before OLT.

RESULTS: Down-staging treatments included trans-arterial chemoembolization (TACE) alone in 15, laparoscopic or open radiofrequency ablation (RFA) alone in 11 patients, TACE combined with percutaneous ablation in 15, laparoscopic RFA plus TACE in 14, and resection in 6 patients. By intention-to-treat analysis, tumor down-staging was successful in 43 patients (70.5%). Thirty-five patients (57.4%) had received OLT, including 2 who had LDLT. Six patients were still awaiting OLT. Follow-up was censored in 2 other patients who had successful down-staging but were excluded for OLT for other reasons. Treatment failure was observed in 18 patients (29.5%), including 3 deaths without OLT, and 15 dropouts due to tumor progression. In the explant of 35 patients who underwent OLT, 13 had complete tumor necrosis, 17 met T2 criteria, and 5 exceeded T2 criteria. The Kaplan-Meier intention-to-treat survival at 1 and 4 years after down-staging was 87.5% and 69.3%, respectively. The 1- and 4-year post-transplant survival rates were 96.2% and 92.1%, respectively. None had HCC recurrence after a median follow-up of 25 months after OLT (range 1.7-57 months). In univariate analysis, the only factor predicting treatment failure was pre-transplant alpha-fetoprotein > 1000 ng/mL (HR 7.8, p<0.001).

CONCLUSION: In carefully selected patients, down-staging of HCC to meet conventional criteria for OLT can be achieved in the majority of patients, and is associated with excellent post-transplant outcome.

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A PROSPECTIVE MULTICENTRIC FOLLOW-UP STUDY ON 105 PATIENTS WITH ACUTE PORTAL VEIN THROMBOSIS (PVT): RESULTS FROM THE EUROPEAN NETWORK FOR VASCULAR DISORDERS OF THE LIVER (EN-VIE)

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Extra hepatic portal hypertension (PHT) and intestinal infarction are potentially lethal complications of acute PVT. Current recommendation for early anticoagulation is based on extremely limited, retrospective data. Aims: to assess prospectively the extent of, and predictive factors for recanalization of acute non-cirrhotic, non-malignant PVT. Between 2003 and 2006, patients were prospectively enrolled in 9 European countries, if there was radiographic evidence for a recent thrombus in the portal vein trunk or one of its main branches, and absence of cavernoma. Expert radiological review and centralised etiological work-up were obtained. Patients were followed from date of diagnosis until death or last visit. End points were (i) relief of extrahepatic PHT (i.e. patency of portal vein trunk and one of its main branches); and (ii) bleeding, intestinal infarction, or death. Results: 105 eligible patients (53 males, median age 49 years) were followed-up a median of 229 days (range 0-904 days). At diagnosis, extrahepatic PHT was present in 91 (87%) patients, and complete obstruction including mesenteric and splenic veins in 29 (28%). A local factor was found in 25% of patients; prothrombin gene mutation in 19%; V617F-JAK2 mutation in 14%; a myeloproliferative disease in 8% of JAK2-wild type patients; factor V Leiden in 14%; protein S deficiency 9%; C677T homozygous MTHFR mutation in 4%, and oral contraceptive use in 22%. Early anticoagulation was given to 97 patients, with a median delay of 0 days (range -7 to 52), and TIPS inserted in 1 patient. At 1-year follow-up, relief of extrahepatic PHT was obtained in 44% of treated patients. Independent predictive factors for the absence of relief of extrhepatic PHT was ascites (assessed with radiological procedure) (HR= 3.0, 95% CI: 1.2-8.1). The one year recanalization rate was of 38% in patients with ascites vs 65% without (p=0.02).Type and number of prothrombotic disorders failed to predict outcome. On anticoagulation, non-lethal bleeding occurred in 9 patients (gastrointestinal 5, nasal 2, procedure-related 2), and intestinal infarction in none. Two patients died, 1 from sepsis and 1 from late malignancy. Conclusion In this cohort, anticoagulation for acute PVT relieved PHT in 44% of patients at one year, and no intestinal infarction was seen in all, independently from causes. Non-lethal bleeding occurred in 9%, and mortality was 2%. These data support a recommendation for early anticoagulation. Nevertheless, at one year 62% of our patients with ascites had persisting extrahepatic PHT despite anticoagulation, and may therefore be candidates to test alternatives procedures.

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THE PLASMA LIPIDOMIC SIGNATURE OF NONALCOHOLIC STEATOHEPATITIS: DIFFERENTIAL LEVELS OF N-3 AND N-6 POLYUNSATURATED FATTY ACIDS AND THEIR LIPOXGENASE PRODUCTS

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BACKGROUND: Nonalcoholic steatohepatitis (NASH) is characterized by increased hepatic triacylglycerol, free cholesterol (FC):phosphatidylcholine (PC) and n-6-n-3 polysaturated fatty acid (PUFA) ratio (Puri et al, Hepatol, 2007 in press). The plasma lipidomic signature and its diagnostic utility in NASH are unknown. AIMS: To define (1) the plasma lipidomic profile in subjects with NASH and compare it to nonalcoholic fatty liver (NAFL) and lean normal controls, (2) the basis for changes in plasma PUFA, and (3) the diagnostic utility of these changes for NASH. METHODS: NAFL and NASH were identified by liver biopsy and clinical assessment. Plasma lipid classes and their fatty acid composition were measured by GC-FID and inflammatory lipids including products of cyclooxygenase (COX), lipoxgenase (LOX) and cytochrome p-450 (Cyp) activities were measured by LC-MS. RESULTS: A total of 52 subjects were studied (normal:NAFL:NASH 12:10:30). Lipidomic profile: NASH was associated with significantly decreased PC (2165:1838:1787 nmole/g, p< 0.001) and higher FC:PC ratio (0.6:0.6:0.7, p< 0.02) relative to normal. Total n-3 PUFA (nmole/g) were specifically depleted in NASH (428:491:340, p< 0.02 NASH vs normal and NAFL) resulting in increased n-6:n-3 ratio in NASH (10.7:9.5:12.8, p< 0.04). PUFA metabolism: The linoleic acid (LA):α-linolenic acid (ALA) ratio in free fatty acid was significantly increased in NASH (11.7:13:14.9, p<0.0005 NASH vs normal). Although ALA was decreased, mead acid was not increased indicating an absence of dietary deficiency. N-6 changes: Arachidonic acid (AA):dihomogamalaminolenic acid (DGLA) was decreased in NASH suggesting decreased Δ5-desaturase activity or increased utilization. Pro-inflammatory LOX products of AA (hydroxyeicosatetraenoic acids [HETE]; 8HETE, 9HETE, 11HETE, 15HETE) were increased (p< 0.05 for all) in NASH; COX products were
unchanged and 8.9 diHETE, a Cyp product of AA was increased. N-3 changes: ALA levels were decreased in NASH while eicosapentaenoic acid was unchanged. Docosahexanoic acid (DHA), a downstream n-3 PUFA, was decreased significantly in all lipid classes in NASH. 19,20 diHDPA, a Cyp product of DHA, was also decreased in NASH. Diagnostic utility: An n-6:n-3 ratio ≥ 12.5 separated NASH from other groups (AUROC 0.77, + likelihood ratio 11.4, p< 0.0001). CONCLUSIONS: NASH is associated with increased FC:PC and n-6:n-3 PUFA in plasma. N-3 changes: ALA levels were decreased in NASH. Diagnostic utility: An n-6:n-3 ratio may be useful as a diagnostic tool for NASH.

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167 ROBUST SYNERGISTIC ANTIVIRAL EFFECT OF R1626 IN COMBINATION WITH PEGIFN-ALFA-2A (40KD), WITH OR WITHOUT RIBAVIRIN – INTERIM ANALYSIS RESULTS OF PHASE 2A STUDY

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The novel nucleoside, R1626, is a potent inhibitor of the HCV RNA polymerase. When administered as monotherapy to patients with chronic hepatitis C, R1626 demonstrates dose-dependent decreases in HCV RNA blood levels. This Phase 2a study evaluated the safety and efficacy of R1626 administered for 4 weeks in combination with 180 µg peginterferon alfa-2a (PEG-IFNα-2a) ± 1000–1200 mg ribavirin (RBV) in HCV genotype 1 treatment-naïve patients. 104 patients were randomized to: Dual Low: 1500 mg bid + PEG-IFNα-2a (n=21); Dual High: 3000 mg bid + PEG-IFNα-2a (n=32); Triple Low: 1500 mg bid + PEG-IFNα-2a + RBV (n=31); SOC: PEG-IFNα-2a + RBV (n=20). Virological response was measured by Roche COBAS TaqMan (undetectable <15 IU/mL). At 4 weeks HCV RNA was undetectable in 81% of patients treated with Triple Low (mean reduction of 5.2 log10 IU/mL), 69% treated with Dual High (mean reduction of 4.5 log10 IU/mL), and 33% treated with Dual Low (mean reduction of 3.6 log10 IU/mL), compared to only 5% of patients treated with SOC (mean reduction of 2.4 log10 IU/mL). Synergy was observed between R1626 and SOC (additional 2.8 log10 IU/mL, Triple Low vs. SOC), and between R1626 and RBV (additional 1.6 log10 IU/mL, Triple Low vs. Dual Low) (Figure). ALT normalized in approximately 50% of patients in R1626 treatment groups. Most adverse events (AEs) were mild or moderate. Six patients had 8 serious AEs during the 4-week period: 4 in Dual High, 1 in Triple Low and 1 in SOC. The incidence of Grade 4 neutropenia was 48%, 78%, 39% and 10% in Dual Low, Dual High, Triple Low and SOC, respectively and was the main reason for dose reductions. In conclusion, a robust synergistic antiviral effect was observed when R1626 is combined with PEG-IFNα-2a ± RBV, with up to 81% of patients undetectable by week 4. Dosing of R1626 may be limited mainly by neutropenia; additional studies of different dosages of R1626 in combination with PEG-IFNα-2a and RBV are underway.

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Anna Chan - Employee: Roche

168 THE DIRECT TRIAL (DAILY-DOSE CONSENSUS INTERFERON AND RIBAVIRIN: EFFICACY OF COMBINED THERAPY): TREATMENT OF NON-RESPONDERS TO PREVIOUS PEGYLATED INTERFERON PLUS RIBAVIRIN: SUSTAINED Virologic RESPONSE DATA

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Background: Approximately 50% of HCV treatment-naïve patients treated with pegIFN/RBV fail therapy. A controlled trial of re-treatment of pegIFN/RBV non-responders with a subsequent course of pegIFN/RBV resulted in only a 3% sustained response. Daily use of consensus interferon (CIFN), which in vitro has been shown to be more effective than IFN alfa-2a and IFN alfa-2b, allows constant pharmacologic pressure on HCV and, when combined with RBV, may be an effective treatment option for previous pegIFN/RBV non-responders. Methods: The DIRECT clinical trial was a Phase 3, open-label, multi-center US-based study that enrolled 343 patients who were previous non-responders to pegIFN/RBV therapy. Patients were randomized to receive 48 weeks of CIFN 9 µg/day or CIFN 15 µg/day both with RBV (1.0-1.2 g/day). Prior to study entry, patients had to have <2 log10 decline in viral load (VL) while undergoing
at least 12 weeks of previous pegIFN/RBV therapy or have detectable VL at end of treatment after completing at least 24 weeks of therapy with ≥80% adherence. Patients had a negative VL if virus was undetectable by both bDNA and TMA assays. No adjunctive growth factors were used. Results: Patient demographics included mean age 50 yr, 71% male, 68% high viral load, 94% genotype 1, mean weight 89 kg, 17% African American, and 67% Caucasian. Other important patient characteristics included 59% with evidence of bridging fibrosis or cirrhosis on biopsy and 79% null responders (<2log_{10} drop in VL during their previous pegIFN/RBV). In the intent-to-treat analysis, SVR rates were 5% and 10% for CIFN 9µg/day and CIFN 15µg/day, respectively. SRV rates by previous response to therapy and fibrosis score for available patients are listed in Table 1. Discontinuations due to adverse events occurred in 11% and 17% in the CIFN 9µg/day and CIFN 15µg/day arms, respectively. Conclusions: Use of daily CIFN plus RBV in patients with advanced liver disease who were primarily null responders to previous pegIFN/RBV therapy resulted in viral clearance in a dose-dependent manner. Non-cirrhotic patients who achieved a partial response on their previous pegIFN/RBV treatment achieved the best outcomes, which approached 30% SVR. Daily CIFN up to 15µg/day and RBV was well tolerated in this population of pegIFN/RBV non-responders.

Table 1: Sustained Virologic Response (%) By Previous Response to Therapy and Fibrosis Score

<table>
<thead>
<tr>
<th>Fibrosis Score (within 3 yr of study entry)</th>
<th>F0-F1</th>
<th>F2-F3</th>
</tr>
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<tbody>
<tr>
<td>Previous Response to Therapy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;2 log_{10}</td>
<td>6/18 (33%)</td>
<td>11/98 (11%)</td>
</tr>
<tr>
<td>≥2 log_{10}</td>
<td>1/18 (5%)</td>
<td>4/14 (29%)</td>
</tr>
<tr>
<td></td>
<td>0/100 (0%)</td>
<td>6/37 (16%)</td>
</tr>
</tbody>
</table>

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169 CONFIRMATION OF THE GENES ENCODING THE BILARY CHOLESTEROL TRANSPORTER ABCG5/ABCG8 AS GALLSTONE SUSCEPTIBILITY (LITH) GENES

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Introduction: Employing quantitative trait locus (QTL) mapping in the inbred mouse model of cholesterol cholelithiasis, we identified the Lith9 locus on murine chromosome 17. Lith9 co-localized with the positional candidate genes Abcg5 and Abcg8 and Lith9-susceptible mouse strains displayed higher hepatic mRNA expression levels of both genes compared with Lith9-resistant strains. The ABCG5/ABCG8 heterodimer is expressed in liver and small intestine and limits intestinal absorption and promotes biliary secretion of cholesterol and plant sterols. Methods: To confirm Abcg5/Abcg8 as Lith genes, we performed a QTL analysis for plasma plant sterol levels in F2 progeny harboring a susceptible Lith9 allele displayed significantly lower plasma plant sterol levels (P < 0.001). In congenic mice carrying a resistant Abcg5/Abcg8 allele, plasma cholesterol and phytosterol levels were significantly higher compared with C57BL/6 controls. Lower hepatic expression levels of Abcg5 (P < 0.001) correlated with lower biliary cholesterol levels (P < 0.05) in congenic mice compared with C57BL/6 controls substantiating Abcg5/Abcg8 as Lith genes. However, lower intestinal expression of Abcg5 and higher plasma phytosterol levels indicated more efficient cholesterol absorption that resulted in substantial macrovesicular hepatic steatosis in Lith9-resistant congenic mice. Conclusion: Our data validate lithogenic alleles of Abcg5/Abcg8 that contribute to lower serum phytosterol levels and decrease hepatic steatosis but increase the risk of cholesterol gallstone formation in mice. The findings underscore the concept of Abcg5/Abcg8 as a principle cause of cholesterol gallstone susceptibility and indicate complex consequences of lipid metabolism from dys-regulated canalicular and intestinal cholesterol transport.

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170 A NOVEL ROLE OF TRANSFORMING GROWTH FACTOR β 1 IN TRANSCRIPTIONAL REPRESSION OF THE CHOLESTEROL 7 α -HYDROXYLASE GENE IN HUMAN HEPATOCYTES

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Bile acids are synthesized from cholesterol exclusively in the liver. Cholesterol 7 α-hydroxylase (CYP7A1) is the rate-limiting enzyme in the bile acid biosynthetic pathway and plays a key role in maintaining bile acid homeostasis. Inhibition of CYP7A1 by bile acids and inflammatory cytokines provides an important mechanism for protecting hepatocytes from bile acid toxicity during cholestatic liver diseases. Transforming growth factor β 1 (TGF β 1) released by hepatic stellate cells during chronic liver injury plays a critical role in liver inflammation and fibrogenesis. Objective: to investigate the role of TGF β 1 in hepatic bile acid synthesis. Methods & Results: Quantitative real-time PCR analyses showed that TGF β 1 strongly inhibited the mRNA expression of CYP7A1 in primary human hepatocytes. Reporter assays showed that Smad3, a downstream transcription factor mediating TGF β receptor signaling, inhibited CYP7A1 promoter activity by reducing hepatocyte nuclear factor 4 α (HNF4 α ) binding to the CYP7A1 gene. Adenovirus-mediated Smad3 gene transfer inhibited the CYP7A1 mRNA expression. The histone deacetylase (HDAC) inhibitor Tricostatin A partially blocked the TGF β 1 inhibition of CYP7A1 mRNA expression. Chromatin immunoprecipitation (ChIP) assays showed that TGF β 1 decreased acetylation of histone H3 in the CYP7A1 chromatin. ChIP assays also revealed that TGF β 1 treatment or adenovirus-mediated Smad3 gene transfer reduced HNF4 α and co-activator CBP binding but increased the recruitment of Smad3, HDAC1 and a repressor mSin3A to the CYP7A1 chro-
matin. Conclusion: This study provides the first evidence that TGF β 1 represses CYP7A1 gene transcription in human hepatoctyes by a mechanism involving Smad3-dependent inhibition of HNF4α and recruitment to CYP7A1 chromatin. The TGF β 1 /Smad3 signaling may reduce bile acid synthesis in the liver and prevent hepatocyte injury in obstructive liver diseases.

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171 MECHANICAL STRETCH STIMULATES CHOLANGIOCYTE PROLIFERATION AND PROFIBROTIC GENE EXPRESSION VIA THE ANGIOTENSIN TYPE I RECEPTOR: A NOVEL MECHANISM FOR DUCTAL PROLIFERATION DURING OBSTRUCTIVE CHOLELITHIASIS

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The angiotensin type I receptor (AT1) plays a critical role in pressure-induced vascular growth. In addition to ligand-dependent activation, AT1 also functions as a mechanoreceptor. Although marked cholangiocyte proliferation occurs during obstructive cholestasis, a condition associated with increased biliary pressure (Gastro 1990;466:77), the role of AT1 in this process remains unclear. AIM: To address role of AT1 stimulation in the regulation of cholangiocyte growth. METHODS: In vivo, normal and bile duct ligated (BDL) rats were treated after surgery with angiotensin II (Ang II; 50 ng/Kg BW/min), a potent ligand for AT1, by osmotic minipump for 2 wks. Biliary growth was assessed by evaluation of PCNA immunohistochemistry and the number of bile ducts in liver sections.

172 PI3K-INDEPENDENT ACTIVATION OF P38MAPK IS REQUIRED FOR CAMP-STIMULATED MRP2 TRANSLLOCATION IN HEPATOCYTES

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Cyclic AMP stimulates translocation of bile salt export pump (Bsep) and multidrug resistance associated protein 2 (Mrp2) to the canalicular membrane in rat hepatocytes; the effect of cAMP on Bsep translocation is phosphoinositide-3-kinase (PI3K) independent. A PI3K-independent activation of p38 MAPK may be involved in taurolursodeoxycholate (TUDC)-induced increases in bile acid secretion and Bsep translocation. Since, TUDC stimulates Mrp2 translocation and cAMP activates p38 MAPK in hepatocytes, we hypothesized that p38 MAPK may mediate cAMP-induced Mrp2 translocation. This hypothesis was tested in the present study conducted in isolated rat hepatocytes and in the human hepatoma cell line HuH-7. Cells were treated with 100 μM chlorophenylthio-cAMP (CPT-cAMP) for 15 min followed by determination of Mrp2 translocation and p38 MAPK activity. Mrp2 translocation was determined using a biotinylation method to label membrane Mrp2 followed by streptavidin precipitation of biotin-labeled proteins and immunoblotting with Mrp2 antibody. The activity of p38 MAPK was assessed from phosphorylation of p38MAPK ([I]180/182) in cell lysates. In isolated rat hepatocytes, CPT-cAMP increased Mrp2 translocation and p38 MAPK activity by 65±10% and 80±11% (mean±SEM), respectively. These effects of CPT-cAMP were almost completely inhibited by SB203580 (1 μM), an inhibitor of p38 MAPK. Wortmannin (50 nM), a specific inhibitor of PI3K, did not inhibit CPT-cAMP-induced activation of p38 MAPK, indicating PI3K-independent activation of p38 MAPK by cAMP. To further define the role of p38 MAPK, HuH-7 cells were transiently transfected with constitutively active (CA) or dominant negative (DN) Flag-cAMP kinase 6 (M KK6), an upstream kinase responsible for the activation of p38 MAPK. Transfection using LipofectAMINE was confirmed by expression of M KK6 using an antibody against Flag. CA-MKK6 increased p38 MAPK activity and Mrp2 translocation by 150±45% and 49±8%, respectively, compared to the empty vector, and these effects were not further augmented by CPT-cAMP. Basal and CPT-cAMP-induced p38 MAPK activity was not affected in cells transfected with DN-MKK6. Since p38 MAPK can also be activated by M KK3, any inhibition by DN-MKK6 may have been compensated by M KK3-mediated activation. In agreement with this possibility, basal and CPT-cAMP induced activation of p38 MAPK was inhibited in DN-MKK3/6 transfected cells. DN-MKK3/6 also inhibited CPT-cAMP-induced Mrp2 translocation. Taken together, these results suggest that PI3K-independent activation of p38 MAPK by cAMP is required for Mrp2 translocation in hepatocytes.
173 THE STEROID RECEPTOR CO-ACTIVATOR 2 (SRC2) REGULATES THE EXPRESSION OF THE FXR TARGET GENES, BILE SALT EXPORT PUMP (ABC1B1) AND SMALL HETERO DIMER PARTNER (SHP)

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The steroid receptor coactivators, including SRC-2, also known as GRIP1/TIF2, are well established nuclear receptor (NR) coactivators that are known to mediate the transcriptional effects of liganded NRs by directly recruiting other accessory factors and by their ability to modify histones by acetylation. To assess the independent utilization of these co-activators for FXR-mediated transcription, we studied whether FXR can utilize either SRC1 or SRC2 in gene activation within the context of the loci of the Bile Salt Export Pump (BSEP) and the small heterodimer partner (SHP). HepG2 cells were transfected with a plasmid for mammalian expression of FXR and siRNAs for SRC1 and SRC2 (Dharmacon/Pierce). There was a significant decrease in the amount of endogenous levels of SRC1 and SRC2 mRNAs measured by qRT-PCR upon transfection with siRNA for each co-activator. Protein levels were also markedly reduced on immunoblot analysis with antisera directed against SRC1 and SRC2 (Bethyl Labs). To determine the effect on specific gene expression associated with regulation by FXR, we measured BSEP and SHP mRNAs from cells transfected with FXR and treated with CDCA. Our results demonstrate that endogenous levels of BSEP and SHP mRNA are reduced in parallel with the loss of SRC2 induced by siRNA whereas, the loss of SRC1 by siRNA had an insignificant effect on the endogenous levels of BSEP and SHP. In accordance with the results showing that SRC2 is specific for FXR-mediated control of BSEP, we examined chromatin of the HepG2 cells targeted by siRNA of SRC1 and SRC2 for the occupation of the BSEP promoter by the co-activators. The loss of SRC2 at the BSEP and SHP loci on chromatin immunoprecipitation analysis is consistent with the knockdown of SRC2 expression by siRNA. On Chip, siRNA knockdown of SRC2 also depletes several histone modifications essential for the function of FXR including H3R17 and H3K4 methylation and H3K9 acetylation. These data are supported further by the downregulation of BSEP mRNA on microarray analysis of src2/- mouse livers (Jeong, et al Mol.Endo.20:1138, 2006). We conclude that SRC2 is critical for ligand-induced activation of FXR target genes, and selectively regulates the expression of BSEP and SHP.

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174 CHARACTERIZATION OF MICE NULL FOR LIVER-SPECIFIC UPTAKE TRANSPORTER ORGANIC ANION TRANSPORTING POLYPEPTIDE 1B2 (OATP1B2)

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Introduction. The liver-specific basolateral uptake transporter Oatp1b2 transports a large variety of substrates including organic anions, organic cations, and neutral chemicals. It has been perceived that Oatp1b2 may be essential for hepatic uptake of endobiotics and xenobiotics. To elucidate the in vivo function of Oatp1b2, we produced Oatp1b2-null mice and characterized their phenotype. Methods. Oatp1b2 gene was disrupted by homologous recombination and backcrossed into C57BL/6 mice for 6 generations. Hepatic mRNA and protein expression of transporters were determined with branched DNA signal amplification assay and Western blot, respectively. Results. Oatp1b2 protein is absent in liver of Oatp1b2-null mice. Compared to wild-type mice, Oatp1b2-null mice had higher hepatic mRNA expression of the uptake transporter Oatp1a4 and lower organic anion transporter 2. Oatp1b2-null mice were generally healthy and physically indistinguishable from wild-type mice. However, Oatp1b2-null mice developed moderate conjugated hyperbilirubinemia. After intra-arterial injection of unconjugated bilirubin, compared to wild-type mice, Oatp1b2-null mice had as much as 5-fold higher blood levels of conjugated bilirubin, which persisted at least 50 min. Oatp1b2-null mice were completely resistant to hepatotoxicity induced by the mushroom toxicant phalloidin, an Oatp1b2-specific substrate identified in vitro, whereas Oatp1b2-null mice and wild-type mice were similarly sensitive to hepatotoxicity induced by the mushroom toxicant alpha-amanitin. The critical role of Oatp1b2 in mediating hepatotoxicity of phalloidin was further confirmed by the lack of cholestasis in Oatp1b2-null mice after intra-arterial injection of phalloidin (2 mg/kg). After intra-arterial injection of dibromosulphosphate (DBSP), a non-metabolizable organic anion used as a liver function assay, blood concentrations of DBSP were 11-fold higher, and accumulative biliary excretion of DBSP was 41% lower, in Oatp1b2-null mice compared to wild-type mice. Conclusion. Oatp1b2 plays a key role in determining hepatic uptake of conjugated bilirubin, phalloidin, and DBSP, whereas biliary excretion of these chemicals is a function of hepatic uptake, intrahepatic biotransformation, and biliary efflux. Oatp1b2-null mice will be a powerful tool to elucidate the physiological role of Oatp1b2 and its human orthologs OATP1B1 and OATP1B3 (supported by NIH grant SRO1ES009649).

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175 FINAL RESULTS OF PATIENTS TREATED WITH PEG-INFON-ALFA-2A (PEG-IFN) AND RIBAVIRIN (RBV) FOLLOW-ON THERAPY AFTER 28-DAY TREATMENT WITH THE HEPATITIS C PROTEASE INHIBITOR TELAPREVIR (VX-950), PEG-IFN AND RBV

Maribel Rodriguez-Torres1, Eric J. Lawitz2, John G. McHutchison3; 1Fundacion de Investigacion de Diego, Santurce, PR; 2Alamo Medical Research, San Antonio, TX; 3Duke Clinical Research Institute & Division of Gastroenterology, Duke University, Durham, NC

Purpose: Telaprevir (TVR, VX-950) is an orally administered, highly selective peptidomimetic inhibitor of the Hepatitis C virus (HCV) NS3-4A protease. The VX05-950-102 study was designed to assess the safety of telaprevir when given in combination with Peginterferon alfa-2a (Peg-IFN) and ribavirin (RBV) and to evaluate the antiviral response during 28 days of dosing. After completion of the 28-day study, all subjects received off-study therapy with Peg-IFN/RBV under the clinical care of their physicians. Here we report the outcome of treatment after this post-study therapy. Methods: This study included 12 treatment-naive, mostly Latino (10/12), patients infected with...
176 PRE-TREATMENT PD-1 EXPRESSION ON HCV-SPECIFIC CTLS PREDICTS EARLY AND LONG-TERM RESPONSE TO COMBINATION THERAPY IN AFRICAN AMERICANS BUT NOT CAUCASIAN AMERICANS

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African Americans (AA) with chronic HCV are less likely to have a sustained virological response (SVR) to IFN-based antiviral therapy than Caucasian Americans (CA), but mechanisms for these differences are not fully elucidated. Host immune responses are postulated to affect viral kinetics and ultimate outcome of treatment. Aim: To assess the association of PD-1, recently shown to be an important marker for CTL exhaustion, with early viral decline and ultimate response to treatment in AA and CA with chronic HCV. Methods: 72 genotype 1 patients (30 AA, 42 CA) with chronic HCV were selected from prospectively characterized patients, pre-treatment PD-1 expression on HCV-specific CTLs was significantly, inversely associated with SVR (RR=0.95; 95% CI 0.93-0.97; p<.0001). This association, however, was not significant in CA (Figure). Conclusions: In this large cohort of prospectively characterized patients, pre-treatment PD-1 expression on HCV-specific CTLs was associated with early and long-term virologic response to combination antiviral therapy in AA but not CA patients. These findings suggest that immune factors may contribute to the poor antiviral responses found among AA patients with hepatitis C and provide potential immune targets for manipulation.

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177 INTERIM ANALYSIS RESULTS FROM A PHASE 2 STUDY OF TELAPREVIR WITH PEGINTERFERON ALFA-2A AND RIBAVIRIN IN TREATMENT-NAIVE SUBJECTS WITH HEPATITIS C

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Background: The VX05-950-104 study (PROVE1) is a randomized, placebo-controlled Phase 2 study of telaprevir (TVR, VX-950), an HCV protease inhibitor, with Peg-IFN-2a and RBV, in naive subjects with genotype 1 hepatitis C. We report the...
results of a planned interim analysis. Methods: Subjects were randomized into 4 groups; 3 groups received TVR 750 mg q8h, Peg-IFN-2a 180 µg/week, and RBV 1000-1200 mg/day for 12 weeks followed by 0 (n=20), 12 (n=80) or 36 (n=82) weeks of Peg-IFN-2a and RBV (TVR/PR groups). The control group (n=81) received up to 48 weeks of Peg-IFN-2a/RBV (PR). This analysis was performed when all treated subjects had completed 12 weeks of dosing. Follow-up information was also obtained for the subjects who completed the 12-week dosing arm. Plasma HCV RNA was isolated for viral sequencing at baseline and at each HCV RNA assessment in samples that contained greater than 1,000 IU/mL. Results: The proportion of subjects with undetectable HCV RNA (LOD 10 IU/mL) at Week 4 was 79% (TVR/PR group), and 11% (control group) (p<0.001), and at Week 12 was 70% (TVR/PR group), and 39% (control group) (p<0.001). Viral breakthroughs (increase of >1-log above HCV RNA nadir) with previously described TVR resistant variants were observed in 12 of 175 (11 genotype 1a and 1 genotype 1b) subjects in the TVR/PR groups. In the group assigned to 12 weeks of treatment, 6 of 9 subjects with RVR and completing 12 weeks of treatment achieved SVR. The remaining 3 subjects relapsed with TVR resistant variants (genotype 1a: R155T/I). Discontinuations due to adverse events were more frequent in the TVR/PR group (11% vs. 3%). Rash, gastrointestinal events and anemia were more common, and rashes more severe in the TVR/PR groups. An additional interim analysis will be conducted when all subjects have completed 36 weeks on-study, including 12-week post-treatment follow-up for the 24-week study arm subjects. Conclusions: Compared with standard therapy, significantly more subjects receiving a TVR/PR regimen achieved undetectable HCV RNA at Weeks 4 and 12, and some subjects achieved an SVR after 12 weeks of TVR/PR. Other patients relapsed with TVR-resistant variants, suggesting that 12 weeks of TVR/PR may be adequate to eliminate wild-type virus. The post-treatment follow-up results in the 24-week study groups will evaluate whether the additional 12 weeks of PR is sufficient to eliminate the remaining TVR-resistant variants. * for the PROVE1 study team

Disclosures:
Ira M. Jacobson - Consultant/Adviser: Vertex; Grant/Research Support: Vertex; Consultant/Adviser: Schering-Plough; Speakers Bureau: Schering-Plough; Consultant/Adviser: Roche; Consultant/Adviser: Schering-Plough
Gregory T. Everson - Grant/Research Support: Schering-Plough; Speakers Bureau: Schering-Plough; Speakers Bureau: Roche; Consultant/Adviser: Roche; Speaker’s Bureau: Roche
Stuart C. Gordon - Grant/Research Support: Vertex; Consultant/Adviser: Roche; Consultant/Adviser: Vertex; Speakers Bureau: Schering-Plough; Speakers Bureau: Roche
Robert Kaufman - Employee: Vertex
Lindsay McNair - Employee: Vertex
Andrew Muir - Grant/Research Support: Vertex; Consultant/Adviser: Schering-Plough; Speakers Bureau: Schering-Plough
John G. McHutchison - Consultant/Adviser: Vertex; Consultant/Adviser: Roche; Consultant/Adviser: Roche; Consultant/Adviser: Schering-Plough; Consultant/Adviser: Roche
The following people have nothing to disclose: Lindsay McNair

<table>
<thead>
<tr>
<th>Treatment-naive</th>
<th>Treatment-experienced</th>
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<tbody>
<tr>
<td>PegIFN-RBV</td>
<td>RVR</td>
</tr>
<tr>
<td>26/30 (66%)</td>
<td>22/29 (76%)</td>
</tr>
<tr>
<td>NTZ-PegIFN</td>
<td>RVR</td>
</tr>
<tr>
<td>18/29 (67%)</td>
<td>26/29 (96%)</td>
</tr>
<tr>
<td>NTZ-PegIFN-RBV</td>
<td>RVR</td>
</tr>
<tr>
<td>21/22 (72%)</td>
<td>26/29 (96%)</td>
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Disclosures:
180 SUSTAINED VIROLOGIC RESPONSE RATES WITH ALBINTERFERON ALFA-2B PLUS RIBAVIRIN TREATMENT IN IFN-NAIVE, CHRONIC HEPATITIS C GENOTYPE 1 PATIENTS

Stefan Zeuzem1, Eric M. Yoshida2, Yves Benhamou3, Vincent G. Bain4, Daniel Shouval2, Stephen Pianko5, Robert Flisiak7, Mircea Grigorescu5, Vratslav Rehak8, Kelly D. Kaita10, Patrick Cronin11, Erik Pulkstenis11, G. M. Subramanian11, John G. Mchutchison12, 1 J.W. Goethe-University Hospital, Frankfurt, Germany; 2 University of British Columbia, Vancouver, BC, Canada; 3 Hospital Pitie-Salpetriere, Paris, France; 4 University of Alberta, Edmonton, AB, Canada; 5 Hadassah University, Hadassah, Israel; 6 Monash University, Melbourne, VIC, Australia; 7 Medical University of Białystok, Białystok, Poland; 8 Spitalul Clinic, Cluj-Napoca, Romania; 9 Nuselska poliklinika, Prague, Czech Republic; 10 University of Manitoba, Winnipeg, MB, Canada; 11 Human Genome Sciences, Rockville, MD; 12 Duke Clinical Research Institute, Durham, NC

Aim/Background: This Phase 2b study evaluates the efficacy and safety of albinterferon alfa-2b (alb-IFN), a novel recombinant protein consisting of IFNα-2b genetically fused to human albumin, in combination with oral ribavirin (RBV) in IFN-naïve, chronic hepatitis C genotype 1 patients. Methods: 458 patients were randomized to 4 treatment arms: PEG-IFNa-2a 180mcg Q1w (PEG-IFN) or one of 3 alb-IFN arms (900mcg Q2w, 1200mcg Q2w or 1200mcg Q4w), all in combination with weight-based oral RBV1000-1200 mg/d (Table). HCV RNA was measured by real-time PCR (limit of detection [LOD]): 10 IU/ml. The primary efficacy endpoint was sustained virologic response (SVR) defined as HCV RNA<LOD at w24 following treatment completion. Results: By ITT analysis, SVR rates were 58.5% for the 900Q2, 55.5% for 1200Q2, 50.9% for 1200Q4 and 57.9% for PEG-IFN (Table). In patients with ≥80% adherence to therapy, SVR rates were 71.6% for the Q2w alb-IFN arms compared to 66.7% for PEG-IFN. Of note, in heavier patients (≥75 kg) adherent to therapy, SVR rates were maintained for the alb-IFN arms and declined for PEG-IFN. Discontinuations due to adverse events in the alb-IFN arms were 9.3% in the 900Q2, 18.2% in the 1200Q2 and 12.1% in the 1200Q4 compared with 6.1% for PEG-IFN. Hematology reductions were lowest in the Q4w arm and comparable across other arms. Quality-of-life as measured by SF-36v2 and missed work was significantly more favorable for the 900Q2 arm. Conclusions: These data suggest that the Q2w alb-IFN may offer comparable efficacy, with an improved dosing schedule and quality-of-life compared with current standard of care with PEG-IFN.

<table>
<thead>
<tr>
<th>response groups</th>
<th>relative relapse rate (%)</th>
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<td>week 4 response</td>
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<tr>
<td>=&gt; log decline, bDNA positive</td>
<td>36% (all patients)</td>
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<tr>
<td>bDNA negative, TMA positive</td>
<td>38% (all patients)</td>
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<tr>
<td>week 12 response</td>
<td></td>
</tr>
<tr>
<td>=&gt; log decline, bDNA positive</td>
<td>77% (all patients)</td>
</tr>
<tr>
<td>bDNA negative, TMA positive</td>
<td>64% (all patients)</td>
</tr>
<tr>
<td>bDNA negative, TMA negative</td>
<td>20% (all patients)</td>
</tr>
</tbody>
</table>

Disclosures: The following people have nothing to disclose: Thomas Berg, Viola Weich, Gerlinde Teuber, Hartwig Klinker, Bernd Möller, Jens Rasenack, Holger Hinrichsen, Tilman Gerlach, Ulrich Spengler, Peter Buggisch, Heike Balk, Myrga Zankel, Christoph Sarrazin, Stefan Zeuzem

The following people have nothing to disclose: Emmet B. Keeffe, Jeffrey Glenn - Grant/Research Support: Other

IMPORATANCE OF A MINIMAL RESIDENT VIREMIA FOR THE RELAPSE PREDICTION IN HCV TYP1 PATIENTS RECEIVING STANDARD OR INDIVIDUALIZED TREATMENT DURATION

Thomas Berg1, Viola Weich1, Gerlinde Teuber2, Hartwig Klinker3, Bernd Möller4, Jens Rasenack5, Holger Hinrichsen6, Tilman Gerlach7, Ulrich Spengler8, Peter Buggisch9, Heike Balk10, Myrga Zankel10, Christoph Sarrazin10, Stefan Zeuzem10,11

The exact estimation of early virologic response rates in the course of antiviral therapy is an important goal in order to improve individualized therapeutic strategies in HCV infection. The sensitive TMA test could provide better advantage to distinguish at early stages sustained from non-sustained responders. We evaluated HCV type 1 patients who took part in a prospective study asking whether the application of TMA in bDNA-negative patients may be a better indicator to predict long-term outcome of the HCV infection. Methods 433 patients were randomized to receive either 1.5ug/kg PEG-INFα-2b plus 800-1400 mg RBV for 48 weeks (n=225, group A) or an individualized tailored treatment duration (n=208, group B). In the latter group treatment duration was calculated by the time required to become for the first time HCV RNA negative as defined by bDNA assay (detection limit 615 IU/ml) multiplied by the factor 6. HCV RNA levels were quantified weekly until week 8, at week 12 and 24. For all those patients who were bDNA negative the more sensitive TMA test (detection limit 5.3 IU/ml) was also prospectively assessed. The different response groups were classified according to the HCV RNA levels at week 4 and 12 (Table). Results Table shows the relevant data and refers to the relative relapse rates in group A and B at week 4 and 12 in relation to the treatment schedule. There is clear evidence for a high relapse rate in patients with a positive TMA within the first 12 weeks of therapy being more pronounced when treatment duration shortened in the individualized treatment group. In contrast patients shown to respond as early as week 4 evidenced by a negative TMA test had relapse rates below 10% irrespectively from treatment group. Conclusion The application of the highly sensitive HCV RNA tests must be distinguished at early stages sustained from non-sustained response (SVR) defined as HCV RNA<LOD at w24 following treatment schedule and quality-of-life compared with current standard of care with PEG-IFN.
Liver fibrosis was staged according to the METAVIR scoring system (0 = no fibrosis, 1 = slight fibrosis, 2 = significant fibrosis, 3 = cirrhosis). Combinations of these methods were assessed by multivariate regression. Results: Mean age was 47.26 (17-69) years, BMI 27.5 (17.2-57.8), LB length 15 mm (median 22 mm, range 15-50 mm) within one month.

The effect of age, gender, BMI, LB length, activity grade, LSM, and histological assessment were observed in 28 cases (11%). Discordances of at least 2 stages between LSM and histology were observed in 8/127 cases (6.3%) versus 20/127 cases (15.7%), P < 0.02, respectively. In patients with IQR/LSM > 0.2 versus < 0.2, for the diagnosis of liver fibrosis F ≥ 2, F ≥ 3 and cirrhosis, AUROC were 0.77 versus 0.83 (P = 0.18), 0.79 versus 0.91 for (P < 0.01) and 0.86 versus 0.95 (P = 0.11), respectively.

Conclusion: IQR/LSM is a key factor of accuracy of FibroScan for the diagnosis of liver fibrosis. This ratio significantly improves the performance of FibroScan for the diagnosis of extensive fibrosis [F3]. In case of IQR/LSM > 0.2, a new measurement of LS or a LB should be proposed. 1) Castera et al. Gastroenterology 2005;128:343-50.

Disclosures:
The following people have nothing to disclose: Damien Lucidarme, Juliette Foucher, Brigitte Le Bail, Laurent Castera, Sandrine Villan, Gerard Forzy, Bernard Floche, Patrice Couzigou, Victor de Lédinghen
## Table 1

<table>
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<tr>
<th>AUROC</th>
<th>Liver histology (METAVIR)</th>
<th>F0’s score (mean ± 95% CI)</th>
<th>F1’s score (mean ± 95% CI)</th>
<th>F2’s score (mean ± 95% CI)</th>
<th>F3’s score (mean ± 95% CI)</th>
<th>F4’s score (mean ± 95% CI)</th>
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<tr>
<td>Liver histology</td>
<td>0.936</td>
<td>0.837</td>
<td>0.709</td>
<td>0.849</td>
<td>0.985</td>
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<tr>
<td>F0</td>
<td>0</td>
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<td>0</td>
<td>0.849</td>
<td>0.985</td>
<td></td>
</tr>
<tr>
<td>F1</td>
<td>0.936</td>
<td>0.837</td>
<td>0.709</td>
<td>0.849</td>
<td>0.985</td>
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<tr>
<td>F2</td>
<td>0.936</td>
<td>0.837</td>
<td>0.709</td>
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<td>F3</td>
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<td>0.709</td>
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<tr>
<td>F4</td>
<td>0.936</td>
<td>0.837</td>
<td>0.709</td>
<td>0.849</td>
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### 183 TRANSIENT ELASTOGRAPHY IN PATIENTS WITH NON-ALCOHOLIC FATTY LIVER DISEASE (NAFLD)

Masato Yoneda1, Hironori Mawatari1, Takuma Higurashi1, Hiroshi lida1, Yuichi Nozaki1, Hiroki Endo1, Koji Fujita1, Hisayuki Kirikoshi1, Masaya Tamano2, Masashi Yoneda2, Hideyuki Hiraishi2, Kenseke Kubota2, Satoru Saito2, Atsushi Nakajima1; 1Division of Gastroenterology, Yokohama City University School of Medicine, Yokohama, Japan; 2Division of Gastroenterology, Dokkyo Medical School, Mibu, Japan

**Background and aims** The histopathological changes in NAFLD range over a wide spectrum, extending from simple steatosis to nonalcoholic steatohepatitis (NASH). Liver fibrosis is the main predictor of the progression of NAFLD. Transient elastography (FibroScan; EchoSens, Paris, France), which measures liver stiffness, is a novel, noninvasive and rapid method to assess liver fibrosis. Studies have suggested that this technique is useful for the accurate prediction of hepatic fibrosis in patients with chronic hepatitis C. We have previously reported a pilot study that transient elastography have the possibility of the measurement of the fibrosis in patients with NAFLD (GUT 2007). We prospectively investigated the usefulness of liver stiffness measurement (LSM) in the evaluation of liver fibrosis in patients with nonalcoholic fatty liver disease (NAFLD).

**Methods** Transient elastography was performed in LSM in 97 patients with histologically confirmed NAFLD. Moreover, we investigated the relationship between LSM and the serum levels of hyaluronic acid and type IV collagen 7s domain. (Result) The median liver stiffness values (and 95% CI) were F0, 4.907 (4.417 – 5.396) kPa; F1, 6.142 (5.582 – 6.702) kPa; F2, 7.894 (6.384 – 9.404) kPa; F3, 11.027 (8.555 – 13.500) kPa; F4, 26.960 (17.705 – 36.215) kPa. The liver stiffness was well correlated with the stage of liver fibrosis (r ≥ 0.865 for ≥ F 1, r ≥ 0.904 for ≥ F 2, r ≥ 0.991 for ≥ F 3, r ≥ 0.865 for ≥ F 2, r ≥ 0.904 for ≥ F 3, r ≥ 0.991 for ≥ F 3). Liver stiffness was also strongly correlated with the serum levels of hyaluronic acid and type IV collagen 7s domain.

**Conclusion** This is the first study to demonstrate a consistent and profound elevation of the liver stiffness in NASH patients, as confirmed by the results of liver biopsy, which remains the gold standard for evaluation of the severity of liver fibrosis in patients with NASH. Measurement of liver stiffness is a non-invasive and clinically useful method for predicting the severity of liver fibrosis in patients with NASH.

Disclosures:
The following people have nothing to disclose: Masato Yoneda, Hironori Mawatari, Takuma Higurashi, Hiroshi Iida, Yuichi Nozaki, Hiroki Endo, Koji Fujita, Hisayuki Kirikoshi, Masaya Tamano, Masashi Yoneda, Hideyuki Hiraishi, Kenseke Kubota, Satoru Saito, Atsushi Nakajima

### 184 COMPARISON OF MR ELASTOGRAPHY, ULTRASOUND ELASTOGRAPHY AND APRI FOR THE NON-INVASIVE ASSESSMENT OF LIVER FIBROSIS

Laurent Huwart, Christine Sempoux, Najat Salameh, Laurence Annet, Yves Horsmans, Bernard Van Beers; Université Catholique de Louvain, Brussels, Belgium

**Introduction** Recently, magnetic resonance (MR) elastography has emerged as a non-invasive method to assess the fibrosis stage. The purpose of our study was to prospectively compare the diagnostic accuracy of MR elastography, ultrasound elastography, and aspartate aminotransferase to platelets ratio index (APRI) for the staging of fibrosis in patients with chronic liver disease. Materials and methods: Ninety-six patients who had liver biopsy for suspicion of chronic liver disease had MR elastography, ultrasound elastography and APRI within 2 days. Fibrosis stage was assessed by two pathologists according to the METAVIR score (from F0 to F4) that served as reference. MR elastography was performed with a 1.5 Tesla scanner using a motion-sensitized sequence and a transducer that applied mechanical waves into the liver. Ultrasound elastography was performed with the commercially available FibroScan. The measurements of MR elasticity, ultrasound elasticity and APRI were analyzed with receiver operating characteristic curves. Results: The distribution of fibrosis stage was F0 in 22/96 patients (23%), F1 in 22 (23%), F2 in 19 (20%), F3 in 15 (15%) and F4 in 18 (19%). The areas under the receiver operating characteristic curves of MR elastography (0.994 for F ≥ 2, 0.985 for F ≥ 3 and 0.998 for F = 4) were significantly (P < 0.05) larger than those of ultrasound elastography, APRI and the combination of ultrasound elastography and APRI (respectively 0.837, 0.709 and 0.849 for F ≥ 2; 0.906, 0.816 and 0.936 for F ≥ 3; 0.930, 0.820 and 0.944 for F = 4). The most discriminant cut-off values of MR elasticity were respectively 2.5 kPa for F ≥ 2, 3.1 kPa for F ≥ 3, and 4.1 kPa for F = 4. Conclusion: The results of this study show that MR elastography is more accurate than ultrasound elastography and APRI to stage liver fibrosis and suggest that MR elastography can be used for the assessment and follow-up of liver fibrosis.

**Table** Most discriminant MR elasticity cut-off values (kPa) and corresponding sensitivities, specificities, positive and negative predictive values with 95% confidence intervals in parentheses for METAVIR scores F ≥ 2, F ≥ 3 and F = 4.

<table>
<thead>
<tr>
<th>Cut-off (kPa)</th>
<th>F ≥ 2</th>
<th>F ≥ 3</th>
<th>F = 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity</td>
<td>0.91 (0.87-0.95)</td>
<td>0.91 (0.86-0.95)</td>
<td>1.00 (0.91-1.00)</td>
</tr>
<tr>
<td>Specificity</td>
<td>0.97 (0.89-0.99)</td>
<td>0.96 (0.89-0.99)</td>
<td>0.96 (0.89-0.99)</td>
</tr>
<tr>
<td>Positive predictive value</td>
<td>0.95 (0.87-0.99)</td>
<td>0.86 (0.64-0.97)</td>
<td>1.00 (0.95-1.00)</td>
</tr>
<tr>
<td>Negative predictive value</td>
<td>0.97 (0.89-0.99)</td>
<td>0.96 (0.89-0.99)</td>
<td>0.96 (0.89-0.99)</td>
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</tbody>
</table>

Disclosures:
The following people have nothing to disclose: Laurent Huwart, Christine Sempoux, Najat Salameh, Laurence Annet, Yves Horsmans, Bernard Van Beers
FIBROTEST VERSUS LIVER BIOPSY: AN INDEPENDENT MULTICENTER EVALUATION OF PERFORMANCE

Maria Guido1, Alfredo Alberti2, Giorgio Bellati3, Guido Collaredo4, Antonio Craxi2, Vita Di Marco5, Stefano Fagiuoli6, Matteo Fassan1, Luciano Giacomelli1, Alessandra Mangia7, Giada Sebastiani2, Massimo Rugge1; 11. Department of Diagnostic Medical Sciences & Special Therapies, University of Padova, Padova, Italy; 2Department of Clinical and Experimental Medicine, University of Padova, Padova, Italy; 3S. Anna Hospital, Como, Italy; 4Policlinico San Pietro, Bergamo, Italy; 5Department of Gastroenterology, University of Palermo, Palermo, Italy; 6Ospedali Riuniti, Bergamo, Italy; 7IRCSS Casa Sollievo della Sofferenza, S. Giovanni Rotondo, Italy

BACKGROUND AND AIMS: Algorithms based on indirect biochemical markers of fibrosis are becoming increasingly popular. Though the Fibrotest (FT) is accredited for the non-invasive assessment of liver fibrosis, its validation against liver biopsy comes mainly from data generated by the center where it was originally developed. In an independent nationwide multicenter study, we aimed to assess the diagnostic accuracy of FT in a large series of patients with chronic hepatitis B and C undergoing liver biopsy (LB) for disease grading and staging. This is the first multicenter study in which all liver biopsies were reviewed at a single center by the same experienced liver pathologist. METHODS: In all, 496 consecutive patients with chronic HBV (55 cases) or HCV hepatitis were enrolled at five centers where liver biopsies and biochemical markers were collected on the same day. All subjects were HIV negative. The diagnostic value of the FT was assessed, applying logistic regression as a discriminant function, in terms of area under the ROC curve (AUCs), sensitivity, specificity, PPV, NPV, general agreement, and kappa score. Liver biopsies more than 2 cm long & with ≥ 11 complete portal tracts (= 277 cases) were considered the “platinum” standard: all analyses were repeated in these biopsies to test the hypothesis that FT might significantly improve its diagnostic accuracy with a better gold standard, assuming that the discrepancy between FT and LB is due mainly to poor biopsy quality. RESULTS: 73% of the biopsy samples were ≥ 2 cm long (median 2 cm; range: 0.5–6.5). According to the METAVIR score, histological fibrosis was F0 in 8% (39/496) of cases, F1 in 29% (142/496), F2 in 35% (176/496), F3 in 13% (63/496), and F4 in 15% (76/496). FT proved scarcely able to discriminate between single fibrosis stages and 34% of cases had intermediated values and cannot be classified. Table 1 summarizes the diagnostic value of FT in discriminating between absent/mild fibrosis and severe fibrosis/cirrhosis. The results did not change when the analysis was restricted to the “platinum” standard biopsies. CONCLUSIONS: FT performs best in discriminating severe fibrosis/cirrhosis from absent/mild fibrosis, but it does not accurately predict the various intermediate stages of fibrosis. Its performance does not improve significantly with better-quality liver biopsies.

Table 1

<table>
<thead>
<tr>
<th>AUC-ROC</th>
<th>SENS</th>
<th>SPEC</th>
<th>PPV</th>
<th>NPV</th>
<th>Agreement</th>
<th>k</th>
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<tbody>
<tr>
<td>F0/F1 vs. F2/F4</td>
<td>0.77</td>
<td>0.71</td>
<td>0.72</td>
<td>0.59</td>
<td>0.82</td>
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<tr>
<td>F0/F2 vs. F3/F4</td>
<td>0.82</td>
<td>0.84</td>
<td>0.83</td>
<td>0.85</td>
<td>0.86</td>
<td>78.43</td>
</tr>
</tbody>
</table>

Disclosures:
The following people have nothing to disclose: Maria Guido, Alfredo Alberti, Giorgio Bellati, Guido Collaredo, Antonio Craxi, Vita Di Marco, Stefano Fagiuoli, Matteo Fassan, Luciano Giacomelli, Alessandra Mangia, Giada Sebastiani, Massimo Rugge

PROSPECTIVE COMPARISON OF TWO ALGORITHMS COMBINING NON INVASIVE TESTS FOR STAGING OF LIVER FIBROSIS IN CHRONIC HEPATITIS C

Laurent Castera1,2, Giada Sebastiani3, Brigitte Le Bail4, Victor de Ledinghen4, Patrice Couzigou1, Alfredo Alberti3; 1Hepatology, Hospital Haut Leveque, CHU Bordeaux, Bordeaux, France; 2Hepatology, Hopital St Andre, CHU Bordeaux, Bordeaux, France; 3Venetian Institute of Molecular Medicine, Padova, Italy; 4Pathology, Hospital Pellegrin, CHU Bordeaux, Bordeaux, France

Background: Non invasive assessment of liver fibrosis is a challenging area. Numerous markers are available but their performance may be better when combined. Two algorithms have been proposed recently in patients with chronic hepatitis C, combining either transient elastography (Fibroscan) and Fibrotest (Castera et al. Gastroenterology 2005) or Fibrotest and AST to Platelet Ratio (APRI) (Sebastiani et al. J Hepatol 2006). Aim: to prospectively compare the diagnostic performance for significant fibrosis and cirrhosis of these 2 algorithms in the same population. METHODS: 302 consecutive HCV patients (58% male, mean age 52±12 yrs) who undergone liver biopsy (>10mm and 6 portal triads) were studied. Fibroscan, Fibrotest and APRI were performed in the same lab the day of liver biopsy taken as reference. Fibrosis was scored according to the Metavir scoring system. Diagnostic performances were assessed by measuring area under the ROC curve (AUROC). Results: Significant fibrosis (F2-F3-F4) was present in 76% of patients and cirrhosis (F4) in 24%. The mean liver biopsy length was: 20±8 mm. Fibroscan failure was observed in 8 cases (3%). Performance of the 2 algorithms using the cut-offs for APRI and fibrotest of the original studies are shown in the table. For significant fibrosis, Castera algorithm saved 23% more liver biopsies than Sebastiani algorithm but its accuracy was lower (AUROC 0.91 vs. 0.94; accuracy 87.1% vs. 97.0%, respectively). Regarding cirrhosis, Castera algorithm was slightly more accurate (AUROC 0.93 vs. 0.87; accuracy 95.7% vs. 88.7%, respectively) and saved 4% more liver biopsies. However, Castera algorithm was less cost-effective (using both 100% Fibroscan and Fibrotest) than Sebastiani algorithm (using 100% APRI (virtually no cost) and around 50% Fibrotest). Conclusion: Both algorithms are effective for the non invasive staging of liver fibrosis in chronic hepatitis C. Their use in clinical practice could avoid the need for liver biopsy in 48 to 71% of cases for the diagnosis of significant fibrosis and in 74 to 78% of cases for cirrhosis.

<table>
<thead>
<tr>
<th>Castera algorithm</th>
<th>Sebastiani algorithm</th>
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<tbody>
<tr>
<td>End Point</td>
<td>F2-F3-F4</td>
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<tr>
<td>APRI performed</td>
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<tr>
<td>Fibrotest performed</td>
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<td>Sensitivity (%)</td>
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<td>Accuracy (%)</td>
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</tr>
<tr>
<td>AUROC</td>
<td>0.91</td>
</tr>
<tr>
<td>Saved biopsies (%)</td>
<td>71.9</td>
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</table>

Disclosures:
The following people have nothing to disclose: Laurent Castera, Giada Sebastiani, Brigitte Le Bail, Victor de Ledinghen, Patrice Couzigou, Alfredo Alberti
DIFFERENTIATION AND ENRICHMENT OF HEPATOCTYES FROM HUMAN EMBRYONIC STEM CELLS IN VITRO AND IN VIVO

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Background: Human embryonic stem cells (hESC) may provide a cell source of functional hepatocytes for therapeutic use. The aim of this study is to develop viable hepatocytes from hESC that can be used for cell-based therapies. Methods: Using a combinatorial approach, we developed culture conditions that differentiated a percentage of hESC along a hepatocyte lineage. The differentiated hESC were further enriched by transducing with a lentiviral vector containing the human α1-antitrypsin (α1-AT) promoter driving the GFP gene. The GFP+ hESC were then purified by laser microdissection and pressure catapulting. In addition, differentiated hESC that were transduced with a lentiviral triple-fusion vector were transplanted into NOD-SCID mice, and the luciferase-induced bioluminescence in the livers was evaluated by a charged coupled device (CCD) camera. Results: After transduction with a lentivirus containing α1-AT promoter, differentiated GFP+ hESC expressed a large series of liver proteins: α1-fetoprotein (AFP), albumin (ALB), α1-AT, transthyretin (TF), tyrosin aminotransferase (TAT), arginase (ARG), glucose-6-phosphatase, CYP1A1, CYP2B6, CYP1B1, CYP2E1, CYP2C9, and CYP3A. AFP expression decreased with time. Differentiated hESC also had liver-specific functions. They accumulated glycogen, expressed high levels of CYP1A2 activity as well as urea synthesis in the livers was evaluated by a charged coupled device (CCD) camera. Results: After transduction with a lentivirus containing α1-AT promoter, differentiated GFP+ hESC expressed a large series of liver proteins: α1-fetoprotein (AFP), albumin (ALB), α1-AT, transthyretin (TF), tyrosin aminotransferase (TAT), arginase (ARG), glucose-6-phosphatase, CYP1A1, CYP2B6, CYP1B1, CYP2E1, CYP2C9, and CYP3A. AFP expression decreased with time. Differentiated hESC also had liver-specific functions. They accumulated glycogen, showed the cellular uptake of indocyanine green, and expressed high levels of CYP1A2 activity as well as urea synthesis. Quantitative RT-PCR revealed that the expression levels of purified hESC over time, when compared with primary human hepatocytes, ranged from 24 to 55% for ALB, 44 to 57% for α1-AT, 73 to 91% for TF, 13 to 61% for TAT, and 128 to 230% for ARG. When NOD-SCID mice were transplanted with the differentiated hESC transduced with a lentiviral triple fusion vector, positive signals were obtained by CCD camera over time. The differentiated hESC survived and engrafted in mouse livers, and the first bioluminescence imaging of hESC in the liver. Successful transplantation of differentiated hESC into an animal liver, and the first bioluminescence imaging of hESC in the liver. Thus, we exploited a novel system for directing and enriching hepatic differentiation from hESC.

IDENTIFICATION OF ADULT HEPATIC PROGENITOR/oval CELLS CAPABLE OF REPULATING INJURED RAT LIVER

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Adult hepatic progenitor/oval (AHPC/OC) cells appear and regenerate the liver when hepatocyte proliferation is compromised. Many different markers have been attributed to these cells but their nature remains still obscure. This study is a detailed gene expression analysis aimed at revealing their identity and their repopulating capacity after transplantation into injured rat liver. Two models for activation and proliferation of OC were used: 2-acetylaminofluorene treatment in conjunction with partial hepatectomy, and D-galactosamine induced liver injury. One 2-AAF pellet (35 mg/14 day release) was implanted subcutaneously and seven days later two-thirds PH was performed. The rats were sacrificed on day 10. D-glal was injected i.p. at a dose of 130 mg/100g body weight and the liver taken on day 5. Gene expression of purified OC was studied with Affymetrix Rat Expression Array 230 2.0, by RT-PCR and immunofluorescent microscopy (IFM). Two major and distinct populations of cells in the OC compartment were identified: EpCAM+ and Thy-1+ cells. EpCAM+ cells express mRNA for liver enriched transcription factors; known (Afp, Ck19, Dlk1, Ggt etc.) and novel (Aqp5, Cd24, Cd44, claudin-4, Cdk133, cadherin 22, claudin-7, mucin-1 etc.) OC markers, some of which are shared by cholangiocytes and hepatoblasts but others are unique. The co-expression of EpCAM and other OC markers (AFP, Ck19, Dlk1, Ggt, Cd24, alpha6-integrin, claudin-7, Cd24) was confirmed with (IFM). OC do not express hematopoietic stem cell markers: c-kit, SCF, Cd34 confirmed by quantitative RT-PCR but they express a number of mesenchymal markers, including vimentin, mesothelin, BMP7, TWEAK receptor, which reveals their epithelial/mesenchymal nature. Thy-1+ cells do not express any OC markers determined by microarrays of isolated cells and IFM. These cells have the phenotype of activated myofibroblasts: they express collagen type 1, alphaSMA and growth factors (HGF, BMP2), but they also express inflammatory cytokines, like IL6 and TNFα. Transplantation experiments revealed that EpCAM+ can reconstitute completely retorsine/partial hepatectomy preconditioned rat liver, while Thy-1+ cells do not. We conclude that EpCAM+ cells are the true AHPC. These cells have dual epithelial/mesenchymal phenotype and may originate from mesenchoderm. AHPC are a valuable candidate for liver cell therapy. We suggest that Thy-1+ cells are myofibroblasts or transdifferentiated stellate cells, which expand in parallel with activated oval cells. They are major supporting cells producing inflammatory cytokines and growth factors necessary for activation and proliferation of oval cells. Supported by NIH grant R01 DK59321

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189 ISOLATION AND EXPANSION OF EPCAM POSITIVE PROGENITOR CELLS FROM HUMAN FETAL LIVER
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Liver progenitor cells hold tremendous promise in their ability to provide a continual source of cells for wide variety of applications including cell therapy. Human liver progenitor cells are EPCAM positive but isolation and culture of these cells have proven difficult. We report the successful isolation and of EPCAM+ progenitor cells from the human fetal liver that can be expanded in in-vitro culture. Methods: 13 and 18 weeks human fetal liver obtained from abortion under the purview of ethics board approval were treated with standard collagenase digestion. Cells were then sorted using magnetic beads conjugated with anti-EPCAM antibody. Cultures were then performed on plates comparing various extracellular matrices including laminin, collagen, and matrigel, with and without feeder layers using chamber inserts. Cells were repeatedly passaged at confluence and purified with repeated EPCAM magnetic sorting.

Results: EPCAM positive cells were successfully isolated with up to 97% purity by immunofluorescence sampling. They demonstrated 3 distinct phenotype characterised by progenitor (CK19+, CD44+, alb-, AFP+), bipotential progenitors (albumin+/AFP+/CK19+) as well as a committed hepatoblast (albumin+/AFP+/CK19+). Laminin or matrigel coated plates together with conditioned media from EPCAM negative non-parenchymal cell fraction of the same liver were critical for maintaining progenitor phenotype. These cells retained high nuclear-cytoplasmic ratio, had a doubling time of 42 hours and could be expanded up to 6 passages in 3 months and were scaled up to 100 fold expansion in numbers. On differentiation protocols, colonies of hepatocyte-like cells that were strongly albumin and glycogen positive were observed. On collagen gels, tubular structures were obtained that were positive for GGT and CK7. Conclusion: EPCAM positive cells select for progenitor cells that are hepatoblast and biliary progenitors. These cells can be maintained in their progenitor status with high and robust proliferative and expansion potential. Manipulation of culture environment can augment differentiation into both hepatocytic and biliary lineages. Current strategy is aimed at isolating subfraction of these cells. The ability to expand progenitor cells in in-vitro cultures holds promise in providing an additional source for hepatocytes for its many potential applications.

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190 SALL4 REGULATES DIFFERENTIATION AND PROLIFERATION IN HEPATIC STEM/PROGENITOR CELLS
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Hepatic stem/progenitor cells are characterized by high proliferative capacity and bipotential differentiation into both hepatocytes and cholangiocytes. They are highly enriched in the fraction of CD45-Ter119-CD29+CD49f+c-kit- or Dlk+ cells derived from mid-fetal livers. Sall4 is a homologue of the Drosophila homeotic gene spalt and a zinc finger transcription factor required for the embryogenesis and proliferation of embryonic stem cells. We found that Sall4 expression was observed in the Dlk+CD45-Ter119- hepatic stem/progenitor cells from embryonic day 14 fetal liver but not adult hepatocytes. Sall4 expression was gradually decreasing during the development of liver, suggesting that Sall4 might be essential for development of fetal hepatic stem/progenitor cells rather than that of adult hepatocytes. Then, the role of Sall4 regulating fetal liver development is analyzed in this study. We constructed a retroviral vector expressing both Sall4 and enhanced green fluorescent protein using internal ribosomal entry site. We estimated proliferation of hepatic stem/progenitor cells using in vitro single colony assay system. Dlk+CD45-Ter119- hepatic stem/progenitor cells were isolated by FACS, infected with retrovirus expressing mock and Sall4 and cultured for 6 days in low cell density on collagen type I coated dishes. Infected cells were detected by the expression of green fluorescent protein. Colonies derived from hepatic stem/progenitor cells infected with Sall4-expressing retrovirus were clearly smaller than those derived from cells infected with control virus, suggesting that Sall4 inhibits the proliferation in hepatic stem/progenitor cells. We previously reported that hepatic stem/progenitor cells differentiated into mature hepatocytes using in vitro hepatic differentiation assay. Sorted Dlk+ hepatic stem/progenitor cells were infected with retrovirus expressing mock and Sall4 and cultured for 7 days in the presence of oncostatin M and extra-cellular matrices derived from Engelbreth-Holm-Swarm sarcoma. Morphologically, more undifferentiated hepatocytes were observed in the culture overexpressing Sall4. Moreover, the expression of tyrosine aminotransferase and tropohepin-2, 3-dioxogenase, hepatic maturation marker gene, was clearly down-regulated by the overexpression of Sall4. These results demonstrate that Sall4 suppresses maturation of hepatic stem/progenitor cells. Our results suggest that Sall4 may act as a negative regulator in hepatic stem/progenitor cell proliferation and play an essential role for hepatic cell fate determination.

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191 MICRORNA ANALYSIS OF HEPATOCYTIC DIFFERENTIATION OF LIVER STEM CELLS REVEALS UP REGULATION OF MIR23B, 27B, 24 AND DOWN REGULATION OF SMAD3 AND SMAD5 TARGETS IN THE TGFB/β/BMP SIGNALLING PATHWAY
Leslie E. Rogler, Tatayana Tchaikovskaya, Laurenta LeVoci, Raquel Norel, Charles E. Rogler; Hepatology Division Medicine, Albert Einstein College of Medicine, Bronx, NY

MicroRNAs [miRNAs] are a class of endogenous noncoding RNAs of ~22 nucleotide in length, many of which are conserved from C. elegans to man. miRNAs control gene expression by translational inhibition and destabilization of mRNA, acting as molecular rheostats to control important cellular and developmental processes. In order to begin to understand the role of miRNAs in liver stem cell differentiation, we constructed a microRNA microarray consisting of probes for 372 different mouse and human microRNAs. The microRNA array was used to detect changes in miRNAs expressed during DMSO induced hepatocytic differentiation of HBC-3 murine hepatoblast cells.
The data were normalized to U6 expression and significant changes were identified using SAM analysis. Of the miRNAs 31 most highly expressed by HBC-3, 16 were up regulated, 9 were unchanged and 6 were down regulated during hepatic differentiation. The regulation of these genes was confirmed using Northern analysis. Among the miRNAs up regulated during differentiation were three miRNAs MiR-23b, 27b and 24-1, that are clustered on Chromosome 13 of the mouse within an intron of a RIKEN gene, RIKEN 20101101Rik. Bioinformatic analysis revealed two consecutive CAT and TATA box pairs within the sequence 5' of the miRNA cluster and a transcript containing all three miR in the cluster was amplified from HBC-3 cell total RNA using RTPCR indicating that these miRNAs are part of a polycistron. In addition, we were able to identify a putative promoter sequence containing binding sites for several liver enriched transcription factors including HNF1 and HNF4. We next compared lists of bioinformatically identified targets for miR23b, 27b and 24 with our cDNA microarray database to identify genes that were both down regulated and putative targets. Using this approach we found that Smad3 and Smad5 were putative targets of miR23b and were down regulated during hepatocytic differentiation. Further analysis using MicroInspector identified multiple target sites for the microRNAs within the 23b-24 cluster in the 3' UTR regions of both Smad3 and Smad5 including sites that were perfect seed matches and were conserved in mouse, rat, human, chimp and dog genomes. In Situ hybridization revealed that Smads 3 and 5 and miR23b are reciprocally expressed. Smads 3 and 5 expressed in the bile duct and miR23b in hepatocytes. This distribution pattern suggests that the function of miR23b may be to limit TGFβ BMP signaling with in the liver and the miRNAs in this cluster may play a role in cell fate determination in the liver.

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The following people have nothing to disclose: Leslie E. Rogler, Tatayana Chaikovskaya, Lauretta LeVoci, Raquel Noel, Charles E. Rogler

192 PROSTAGLANDIN E2 MODULATES WNT-MEDIATED CONTROL OF LIVER DEVELOPMENT AND REGENERATION IN ZEBRAFISH
Wolfram Goessling1,2, Trista E. North3, Allegra M. Lord3, Sang Lee3, Mark Puder3, Randall T. Moon3, Leonard I. Zon4. Gastrointestinal Unit, Massachusetts General Hospital, Boston, MA; 2Stem Cell Program, HHMI, Children's Hospital, Boston, MA; 3Department of Surgery, Children's Hospital, Boston, MA; 4Department of Pharmacology, HHMI, University of Washington, Seattle, WA

wnt signaling is frequently altered in liver cancers. Recently, we and others described the role of wnt in vertebrate liver development. Using zebrafish, we demonstrated that after endodermal specification, wnt signaling is required for liver growth. In addition, we found that activated wnt signaling, such as in APC+/- embryos, markedly enhanced liver size. Wnt activation also significantly accelerated liver regeneration following partial hepatectomy in both zebrafish and mice. APC+/- zebrafish have increased propensity to develop spontaneous liver tumors, which resemble hepatoblastomas found in humans carrying APC mutations. These data illustrate the central role of wnt signaling in regulating hepatogenesis and in maintaining liver homeostasis in vertebrates. As patients with APC mutations are treated with cyclooxygenase (cox) inhibitors for chemoprevention of colonic polyps, we investigated whether alterations in prostaglandin (PG) signaling could modulate wnt activity in the liver. Using TOP:GFP, wnt reporter zebrafish, we found that PGE2 stimulated wnt signaling in vivo during development; this difference was significant by qPCR. Conversely, the cox inhibitors indomethacin or NS-398 (cox2) significantly decreased wnt activity. By in situ hybridization and confocal microscopy of fluorescent transgenic liver reporter fish, we analyzed the effects of PG modulation on liver development. PGE2 increased liver size and total hepatocyte number compared to controls; this result was confirmed by FACS analysis of individual embryos. Treatment with indomethacin or NS-398 mitigated the effects of wnt activation in APC+/- embryos; histological analysis revealed decreased levels of β-catenin and BrdU incorporation, indicating the inhibition of wnt-mediated cellular proliferation. In adult fish, PGE2 treatment following partial hepatectomy enhanced liver regeneration, as shown in vivo by ultrasound microscopy. Furthermore, indomethacin treatment diminished liver regeneration in APC+/- adults. Induction of the wnt antagonist dickkopf (dkk) or a dominant negative form of the β-catenin nuclear binding cofactor TCF (dnTCF) are detrimental to both liver development and regeneration. PGE2 rescued the effect of dkk induction, but not of dnTCF, suggesting that the interaction of PGE2 with the wnt signaling pathway in vivo occurs in the cytosol, possibly at the level of the destruction complex. The role of PGE2 in modulating wnt activity during liver regeneration was conserved in mice. This work provides in vivo evidence for the validity of pharmacological regulation of PG activity in the treatment of wnt-mediated liver disease and regeneration.

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The following people have nothing to disclose: Wolfram Goessling, Trista E. North, Allegra M. Lord, Sang Lee, Mark Puder, Randall T. Moon, Leonard I. Zon

193 TUMOR NECROSIS FACTOR RECEPTOR 1 AND 2 DEFICIENCY OR ACIDIC SPHINGOMYELINASE ABLATION AMELIORATES ETHANOL-INDUCED LIVER DAMAGE
Anna Fernandez, Anna Colell, Francisco Caballero, Carmen Garcia-Ruiz, Jose C. Fernandez-Checa; Liver Unit, Hospital Clinic, Barcelona, Spain

Tumor necrosis factor (TNF) is a proinflammatory cytokine that induces different cellular responses including inflammation, proliferation and cell death. TNF binds to two different plasma membrane receptors, TNF receptor 1 (TNFR1) and receptor 2 (TNFR2). Evidence from studies using anti-TNF therapy and TNFR1 knockout mice indicated an important role for TNF in the pathogenesis of alcoholic liver disease (ALD). Recent studies in experimental models of alcoholic (ASH) and non-alcoholic steatohepatitis (NASH) questioned the role of TNFR1 in disease progression and hepatic steatosis. Moreover, acidic sphingomyelinase (ASMA) has been shown to play a key role in the hepatocellular death induced by TNF. However, since in the absence of TNFR1 TNF can signal through TNFR2, and because the role of the ablation of both TNFR1 and TNFR2 or ASMA in ALD have not been previously examined, the aim of this study was to examine the contribution of TNFR1/TNFR2 and ASMA in ALD. Methods: 8-weeks-old male C57BL/6 mice deficient in both TNFR1 and TNFR2 (DKO) and ASMase in ALD. Methods: 8 weeks-old male C57BL/6 mice deficient in both TNFR1 and TNFR2 (DKO) and ASMA-/- mice were used. Animals were fed the Lieber-DeCarli diet for up to 4-6 weeks. Animals received a single dose of LPS (5mg/kg) 24 hours before sacrifice. Liver damage was assessed by histochemical hematoxilin/eosin staining and serum transaminase levels. Hepatic steatosis and lipid infiltration were examined by oil red staining and biochemical analysis. Hepatic inflammation was monitored by myeloperoxidase (MPO) staining. Results: the hepatic inflammation and necrosis observed in wild type ethanol-fed mice were attenuated significantly in DKO knockout mice. In vivo LPS injection in ethanol wild animals increased AST serum levels 8 times over pair-fed control mice (1440±210 U/dl vs. 135±14 U/dl) respectively.
This increase was significantly lower in mice lacking both TNF receptors (ethanol DKO +LPS vs. ethanol wild type + LPS: 235±45 U/dL vs. 1320±157U/dL). The increase in hepatic triglycerides, cholesterol and free fatty acids induced by ethanol feeding was also reduced (72±9, 68±11, 59±8%, respectively) in the DKO mice fed ethanol. Moreover, inflammatory cell foci reflected by MPO staining showed a marked reduction in DKO-fed ethanol mice vs wild-type ethanol fed mice following in vivo LPS challenge. The findings with ASMase knockout ethanol-fed mice paralleled those observed with DKO in terms of reduced steatosis, hepatocellular injury and inflammation. Conclusion: These results support the hypothesis that TNF-alpha plays an important role in alcohol-induced liver injury and that the targeting of TNF or its downstream mediator ASMase may be therapeutically useful in this disease.

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195 ETHANOL-INDUCED HISTONE ACETYLATION: A NOVEL MECHANISM FOR ENHANCEMENT OF INFLAMMATION IN ALCOHOLIC HEPATITIS?
Stuart Kendrick, Graeme O'Boyle, Jelena Mann, David E. Jones, Christopher P. Day; Institute of Cellular Medicine, Newcastle University, Newcastle upon Tyne, United Kingdom

Studies in patients and models of acute alcoholic hepatitis (AAH) demonstrate increased release of inflammatory cytokines from Kupffer cells or monocytes in response to bacterial endotoxin (LPS) acting via toll-like receptor 4 (TLR4) after ethanol treatment. This has been described as failure of the normal adaptive mechanism of endotoxin tolerance. Ethanol also increases histone acetylation in the liver. Histone acetylation at gene promoter regions changes chromatin conformation and facilitates transcription. We hypothesised that ethanol enhances cytokine production by increasing histone acetylation of pro-inflammatory genes.

Methods: The human monocyte cell line MonoMac6 was grown in RPMI-1640 medium with and without 0.5% ethanol for 0-6 days. Surface TLR4 was measured by FACS. Cytokine output was stimulated by E. coli LPS 10ng/ml and quantified by multiplex electrochemiluminescent immunoassay and quantitative RT-PCR. Histone modifications were visualised by immunofluorescence and elucidated by acid-urea gel electrophoresis. Promoter-specific changes in histone acetylation were determined by chromatin immunoprecipitation. MntBAP, 4-methylpyrazole and cyanamide were used to clarify the contribution of oxidative stress and ethanol metabolism to the effect.

Results: We observed significant augmentation of IL6 (5160 v 1192pg/ml at 48h p=0.05, Mann-Whitney U test) and IL-10 (1141 v 617pg/ml p=0.05) release in response to LPS in ethanol compared to normal medium. This was accompanied by a significant increase in surface TLR4 in ethanol. Increases in IL-8 (15160 v 14310pg/ml p=0.2) and TNFα (122 v 112pg/ml p=0.05) were more modest, suggesting a differential effect of ethanol on cytokine production by mechanisms not purely dependent on TLR4 number. We tested for failure of endotoxin tolerance but observed that tolerance was maintained - the cytokine response to a second LPS stimulus was strongly suppressed both with and without ethanol. Intriguingly, suppression of the anti-inflammatory IL-10 was more profound with ethanol than without (459 v 667pg/ml p=0.05) which may favour inflammation despite reduction in total cytokine output. We went on to analyse changes in histone modification and observed increased acetylation of histones H3 and H4 in ethanol-exposed cells, mirroring the cytokine observations.

Conclusions: The previously-unreported association between ethanol, histone acetylation and cytokine gene expression reveals a potential component of the mechanism of AAH. This raises the possibility of a novel use for drugs known to modulate histone acetylation, such as theophyllines, an established and safe therapy that remains completely untried in AAH.

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ZEBRAFISH EMBRYOS DEVELOP SIGNS OF LIVER DISEASE FOLLOWING SHORT-TERM EXPOSURE TO ALCOHOL

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Background. Alcoholic liver disease (ALD) is the leading cause of liver cirrhosis in the US and can lead to both acute and chronic hepatitis. In addition, alcohol abuse commonly results in a reversible accumulation of fat in the liver (steatosis), which can progress to irreversible inflammation (steatohepatitis) if drinking continues. Binge drinking, the introduction of a large quantity of alcohol over a short period of time, can also result in a number of ALD symptoms, including steatosis. Many of the molecular mechanisms behind the onset and progression of ALD symptoms remain unclear, in particular, while there is a clear genetic basis to ALD, little is known about the genetic contributions to this disease, and for such studies, a genetically tractable animal model is sought. Zebrafish are renowned for their utility to uncover the genetic basis of embryonic development, and some work using this system as a model to study the effects of alcohol on development have been reported. This system provides the opportunity to carry out alcohol studies on a large scale and, importantly, to address the genes that contribute to ALD onset. Aim. We have developed a delivery system to demonstrate the effects of acute ethanol (EtOH) treatment on a naive, but developed and functional liver in zebrafish embryos. Approach and Results. Exposing day 4 embryos to EtOH concentrations exceeding 3% results in rapid mortality, however, sub-lethal concentrations (2-3% EtOH) for 32 hours resulted in developmental abnormalities, including pericardial edema and malformed musculature, as well as altered swimming behavior. Tissue alcohol measurements indicate that fish treated with 2% EtOH for 32 hours have ~35 mM intracellular EtOH. Importantly, over 50% of embryos that were treated with 2% EtOH for 32 hours (from days 4-5 of development) demonstrate several signs of alcohol mediated liver damage including hepatomegaly and steatosis, as determined by oil red O staining and histological analysis. To determine whether changes in gene expression in accordance with ALD were detected, embryos treated with 1-2% EtOH were analyzed by quantitative real-time PCR. We detected signs of oxidative stress, as indicated by the induction of thioredoxin and cytchrome p450 2e1 (cyp2e1) genes, and endoplasmic reticulum stress as determined by increased bip, chop, gadd45, and atf3 expression. Conclusions. Morphological and molecular data support the idea that EtOH treated zebrafish embryos develop signs ALD, and therefore present a prime candidate for future studies to investigate the genetic factors which contribute to this disease.

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PKCε PLAYS A CAUSAL ROLE IN ETHANOL-INDUCED STEATOSIS

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Insulin resistance is a known independent risk factor for alcoholic liver disease. Whereas ethanol metabolism has been shown to cause hepatic steatosis, recent studies have suggested that insulin resistance exacerbates fat accumulation caused by ethanol. PKCε has been shown to cause hepatic insulin resistance and steatosis in experimental (NAFLD); however, the role of PKCε in ethanol-induced steatosis has not been determined. The purpose of this study was to therefore test the hypothesis that PKCε contributes to ethanol-induced steatosis. Accordingly, the effect of acute ethanol on indices of hepatic steatosis and insulin signaling were determined in wild-type and PKCε-/- mice. Acute ethanol (6 g/kg i.g.) caused a robust increase in hepatic non-esterified free fatty acids (NEFAs), which peaked peaked (5-fold) 1 h after ethanol exposure; a concomitant activation of PKCε was observed under these conditions. Acute ethanol also changed the pattern of expression of insulin-responsive genes (e.g., glucose-6-phosphatase; G6Pase), indicative of impaired insulin signaling. Lastly, acute ethanol exposure caused a >20-fold increase in hepatic triglycerides, peaking 12 hrs after ethanol administration. The alteration in G6Pase and later triglyceride accumulation caused by ethanol was blunted in PKCε-/- mice. In contrast, the increase in NEFAs caused by ethanol was not attenuated in PKCε-/- mice. Taken together, these data suggest that the increase in NEFAs caused by hepatic ethanol metabolism activates PKCε, which then exacerbates hepatic lipid accumulation by inducing insulin resistance. These data also suggest that PKCε plays a causal role in at least the early phases of ethanol-induced liver injury. [supported by NIAAA].

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and fibrosis were not seen. LCFA Uptake Kinetics: The hepatocellular LCFA uptake Vmax was increased vs control (1.1±0.07 pmol/sec/50,000 cells) in dose dependent fashion in the 10, 14, and 18% EtOH-fed groups (2.3±0.1, 2.8±0.2, and 3.1±0.4 pmol/sec/50,000 cells, respectively; p<0.001). While none of the EtOH-fed groups gained weight, epididymal fat pad weights increased, suggesting loss of muscle mass, and, although neither glucose nor LCFA levels were elevated, adipocyte LCFA uptake Vmax increased ~1.8-fold (p<0.05). Cardiac Histology: Control hearts showed occasional ORO+ droplets within cardiomyocytes. EtOH produced a dose-dependent increase in intra-cardiomyocyte lipid droplets that was striking in the 18% EtOH group. Conclusions: EtOH produces significant, dose dependent changes in LCFA disposition in liver, fat, and myocardium. Observed changes in adipocyte LCFA disposition may result in an increased LCFA load to the liver, contributing to HS, and increased cardiac TG accumulation, reflecting increased LCFA uptake, may contribute to alcoholic cardiomyopathy as it does in other settings. Simple mouse models of EtOH consumption can elucidate human pathophysiology and disease.

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199 CONTRIBUTION OF THE SYMPATHETIC HORMONE EPINEPHRINE TO THE SENSITIZING EFFECT OF ETHANOL ON LIPOPOLYSACCHARIDE-INDUCED LIVER DAMAGE
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It is well-known that ethanol pre-exposure sensitizes the liver to LPS hepatotoxicity. The mechanisms of this effect of ethanol on liver injury owing to LPS are not completely elucidated, but are known to involve an enhanced inflammatory response. It has been shown that the metabolic effect of ethanol on liver is mediated at least in part, by the sympathetic hormone, epinephrine. However, whether or not the sympathetic nervous system also contributes to the sensitizing effect of ethanol preexposure on LPS-induced liver damage has not been determined. The purpose of this study was therefore to test the hypotheses that (i) epinephrine preexposure enhances LPS induced liver damage (comparable to that of ethanol preexposure) and that (ii) the sympathetic nervous system contributes to the sensitizing effect of ethanol. Accordingly, male C57BL/6j mice were administered epinephrine for 3 days (2mg/kg/d) via osmotic pumps or bolus ethanol for 3 days (6 g/kg/d) by gavage. Twenty-four h later, mice were injected with LPS (10 mg/kg i.p.). Both epinephrine and ethanol preexposure exacerbated LPS-induced liver damage and inflammation. Concomitant administration of propranolol with ethanol (3x or 1x with the last dose of ethanol), significantly attenuated the sensitizing effect of ethanol on LPS-induced liver damage. These data support the hypothesis that the sympathetic nervous system contributes, at least in part, to the mechanism of the sensitizing effect of ethanol. These results also suggest that sympathetic tone may contribute to the initiation and progression of alcoholic liver disease. (Supported, in part, by NIAAA).

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200 ENHANCEMENT OF FIBRIN DEPOSITION CONTRIBUTES TO THE SYNERGISTIC EFFECT OF ETHANOL ON LPS-INDUCED LIVER INJURY
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It is well-known that ethanol enhances liver injury caused by bacterial lipopolysaccharide (LPS). Work by others has shown that LPS-induced liver injury involves, at least in part, activation of the coagulation cascade (e.g., factor X) leading to fibrin deposition. Recent work by this group has shown that ethanol induces activation of plasminogen activator inhibitor-1 (PAI-1), which may cause fibrin accumulation, but by inhibiting degradation of fibrin (fibrinolysis) and not by enhancing deposition via the coagulation cascade. Given that LPS and ethanol both potentially cause fibrin accumulation, but via different mechanisms, it was hypothesized here that fibrin accumulation contributes to the synergistic effect of ethanol on LPS-induced liver damage. Accordingly, the effect of ethanol pretreatment on LPS-induced liver injury and fibrin deposition was determined in mice. Ethanol pretreatment significantly enhanced liver damage caused by LPS, as determined by plasma parameters (AST and ALT) and histologic indices of inflammation and damage. This enhancement of liver damage was concomitant with a significant increase in the induction of plasminogen activator-1. The extracellular deposition of fibrin caused by LPS was also robustly increased by ethanol preexposure. Co-administration of agents that inhibited thrombin (heparin and hirudin) significantly attenuated the enhanced liver damage caused by ethanol preexposure; this protective correlated with a significant blunting of the induction of PAI-1 caused by ethanol/LPS. Furthermore, thrombin inhibition prevented the synergistic effect of ethanol on the extracellular accumulation of fibrin caused by LPS. Taken together, these results suggest that enhanced LPS-induced liver injury caused by ethanol is mediated, at least in part, by the synergistic activation of extracellular fibrin deposition (by LPS) and concomitant inhibition of fibrinolysis (by ethanol) and supports the hypothesis that fibrin accumulation and subsequent hemostasis may play a critical role in the development of alcohol-induced liver injury. (Supported, in part, by NIAAA)

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201 PROSPECTIVE SCREENING OF INFECTION IN PATIENTS WITH SEVERE ALCOHOLIC HEPATITIS TREATED WITH STERODS: EARLY RESPONSE TO THERAPY IS THE KEY FACTOR
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Despite the efficacy of steroids in severe alcoholic hepatitis (SAH) (Maddrey≥32), concerns are still raised with regard to the risk of infection. Aim: using a systematic screening, we prospectively determined the incidence of infection in all patients admitted for SAH before (pre-steroids), during and 2 months after the initiation of steroids (post-steroids). Methods: At admission the systematic screening of infection consisted in...
chest X-ray, blood, ascites and urinary cultures. C reactive protein was monitored weekly. The same screening was performed in the case of any suspicion of infection. All patients were treated with prednisolone 40mg daily during 28 days. Response to steroids was early defined at 7 days using the Lille model [Hepatology May 2007]. Results: At baseline, characteristics of the 189 patients were: age 50.9±0.6 years, ascites 73%, encephalopathy 26%, INR 2.1±0.04, serum bilirubin 183±8 mg/l, MELD 27±0.4, Maddrey 68±2, albumin 26±0.3 g/l, creatinine 12.4±0.9 mg/l, AST 189±22 IU/l. Pre-steroids/47 infections were diagnosed before steroids: 21 (44.7%) spontaneous bacterial peritonitis (SBP), 4 (8.5%) pulmonary (PI), 16 (34%) urinary (UI) and 6 (13%) others (OI). All infected patients were treated by antibiotics. 2 died before steroids initiation. Steroids were initiated 7 days (95%CI: 5-8) after the onset of infection. Patients infected before steroids had similar 2-month survival than others: 68±7% vs 72±4%. Post-steroids/47 infections were diagnosed after steroids: 41 patients developed an infection at a median delay of 14 days (95%CI: 9-24): 12 (29.3%) SBP, 15 (35.6%) PI, 8 (19.5%) UI and 6 (14.5%) OI. Infection occurred more frequently in non-responders than in responders: 9% vs 43%, p<0.000001. In univariate analysis, only the MELD (p=0.005) and the Lille model (p=0.0005) were associated with the probability of being infected. In multivariate analysis, only the Lille model (p=0.0001) independently predicted the probability of being infected whereas MELD not (p=0.13). In survival analysis, 6 variables reached a univariate p value ≤ 0.1: encephalopathy (p=0.0001), ascites (p=0.07). MELD (p=0.000001), Lille model (p=0.000001), AST (p=0.02), and infection (p=0.0004). In multivariate analysis, only the Lille model (p=0.000001) was independently associated with survival whereas the others including MELD (p=0.2) and infection (p=0.5) were not. Conclusion: SAH is associated with a high risk of infection. Infection screening is warranted at the admission but should not contraindicate steroids. In term of mechanisms, non-response to steroids is the key factor explaining the development of infection and is the only independent prognostic factor.

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203 EFFECT OF CHRONIC ALCOHOL CONSUMPTION ON NUCLEAR GENE REGULATORS OF MITOCHONDRIAL FUNCTION
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We have shown that chronic alcohol consumption affects the liver through mitochondrial damage and redox state alteration. Recently, it was found that a NAD dependent deacetylase Sir1 (SIRT1) regulates the activity of the peroxisome proliferator-activated receptor-γ coactivator 1α (PGC-1α), a key regulator of glucose production and energy metabolism, suggesting that these nuclear factors participate in the regulation of mitochondrial biogenesis, while interacting also with the nuclear receptor peroxisome proliferator-activated receptor-γ (PPARγ). The aim of this study was to investigate the role of chronic alcohol consumption on the expression of these proteins in the presence of alcohol-induced liver injury. Accordingly, rats were pair-fed the standard dextrin-maltose or the alcohol-containing Lieber-DeCarli liquid diets for 21 to 28 days. Alcohol significantly increased CYP2E1 mRNA (p=0.0002) and induced microsomal and mitochondrial proteins (p<0.001) with associated mitochondrial lipid peroxidation (p<0.001) and TNF-α mRNA increase (p=0.022). The mRNA expression of SIRT1, PGC-1α and PPARγ genes were assessed by RTPCR in liver lysate. The mRNA and the protein expressions of the transcription factor on age, gender, creatinine, PT and bilirubin selected without knowledge of their survival. Results: 38 patients were included in this study: 19 treated with MARS and 19 paired non-responders. At baseline, there was no significant difference between MARS and control groups for male gender (68.4 vs 68.4%), age (50.8±1.8 vs 51.1±1.4years), percentage of encephalopathy (45.6 vs 44.8%), AST (329±185IU/l vs 149±18IU/l), bilirubin (213±25 vs 192±22mg/l), creatinine (10.4±2 vs 12.5±2mg/l), albumin (26±1.7 vs 26±1.5g/l), PT (26.7±1.6 vs 25±2s), Maddrey function (84±5 vs 80±9) and Lille model (0.73±0.08 vs 0.78±0.06). As planned, the median number of MARS sessions was 3 (95%CI: 2-3, range 1-3). Fibrinogen before MARS was 1.5±0.2 g/l. In terms of safety, fibrinolysis occurred in 6 out of the first 8 patients treated with MARS. In the following 11 patients, during each session, we systematically administered 10mg/kg/hr Exacyl® (tranexamic acid). The decrease of fibrinogen after the first session was drastically lower in the patients treated with Exacyl®: 16±3% vs -76±12%, p<0.001) and coagulation disorders were no longer observed (75 vs 0 %, p=0.0005). On overall patients, each session of MARS induced a significant relative decrease in bilirubin (-18±4%, -6±4%, -15±5%) and creatinine (-20±6%, -13.5±7%, -5±9%) whereas it worsened PT (+19±6%, +11±4%, +16±6%). Patients treated with MARS had similar 1 and 2-month survival than their matched controls: 52.6±11 vs 52.6±11% and 42.1±11 vs 31.5±10.7%. In a sensitivity analysis restricted on patients treated with Exacyl®, there is still no difference in 2-month survival between the MARS patients and controls (46±15 vs 32±11, p=0.25). Conclusion: In the particular subgroup of non responders to steroids characterized by severe liver failure, MARS seems not to be efficient. In terms of safety, administration of Exacyl® is essential to prevent fibrinolysis.

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PPARγ were not affected by alcohol, whereas alcohol significantly reduced hepatic SIRT1 mRNA by 50% (p<0.01) and PGC-1α mRNA by 46% (p<0.01) and it significantly inhibited the protein expression of SIRT1 and PGC-1α (p<0.01) assessed by Western blot and corrected by β-actin as a loading control. In a preliminary attempt to offset these changes, additional groups of rats were fed the alcohol diet in which long-chain triglycerides (LCT) were replaced by medium-chain triglycerides (MCT). After 21 days feeding, MCT significantly restored SIRT1 and PGC-1α mRNA up to control levels (p<0.001 and p<0.01 compared to alcohol respectively), but had no effect on the respective protein expression. Conclusion: 1) for an equivalent dietary intake, chronic alcohol consumption reduces key energy sensing proteins (SIRT1 and PGC-1α) and their mRNAs expression through alterations of the redox state and mitochondrial dysfunction; 2) In the presence of alcohol consumption replacement of LCT by MCT affects transcription of these genes by restoring the mRNA levels to near control values, however MCT was not effective at the translation level since it did not increase the protein expressions after this short period of alcohol consumption. Since there is a pathophysiological link between SIRT1 and PGC-1α and mitochondrial energy the implication of the study is that mitochondrial dysfunction due to alcohol abuse can be treated by dietary modifications. Supported by NIH and VA.

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ALBUMIN AND OTHER ENDOTOxin REMOVAL METH-OIDS RESTORE NEUTROPHIL FUNCTION EX VIVO IN PATIENTS WITH SEVERE ALCOHOLIC HEPATITIS

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Background and Aims: Albumin infusion in patients with spontaneous bacterial peritonitis prevents progression to renal failure. In patients with severe alcoholic hepatitis (AH) ‘immune failure’ contributes to organ failure and mortality. We previously demonstrated that a resting burst of >55% and phagocytosis of <42% was associated with infection risk and mortality. This functional defect in neutrophils could be reproduced by incubating normal neutrophils with endotoxin. This study aimed to test the hypothesis that endotoxin may be responsible for the observed neutrophil dysfunction and that albumin may restore neutrophil function ex vivo. Methods: Isolated peripheral blood neutrophils from healthy volunteers were incubated with plasma from non-infected patients with a resting oxidative burst over 55% or with a resting oxidative burst below 55%. Neutrophil oxidative burst and phagocytosis was assayed by FACs analysis (Phagoburst and Phagotest, Orpegen). Endotoxin was measured using a LAL assay (Charles River). Endotoxin was removed either by passing the plasma over a polymyxin B containing column (Detoxi-Gel, Pierce Biotechnology), incubation with an LPS-neutralizing anti-CD14 antibody (Clone 11D18, Immunotools) or incubating with human albumin (BPL). Results: Patients with high resting oxidative burst had elevated endotoxin levels (0.12±0.04 EU/ml versus 0.01±0.01 EU/ml in controls). When plasma was passed over Detoxi-Gel columns, resting burst was reduced by 32% (p<0.001, n=9) and phagocytosis increased by 31% (p<0.05, n=11), whilst having no effect on neutrophils incubated with low burst (n=8) or healthy control plasma (n=3). Incubation with an anti-CD14 antibody prevented the induction of increased burst in normal neutrophils by high burst patients plasma (p<0.001, n=7) and increased phagocytosis by 20% (p<0.05, n=11), whilst having no effect on neutrophils incubated with low burst (n=8) or healthy control plasma (n=3). Incubation with increasing concentrations of human albumin (10g/L, 20g/L, 40g/L, n=10) caused a dose dependent decrease in resting oxidative burst (19%, 23%, 35%) and an increase in phagocytic capacity (25%, 41%, 41%). Conclusions: Our data indicate that patients with severe AH have endotoxemia and that ex vivo removal/binding of endotoxin or incubation with albumin restores neutrophil function. Albumin and other in vivo and/or ex vivo strategies for endotoxin removal warrant further evaluation in severe AH.

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DIAGNOSTIC ACCURACY OF CARBOHYDRATE DEFICIENT TRANSFERRIN IN PATIENTS WITH CHRONIC LIVER DISEASE. ALCOHOL CONSUMPTION AND LIVER DISEASE SEVERITY EFFECTS

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There are few studies on alcohol screening in a large cohort of patients with chronic liver diseases. G-Glutamyl Transpeptidase (GGT) has a low specificity and Mean Corpuscular Volum (MCV) a low sensitivity. Carbohydrate Deficient Transferrin (CDT) is measured in most clinical laboratories by the BioRad® CDT TIA kit but its specificity is low. Capillary electrophoresis (CE) and HPLC are the 2 confirmatory technics (Helander, Clin Chem 2005). Our aims were to study: 1) diagnostic accuracy of CDT measured by CE (N<1.3%) in 414 patients (HCV=287, HBV=41, Alcohol=19, Others=67), 2) the effect of fibrosis stage on CDT values. We divided the cohort in 2 subgroups of 207 patients by randomisation. The patients were matched by age and gender. Group 1 was the experimental group and Group 2 the validation group. To study the effect of fibrosis on CDT, only HCV patients evaluated with Fibrotest were included. In Group 1 patients, mean age was 53 years and sex ratio 1.3. 15%, 8% and 7% have consumed respectively more than 10g, 20 g of alcohol/day or were heavy drinkers according to the WHO definition. 9% of young men (<50 years) were heavy drinkers. CDT was higher in men than in women but the difference was not statistically different after stratification for alcohol use. CDT was correlated to alcohol ingested in g/day in the last month but was not correlated to transferrin saturation, ferritin, BMI, Metavir grade or stage. CDT, GGT and MCV sensitivity to diagnose heavy drinking was respectively 38%, 94% and 50%. Sp was respectively 96%, 43% and 82%, PPV was respectively 35%, 9% and 15%. Positive likelihood ratio (LR+) was 9.5 for CDT, LR- was 0.7 and Diagnostic Odds ratio (LR+/LR-) 14.6. In patients with chronic hepatitis C (CHC) from Group 1, mean CDT decreased significantly when the fibrosis score (Fibrotest) increased (<0.32 (F0-F1), 0.32-0.58 (F2), >0.58 (F3-F4)). CDT decreased from 0.76 to 0.59 and 0.55 according to the 3 thresholds (p=0.0125, ANOVA test). The same trend was found in Group 2 (p=0.025). In conclusion, CDT decreases with liver disease severity, has an excellent specificity, the best PPV but a diagnostic odds ratio < 30. CDT specificity has clearly increased as compared with the results obtained by other groups with the BioRad® CDT test but
sensitivity and VPP are still low. Young men with liver disease should be frequently screened for alcohol consumption. AUDIT questionnaire should be tested in patients with a liver disease to improve sensitivity. 

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206 PLASMA FROM PATIENTS WITH SEVERE ALCOHOLIC HEPATITIS INDUCES A FUNCTIONAL DEFECT POSSIBLY THROUGH EXPRESSION OF TOLL-LIKE-RECEPTORS 2 AND 9 BUT NOT 4
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In patients with alcoholic hepatitis, organ failure and mortality are often related to infection. Our previous studies have shown that endotoxemia may be related to neutrophil dysfunction and its removal restores neutrophil function but the mechanism of this is unclear. Toll-like receptors (TLR) are responsible for pathogen recognition and play a key role in early inflammatory response. We aimed to investigate the effect of patients plasma on TLR 2, 4 and 9 expression and whether albumin, which prevents induction of neutrophil dysfunction ex vivo, modulates the TLR 2, 4 and 9 expression. Neutrophils were isolated from whole blood of healthy volunteers and incubated with plasma from patients with alcoholic hepatitis (n=10) with or without the addition of human albumin (40 g/L). TLR 2, 4 and 9 expression was analysed by FACS analysis. Incubation of normal neutrophils with patients plasma induced a 71% increase in TLR2 expression and a 174% increase in TLR9 expression (p<0.0001) whereas TLR4 expression was unchanged. Incubation with 40 g/L human albumin reduced TLR2 and 9 expression to values not different from control and did not change TLR4 levels. (Figure 1) Plasma from patients with alcoholic hepatitis increases expression of TLR2 and 9 in normal neutrophils, likely due to the presence of bacterial products. TLR4 surface expression is not altered, which might indicate that TLR4 (the main receptor responding to endotoxin), is constitutively present in neutrophils to ensure immediate response to any endotoxin challenge. The beneficial effect of albumin in preventing the patient plasma induced neutrophil dysfunction together with a reduction in expression of TLR2 and 9 might define the mechanism through which albumin reduces inflammatory response and improves clinical outcome.

Expression of TLR 2, 4 and 9 on neutrophils incubated with plasma from alcoholic hepatitis patients with or without added albumin. PP: patients plasma, *** p<0.001

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207 NUTRITIONAL AND METABOLIC SIMILARITIES BETWEEN ALCOHOLIC AND NON-ALCOHOLIC STEATOHEPATITIS: A GENERAL POPULATION ASSESSMENT
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Objectives: Despite very similar histopathologic appearances, alcoholic liver disease (ALD) and non-alcoholic fatty liver disease (NAFLD) have presumably different etiologies reflected in their respective names. This study was designed to determine what metabolic and nutritional factors, aside from alcohol consumption, might predispose to steatohepatitis of either etiology in the general population of the US. Methods: The National Health and Nutritional Evaluation Survey (NHANES) conducted from 1999-2002 was analyzed to compare alcoholic liver disease (ALD) to non-alcoholic fatty liver disease (NAFLD) patients. All non-pregnant, adult subjects with negative viral serologies for hepatitis B and C with an elevated serum alanine aminotransferase (ALT) level were included in the analysis. Population-adjusted multivariate regression models were developed to compare metabolic and nutritional characteristics between the populations of ALD and NAFLD. Results: Of 6,653 participants, 293 (4.4%) subjects had elevated ALT values and endorsed excessive alcohol consumption (ALD group). 479 (7.2%) subjects had elevated ALT values without a definite alternative etiology and endorsed minimal alcohol consumption (NAFLD group). All non-pregnant, adult subjects with negative viral serologies for hepatitis B and C with an elevated serum alanine aminotransferase (ALT) level were included in the analysis. Population-adjusted multivariate regression models were developed to compare metabolic and nutritional characteristics between the populations of ALD and NAFLD. Results: Of 6,653 participants, 293 (4.4%) subjects had elevated ALT values and endorsed excessive alcohol consumption (ALD group). 479 (7.2%) subjects had elevated ALT values without a definite alternative etiology and endorsed minimal alcohol consumption (NAFLD group). Males were more frequently represented in the ALD group (79.8%) versus the NAFLD group (43.9%) (p=0.0009). Females were 5.2 times (OR 5.2, 95% CI 2.4-11.6) more likely to be in the NAFLD group. Age and ethnicity were not significantly different between the groups. Those with excess abdominal adiposity were 6.9 times (95% CI 2.2-21.2) more likely to be in the ALD group. The diagnosis of diabetes was 5.6 times
of OATPC was also diminished. In summary, the plasma mem-

branous localization of human OATPC is mediated by Golgi complex and vacuolar H+–ATPase vesicle-mediated membrane sorting pathways. cAMP-PKA regulates sorting process through the Golgi complex but not the vacuolar H+–ATPase associated vesicular pathway.

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209 CHEMICAL CHAPERONES PARTIALLY REVERSE THE MIS-

PROCESSING OF A BRIC2 MUTANT OF THE BILE SALT

EXPORT PUMP, ABCB11

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Background: Bile salt export pump (BSEP) is an ATP-dependent transporter that is expressed at the liver canalicular membrane. Abnormal retention of BSEP in intracellular compartments may be a cause of the human progressive and recurrent intrahepatic cholestasis (PFIC-2 and BRIC-2). PFIC-2 and BRIC-2 diseases represent a spectrum of intrahepatic phenotypes with PFIC-2 patients generally presenting a more progressive and severe phenotype than BRIC-2 patients. Chemical chaperones have been used successfully as a strategy to overcome misfolding of other mutant membrane proteins. We identified a BRIC-2 mutant that is misprocessed and hypothesized that this protein is misfolded and that chemical chaperones may correct the processing defect. Aim: To investigate the ability of chemical chaperones to reverse the misprocessing of a Bsep mutant R1128H. Methods: Mutant pEGFP-Bsep constructs were prepared from rat Bsep and stably transfected into HEK293 cells. Processing of Bsep is studied by endoglycosidases sensitivity and resolving the mature fully glycosylated and immature core-glycosylated form of the protein by Western immunoblotting. Biotinylation of surface proteins was used to detect cell surface expression of Bsep. Different compounds were added to stabilize R1128H protein and the effect on protein expression and processing was monitored by Western immunoblotting. Results: The R1128H is misprocessed leading to absence of protein expression at the cell surface. The mutant protein is localized intracellularly and co-localized with markers for the ER (Calnexin), ER-Golgi intermediate compartment (ERGIC-53) and Golgi marker (Golgin) as shown by confocal fluorescent microscopy, indicating that it may be recycling within ER and Golgi compartments. By Western immunoblotting, R1128H is expressed mostly as an immature core-glycosylated band. Inhibition of degradation of R1128H protein by proteasome inhibitor, MG-132 leads to accumulation in a perinuclear aggresome-like structure suggesting that the protein is abnormally folded. Reduced temperature (27°C), sodium butyrate, 4-

phenylbutyrate, glycerol or cyclopentamide (Csa) improved the processing of the core-glycosylated form into the fully glycosy-

lated form. Lower temperature and sodium butyrate can rescue and stabilize Bsep misfolded proteins at the cell surface.

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The following people have nothing to disclose: Ping Lam, Carol J. Soroka, James L. Boyer
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5' UNTRANSLATED REGIONS (UTRS) OF HUMAN MULTIDRUG RESISTANCE PROTEIN 2 (MRP2) GENE REGULATE IN VITRO TRANSLATION
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Human multidrug resistance protein 2 (MRP2; ABCG2), an ATP-binding cassette transporter, effluxes organic anions into bile. The absence of functional MRP2 causes Dubin-Johnson syndrome associated with conjugated hyperbilirubinemia. Three transcription initiation sites of the MRP2 gene in liver have been identified at -245, -204 and -99 nucleotide (nt) relative to the MRP2 AUG (numbered +1, +2 and +3 nt). (Hepatology 30:1507). We investigated the distribution of MRP2 transcription initiation sites in human liver and HepG2 cells, and the effect of the untranslated regions (5'UTRs) on in vitro translation. Among the seven upstream start codon AUGs in the MRP2 gene, only the AUG at -105 nt has a perfect Kozak motif and encodes a 22 amino acid peptide from -105 nt to -37 nt. We also studied whether translational regulation is dependent on this putative peptide sequence. Methods and results: 1. Ribonuclease Protection Assay. Human liver and HepG2 cell RNAs were used to detect MRP2 transcription initiation sites. We found the transcription initiation sites at -245, -204, and -99 nt. The distribution patterns of these sites in liver and HepG2 cells were similar, with the -204 nt and -99 nt 5'UTR transcripts were expressed equally and the -245 nt 5'UTR transcript expression was at low level. 2. Transient co-transfection assay in HepG2 cells. pNL3 constructs containing the 5'UTRs of -245(Hu-L), -204(Hu-M) and -99(Hu-S) nt upstream of the luciferase reporter gene were cotransfected with pSV40-Ren to determine the effect of the 5'UTRs on firefly luciferase expression. In transfected HepG2 cells, the ratios of Fire/Ren luciferase activities of Hu-L, Hu-M, and Hu-S constructs were 1.2, 1.3, and 3.5, respectively. 3. In vitro translation assay. The 5'UTRs (-204(M) and -99(S) nt) were cloned into T7Luc control vector. A mutation at -105GAG→AAG was introduced into mutM construct to disrupt the upstream open reading frame (uORF). ΔhMRP2 construct was prepared to scramble the peptide sequence encoded from -105 nt to -37 nt in hMRP2 construct. Capped reporter messages were prepared from linearized constructs, and in vitro translation rates were determined in Rabbit Reticulocyte Lysate. The translation efficiencies of M, S, and mutM constructs relative to the T7Luc control vector were 0.27, 1.2, and 2.2, respectively. The relative translation efficiencies of hMRP2 and ΔhMRP2 constructs were not different (0.52 and 0.68, respectively). Conclusions: The uORF at -105 nt of the 5'UTR of MRP2 has a significant inhibitory effect on in vitro translation, but this inhibitory effect is not dependent on the sequence of the peptide encoded by this uORF. (GM55343)

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The following people have nothing to disclose: Yuanyuan Zhang, Wei Li, Mary Vore

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FOX2A REGULATES BILE ACID METABOLISM AND PREVENTS ER STRESS IN THE LIVER
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Purpose: Bile acids, synthesized in the liver, are potent detergents, crucial for the absorption of lipophilic nutrients. In addition, synthesis of bile acids is the predominant mechanism for the excretion of excess cholesterol. Dysregulation of bile acid homeostasis leads to cholestatic liver disease and induces cellular stresses, including ER stress. We investigated the role of Foxa2 in bile acid metabolism. Methods: Conditional gene ablation was used to derive mice deficient for Foxa2 in hepatocytes. Global location analysis ("ChIP-on-Chip") was employed to determine Foxa2 targets in the mouse liver. Labeled DNA samples were hybridized to two arrays, the Mouse PromoterChip BCBC-5A and the Mouse PromoterChip BCBC-5B. Mice were fed either normal chow or 0.5% cholic acid diet. Livers were analyzed for bile, cholesterol, triglyceride, and non-esterified fatty acid content. Serum ALT and AST levels were determined. Liver RNA was isolated from Foxa2loxP/loxP;Alfp.Cre and control littermates, and quantitative reverse transcription-PCR were performed for messenger RNA of genes crucial to bile acid homeostasis. Results: Deletion of Foxa2 in hepatocytes leads to a decrease in expression of conjugation (Slc27a5) and glutathione S-transferase enzymes (Gsta1, Gsta2, and Gstm2) and bile acid transporters (Oatp2, Mrp2, Mrp3, and Mrp4), both on the basolateral and canalicular membranes, resulting in reduced serum bile acid levels and mild cholestasis. Transcription of several of these genes is dependent directly on Foxa2. Challenging Foxa2 mutants with a diet containing cholic acid leads to severe intrahepatic cholestasis and a disproportionate rise in serum bile acids. Accumulation of hepatic bile acids is toxic, exacerbated by reduced expression of detoxification enzymes, and leads to ER stress and severe liver injury in Foxa2loxP/loxP;Alfp.Cre mice. Conclusions: We have found that Foxa2 regulates multiple genes involved in hepatic bile acid homeostasis. We demonstrate further that Foxa2 is required to prevent hepatic cholestasis, liver injury, and ER stress, when mice are fed a diet containing cholic acid. Thus, we have identified a novel role for Foxa2 in hepatic bile acid homeostasis and in the prevention of cholestatic liver injury.

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THE MEMBRANE-BOUND BILE SALT RECEPTOR TGR5 IS EXPRESSED IN NON-PARENCHYMAL CELLS OF RAT LIVER
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The G-protein coupled receptor TGR5 (Mbar, Gpbar-1) is the first plasma membrane-bound bile salt receptor described to date. We have recently demonstrated TGR5 expression in sinusoidal endothelial cells (SEC) and Kupffer cells (KC) of rat liver. Stimulation of TGR5 in SEC activated eNOS and increased NO production. Aim of this study was to investigate the expression of TGR5 in different liver cells in normal and diseased rat liver. Methods: An anti-rat TGR5 antibody was developed in our lab. Immunofluorescence staining of normal rat liver revealed that TGR5 is not only expressed in SEC and hepatic stellate cells. TGR5 specific agonists BR26 and tetrachloride treated cirrhotic rats (12 weeks) were stained with the anti-TGR5 antiserum. Antibodies directed against KC (ED2), bile duct epithelial cells (CK19) and cholangiocytes (Taq) were used to identify the non-parenchymal liver cells. Results: Immunofluorescence staining of normal rat liver sections revealed that TGR5 is not only expressed in SEC and...
EXENDIN-4 PROTECTS CHOLANGIOCYTES FROM APOPTOSIS, BOTH IN VITRO AND IN VIVO

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Progression of chronic cholestatic disorders towards ductopenia results from the dysregulation of cholangiocyte survival, with cell death by apoptosis prevailing over compensatory proliferation. Currently, no therapy is available to sustain cholangiocyte survival in the course of those disorders. Cholangiocyte biology and response to injury is regulated by neuropeptides and neuroendocrine hormones. Glucagon-Like Peptide-1 (GLP-1) is secreted by enteroendocrine cells and acts as growth regulator in many cells and tissues. We have recently shown that cholangiocytes express the GLP-1 receptor (GLP-1R), the activation of which results in a significant enhancement of the cellular adaptive reaction to cholestasis. The GLP-1R selective agonist exendin-4 sustains pancreatic beta-cell proliferation and prevents cell death by apoptosis. For such properties, exendin-4 is now employed in humans as a novel therapy for diabetes. In cholangiocytes, we have shown that GLP-1R activation by exendin-4 enhances the activity of PI3K, cAMP/PKA and Ca2+-CamKinase-alpha pathways. Aim: to verify whether exendin-4 is effective in preventing cholangiocyte apoptosis. Methods: in vitro, we tested if exendin-4 (100 nM) is able to prevent apoptosis of cholangiocytes isolated from normal rats induced by glycochenodeoxycholic acid (GCDCA, 400 microM). Cholangiocyte apoptosis was also induced in vivo by the administration, 7 days after bile duct ligation (BDL), of a single IP injection of CCl4. Animals were also treated with either control or exendin-4 injections for 3 days. Results: GCDCA incubation enhanced bax mitochondrial translocation, cytochrome c release and caspase 3 activity; such changes were prevented by the pre-incubation with exendin-4. PI3K, but not cAMP/PKA and Ca2+-CamKinase-alpha, inhibitors neutralized the effects of exendin-4 on caspase 3 activity, bax mitochondrial translocation and cytochrome c release. In vivo, exendin-4 administration prevented the increase in TUNEL positive cholangiocytes observed in BDL rats treated with CCl4. Exendin-4 treatment also prevented the loss of bile duct mass induced by CCl4 in BDL rats. Summary/conclusion: exendin-4 prevents cholangiocyte apoptosis both in vitro and in vivo; such an effect is due to the ability of exendin-4 to counteract the activation of the mitochondrial pathway of apoptosis. Exendin-4 anti-apoptotic properties are mediated by PI3K. These novel data indicate that exendin-4 is effective in protecting cholangiocytes from apoptosis associated with chronic cholestasis. These findings support the hypothesis that exendin-4 may be effective in relenting the progression of cholangiopathies towards ductopenia.

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214 EXPRESSION OF SOLUTE TRANSPORTERS AND WATER CHANNELS IN ARPKD: IMPLICATIONS FOR HEPATIC CYSTOGENESIS

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BACKGROUND: In Autosomal Recessive Polycystic Kidney Disease (ARPKD), kidney and liver cysts progressively grow and expand over time, likely because of disturbances in cell proliferation, apoptosis, and/or water and solute transport. We recently reported that in the PCK rat, an animal model of ARPKD, liver cysts are derived from cholangiocytes, the epithelial cells that line bile ducts. We also showed that in 3-D culture, PCK bile duct explants expand at a 3-fold greater rate than normal explants. Finally, we demonstrated that cholangiocytes contain organelles that sequester functionally related transport proteins (the water channel, aquaporin-1 [AQP1]; the chloride channel, CFTR; and the anion exchanger, AE2) that account for ion-driven ductal water transport. Since abnormalities in fluid movement may play a role in cyst expansion in ARPKD, we tested the hypothesis that the relative expression and topographic locations of AQP1, CFTR, and AE2 are abnormal in PCK cholangiocytes. METHODS: Expression of AQP1, CFTR, and AE2 was assessed by quantitative RTPCR and Western-blots on tissue sections from both freshly isolated and cultured cholangiocytes from normal and PCK rats, and by immunofluorescent confocal microscopy in liver sections. Topographic distribution of the proteins within cholangiocytes was assessed by immunofluorescent confocal microscopy in liver sections. Topographic distribution of the proteins within cholangiocytes was assessed by confocal microscopy in whole liver sections. RESULTS: mRNA levels for AQP1, CFTR, and AE2 are abnormal in PCK cholangiocytes. METHODS: Expression of AQP1, CFTR, and AE2 was assessed by quantitative RTPCR and Western-blots using both freshly isolated and cultured cholangiocytes from normal and PCK rats, and by immunofluorescent confocal microscopy in liver sections. Topographic distribution of the proteins within cholangiocytes was assessed by immunofluorescent confocal microscopy in liver sections. Topographic distribution of the proteins within cholangiocytes was assessed by confocal microscopy in whole liver sections. RESULTS: mRNA levels for AQP1, CFTR, and AE2 were increased (8, 4 and 3-fold, respectively) in PCK cholangiocytes (p<0.05) compared to normal. By Western-blots, AQP1, CFTR and AE2 were also increased (4, 3 and 3-fold, respectively) in PCK cholangiocytes compared to normal (p<0.05). Moreover, in PCK bile duct explants grown in 3D-culture, levels of expression of the three proteins correlated with cyst expansion. Over-expression of the three proteins was confirmed by immunofluorescent confocal microscopy and by immunoelectron microscopy in the whole liver. In cholangiocytes in whole normal liver, AQP1, CFTR and AE2 are preferentially localized to the apical membrane; in contrast, in cholangiocytes in PCK...
livers, the three proteins maldistribute to the basolateral membrane. CONCLUSION: Cholangiocytes from an animal model of ARPKD are characterized by over-expression and altered intracellular topography of three proteins (AQP1, CFTR, and AE2) critically involved in ductal bile formation. The data are consistent with the possibility that abnormalities in the function of these three proteins may contribute to hepatic cystogenesis.

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NM23-H2 INHIBITS CHOLANGIOCARCINOMA GROWTH BY BINDING TO AND DOWNREGULATING PPARδ

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Background & Aims: The nuclear receptor peroxisome proliferator-activated receptor δ (PPARδ/δ) has been linked colorectal, breast, hepatocellular and prostate cancers. The mechanism for PPARδ-induced carcinogenesis is unknown. Our previous studies showed PPARδ is overexpressed in cholangiocarcinoma and PPARδ agonists increase cholangiocarcinoma growth. We hypothesized that deregulation of coregulators of PPARδ may lead to its overexpression in cancer. To identify candidate proteins, we performed a yeast two-hybrid screen using mouse PPARδ, where we identified a novel PPARδ-binding protein, NM23-H2, also known as NME2, NM23B and NDKB, which is an isoform of multifunctional proteins involved in a variety of cellular activities including cell proliferation, development, motility, adhesion, and differentiation. Aims: We determined if NM23-H2 binds to and regulates PPARδ expression and PPARδ-dependent cholangiocarcinoma growth. Methods: Yeast two-hybrid screening was performed using mouse PPARδ bait vector which was transformed with mouse kidney cDNA library. PPARδ and NM23-H2 expression were determined by real-time RT-PCR and western immunoblots in cultured normal rat cholangiocytes (NRC) and human cholangiocarcinoma cell lines. GFP-tagged PPARδ and RFP-tagged NM23-H2 were used for colocalization by immunofluorescence microscopy. PPARδ promoter activity was determined by dual luciferase assays. Cell cycle analysis was performed by FACS. Results: Yeast two-hybrid screening identified NM23-H2 as a binding protein, which interacts with C-terminal region of PPARδ. PPARδ and NM23-H2 interaction was confirmed by communoprecipitation, GST pull-down assays and colocalization in the cytoplasm by fluorescence microscopy. Overexpressed PPARδ or treatment with the PPARδ agonist GW501516 (GW) resulted in increased cell proliferation and expression of the proliferation marker Cdk2 in cholangiocarcinoma cells. Loss of NM23-H2, due to siRNA, activated PPARδ luciferase promoter activity, upregulated PPARδ RNA and protein expression and increased GW-stimulated cholangiocarcinoma growth. Overexpression of NM23-H2 inhibited the PPARδ luciferase promoter activity, downregulated PPARδ RNA and protein expression, induced apoptosis, and reduced GW-stimulated cholangiocarcinoma growth. Summary and Conclusions: We report the novel observation that the association of NM23-H2 with PPARδ negatively regulates PPARδ expression by binding to C-terminal region of PPARδ. Our observations provide the first evidence that the metastasis suppressor, NM23-H2, is involved in the regulation of PPARδ-mediated proliferation and PPARδ-induced carcinogenesis.

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SMALL CHOLANGIOCYTES PROLIFERATE IN RESPONSE TO H1 HISTAMINE RECEPTOR STIMULATION VIA ACTIVATION OF THE IP3/CA2⁺/CAMK/CREB-DEPENDENT PATHWAY

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Cholangiopathies are characterized by the heterogeneous proliferative responses of different sized bile ducts. Constitutively dormant, small cholangiocytes proliferate de novo only in pathological conditions of damage of large ducts (e.g., by CCl4 treatment). Histamine differentially regulates cholangiocyte growth by increasing proliferation by interaction with H1/H2 histamine receptors (H1/H2R), but decreasing mitosis via activation of H3/H4R. Binding of H1 agonists to H1R increases intracellular Ca2⁺ levels, which are coupled with calmodulin-dependent stimulation of CaM kinase, and activation of the transcription factor, CREB. We have shown that: (i) small and large murine cholangiocytes express H1-H4R; and (ii) the H1R agonist, HTMT dimaleate, increases the growth of small but not large murine cholangiocytes. We aim to expand our earlier studies and further elucidate the intracellular mechanisms by which H1R stimulates the growth of small murine cholangiocytes. Methods: We used our immortalized small and large murine cholangiocyte lines that were stimulated with 0.2% BSA or HTMT dimaleate (a H1R agonist, 10 × 10⁻⁶ M) for 24 and 48 hours in the absence or presence of: terfenadine (H1R antagonist, 10 μM); BAPTA/AM (intracellular Ca2⁺ chelator, 1 μM); and W-7 (CaMK inhibitor, 10 μM). Proliferation was determined by MTs assay. IP₃ and cAMP levels were determined in small and large cholangiocytes treated with BSA or HTMT dimaleate. Phosphorylation of CaMK I (Thr 177) was measured in small cholangiocytes treated with HTMT dimaleate. We evaluated CREB activation in nuclear extracts from small cholangiocytes treated with BSA or HTMT dimaleate with/without W-7. Knock-down experiments were performed with shRNA plasmid for CaMK. Real time PCR was used to determine CaMK knock-down efficiency and MTs (in the absence or presence of HTMT dimaleate) was performed. RESULTS: Small but not large cholangiocytes proliferate in response to the H1R agonist, proliferation that was blocked by terfenadine, BAPTA/AM and W-7. IP₃ (but not cAMP) levels were increased in small cholangiocytes treated with HTMT dimaleate. HTMT dimaleate increased the phosphorylation of CaMK I compared to basal levels. H1 stimulation induced activation of CREB that was blocked by W-7. shRNA experiments resulted in an ablation of the effects of H1 stimulation on small cholangiocyte growth. Conclusion: Activation of the H1R induces an increase in small cholangiocyte growth by IP₃/CA2⁺/CaMKInase I-dependent phosphorylation of CREB. Differential regulation of small and large cholangiocyte growth by histamine may be important in the treatment of cholangiopathies targeting specific-sized ducts.
PROGESTERONE REGULATES CHOLANGIOCYTE PROLIFERATION DURING CHOLESTASIS BY AUTOCRINE SIGNALING MECHANISMS

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Cholangiocytes possess the pathway for progesterone (PROG) levels are increased with bile duct ligation (BDL) suggesting that PROG may affect biliary growth during cholestasis. We examined the following: Do cholangiocytes express receptors for PROG? Does PROG regulate cholangiocyte growth and Do cholangiocytes possess the pathway for PROG steriodogenesis? Methods: The studies were performed in female and male normal and 1-wk BDL rats. Nuclear (PR-A and PR-B) and membrane (PRGMC1 and PRGMC2) PROG receptor expression was determined by: (i) immunofluorescence in liver sections and NRIC; and (ii) RT-PCR in isolated cholangiocytes and NRIC. The in vivo effects of PROG on cholangiocyte growth were determined by administration of PROG (50 mg/kg/day IP) to normal rats for 1 week. Also, immediately after BDL rats were treated with a neutralizing PROG antibody (6.5 nmol/day IP) or non-immune serum for 1 week. Subsequently, cholangiocyte growth was measured by evaluation of the number of bile ducts in liver sections.

The biosynthesis pathway for the steroidogenesis of progesterone consists of three key enzymes: steroidogenic acute regulator, cytochrome p450 side-chain cleavage, and 3 β-hydroxysteroid dehydrogenase. The expression of the three enzymes was evaluated by: (i) immunofluorescence in liver sections and NRIC; and (ii) RT-PCR in isolated cholangiocytes and NRIC. Cholangiocyte PROG secretion was evaluated in primary cultures (6 hours) of isolated normal and BDL cholangiocytes. The effect of PROG on cholangiocyte proliferation was evaluated in NRIC stimulated in vitro with PROG (10³ M), and supernatant (containing progesterone) from cholangiocytes in the presence/absence of anti-progesterone antibody. Results: Cholangiocytes and NRIC express the PR-A and PR-B nuclear receptor and PRGMC1 and 2. In vivo, PROG increased the number of bile ducts of normal rats. Anti-PROG inhibited cholangiocyte growth stimulated by BDL. Normal and BDL cholangiocytes expressed the biosynthetic pathway for PROG production and secrete PROG. In vitro, PROG increased the proliferation of NRIC and the supernatant from cholangiocytes increased biliary proliferation, which was partially inhibited by preincubation with anti-progesterone suggesting an autocrine pathway in the regulation of cholangiocyte growth.

CONTRIBUTION OF COMBINATORIAL LIGAND LIBRARIES TO THE HUMAN BILE PROTEOME: NEW IDENTIFICATIONS AND PROMINENCE OF BINDING PROTEINS

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Background: Proteins in bile may have important physiological functions and serve as disease biomarkers. The aim of this study was to identify proteins in normal human gallbladder bile, using a technology of combinatorial ligand libraries, capable to enhance the signal of hidden or low abundance species. Methods: Gallbladder bile was collected from 13 subjects without biliary tract disease who underwent cholecystectomy during liver/pancreas surgery. The proteins present in 275 mL of pooled bile fluid were treated with two serially connected hexapeptide ligand libraries with physicochemical complementary properties. The first library had a primary amine as a terminal group while the second one, had a carboxyl terminal group. The recovery of captured proteins was performed by three elution steps addressing different types of protein interactions (hydrogen bonding, ion exchange and low interaction forces, hydrophobic associations). The analysis of resulting protein mixture was performed by LC-MS/MS. Results: Overall 222 gene products were found; 143 of them were never reported before in bile proteomics studies. Ligand libraries by themselves contributed to find 81 new gene products distributed throughout different categories. The classification into functional categories showed that the proportion of binding proteins was the highest (more than 50 %). This category included immunoglobulin chains, complement precursors and other immune defense proteins. Different newly identified proteins participate in the biliary antimicrobial defense, e.g. azorucidin, or defensin, produced by cholangiocytes. Three major apomucins produced by the gallbladder epithelium (MUC-1, MUC-SAC, MUC-SB) were also detected. The next most frequent functional classes included transport and structural proteins (e.g. actin and actin-binding proteins of the Ezrin-Radixin-Moesin family), catalytic and enzyme regulator activities (e.g. dipeptidyl peptidase 4, an enzyme localized in the apical membrane of hepatocytes and cholangiocytes). We also identified detoxifying enzymes derived from hepato-biliary lining cells (i.e. glutathione S-transferases, of three types, microsomal, alpha and pi, and superoxide dismutase,...) which may provide protection against toxicants eliminated in bile. Conclusions: As a global view from known proteomics studies performed on bile, the number of known species from bile fluid reaches 288 gene products. The present investigation contributes for 143 proteins, about half of them being discovered, as a result of peptide ligand libraries. The described method opens new perspectives for the discovery of markers for specific biliary tract diseases.

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EXTRACELLULAR ATP INDUCES IL-6 TRANSCRIPTION IN BILE DUCT EPITHELIAL CELLS VIA THE P2Y11 RECEPTOR
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Background. Bile duct epithelia (BDE) release the pro-inflammatory cytokine IL-6, which is upregulated in biliary cirrhosis. Recently, we demonstrated that release of IL-6 induces down-regulation of the ecto-nucleotidase NTPDase2, a critical regulator of bile ductular proliferation, by portal fibroblasts. However, the mechanisms regulating release of IL-6 by BDE have not been defined. Several groups have identified multiple P2Y receptors for ATP and other nucleotides expressed by BDE. Thus, the aims of this study were to determine whether extracellular ATP regulated BDE IL-6 upregulation and to identify the molecular mechanisms regulating this process. Methods. Immuno-isolated rat BDE were used for all studies. The effects of the following nucleotides (ATP, ADP, AMP, UTP, and UDP; 100 µM each) on IL-6 mRNA expression were determined by quantitative RT-PCR. The effect of cytosolic Ca²⁺ was determined using the Ca²⁺ chelator BAPTA/AM, and the effect of cAMP was determined using the cAMP-dependent protein kinase inhibitor Rp-cAMP. The effects of ATP on BDE cAMP levels was determined by ELISA. Results. ATP upregulated IL-6 mRNA expression to 2.18 ± 0.37-fold of control (p < 0.0001), yet ADP, UTP, UDP did not upregulate IL-6 transcription. No effect was noted with AMP (negative control), and the effect of ATP was blocked by the P2Y inhibitor suramin (50 µM; p = 0.0001 vs. ATP). The pharmacologic profile for IL-6 upregulation was most consistent with the newly identified P2Y₁₁ receptor. Since the P2Y₁₁ receptor is coupled to both cytosolic Ca²⁺ and cAMP generation, we assessed the effect of inhibition of Ca²⁺ signals and cAMP on ATP-dependent IL-6 upregulation. Both BAPTA/AM and Rp-cAMP inhibited the IL-6 upregulation, consistent with the known pharmacology for P2Y₁₁. Furthermore, extracellular ATP upregulation of BDE cAMP levels (from 1.82 ± 0.59 to 3.14 ± 0.24 fmol/10⁴ cells; p < 0.05). Conclusions. This is the first description of the novel P2Y receptor subtype P2Y₁₁ in BDE. Activation of the P2Y₁₁ receptor transcriptionally upregulates IL-6 via Ca²⁺- and cAMP-dependent pathways. These experiments suggest that purinergic regulation of cytokine release is a novel mechanism that is important in the pathogenesis of biliary cirrhosis.

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MORPHOLOGICAL AND FUNCTIONAL HETEROGENEITY OF THE MURINE INTRAHEPATIC BILIARY EPITHELIUM
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The rat and human intrahepatic biliary epithelium is morphologically and functionally heterogeneous. Since no information exists on the heterogeneity of the murine intrahepatic biliary epithelium and with increased usage of transgenic mouse models to study liver disease pathogenesis, we sought to evaluate the morphological, secretory, proliferative and apoptotic phenotypes of small and large bile ducts in normal and cholestatic models of liver injury. Methods: For morphometry, two normal mouse livers (C57/BL6) were dissected into blocks of 2-4 mm², embedded in paraffin, sectioned, and stained with H&E. Sizes of bile ducts and cholangiocytes were evaluated by using SigmaScan to measure the diameters of a total of 38 bile ducts and 250 cholangiocytes. In small and large normal cholangiocytes, we evaluated the mRNA expression of cholangiocyte specific markers, cytokeratin-19 (CK-19) and secretin receptor (SR) by real time PCR; and cAMP levels in response to secretin (100 nM). To evaluate cholangiocyte proliferative responses after bile duct ligation (BDL), small and large cholangiocytes were isolated from 3-day BDL mice, and proliferation was analyzed by PCNA expression and by the number of CK-19-positive bile ducts in liver sections. We treated normal mice with a single dose of carbon tetrachloride (CCL₄; 0.5 mg/Kg BW, IP) and evaluated the proliferation of: small and large ducts by PCNA and CK-19 immunohistochemistry; and apoptotic small and large cholangiocytes by TUNEL analysis in liver sections. Results: In situ morphometry established that mean minor x major diameters for bile ducts classified as small and large were approximately 12 x 26 µm and 284 x 643 µm, respectively. The diameter of cholangiocytes lining small ducts was 7.3 ± 1.6 µm, and of those lining large ducts was 15.4 ± 2.6 µm. Regression analysis showed that a significant relationship exists between cholangiocyte area and bile duct diameter (R = 0.82, p < 0.001). We isolated small (≈7.3 µm diameter) and large (≈15.6 µm diameter) cholangiocytes. Both small and large cholangiocytes express CK-19 and only large cholangiocytes express SR mRNA and respond to secretin with increased cAMP levels. Following BDL in mice, only large cholangiocytes proliferate leading to increased ductal mass. Acute CCL₄ induced the necrosis of centrilobular hepatocytes and apoptosis of only large cholangiocytes, associated with decreased large bile duct mass and increased numbers of small ducts. Conclusion: Similar to rats, mouse intrahepatic biliary epithelium is morphologically and functionally heterogeneous. The mouse is a suitable model for defining the heterogeneity of the biliary tree.

Disclosures:
HEDGEHOG SIGNALING AND EPITHELIAL MESENCHYMAL TRANSITIONS DURING BILARY FIBROSIS
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Background Epithelial-mesenchymal transitions (EMT) may occur in primary biliary cirrhosis (PBC) (Hepatology 45:977-81, 2007), but the mechanisms involved are unknown. The Hedgehog (Hh) signalling pathway regulates EMT. We reported that portal tracts in PBC livers accumulate Hh-responsive cells, and showed that paracrine Hh-signaling between co-cultured bile ductular cells (BDC) and myofibroblasts (MF) modulates the growth and viability of both cell types. Aim To determine if BDC near Hh-producing MF undergo EMT. Methods In vitro An immortalized murine BDC line (H03B) and a clonally derived rat MF cell line (BB) were placed in monocultures or co-cultured for 6 days in a transwell system that increases Hh in the medium. BDC mRNA was harvested and pooled from 6 wells/plate/experiment, and used to make RNA probe for microarray analysis (Affymetrix Mouse 430-2 GeneChips® (N=3 experiments). Selected microarray results were confirmed by QRT PCR. In vivo Biliary fibrosis was induced in 32 rats by bile duct ligation (BDL). Bilio-jejunal Roux-en-Y (RY) anastomosis was performed 4 weeks post-BDL, and liver samples were collected during 12 weeks of recovery and processed for RNA analysis. Expression of the EMT marker, S100A4, was co-localized with vimentin by immunohistochemistry (IHC) in human PBC samples. Results Compared to BDC monocultures, BDC co-cultured with MF exhibited significant up-regulation and down-regulation of 965 and 235 genes, respectively. The most over-expressed gene, Lipocalin2 (Lcn2, +3665%, p < 0.001), is involved in EMT. Ontogeny analysis of Cell differentiation (57 up- and 26 down-regulated genes), Development (129 up- and 55 down-regulated genes) and Cytoskeleton (55 up-regulated genes) gene sets confirmed an induction of various mesenchymal genes, including fibronectin, α(I) (+6000%). Gene expression normalized by 12 reference genes, showed that paracrine Hh-signaling between co-cultured bile ductular cells (BDC) and myofibroblasts (MF) modulates the growth and viability of both cell types. Aim To determine if BDC near Hh-producing MF undergo EMT. Methods In vitro An immortalized murine BDC line (H03B) and a clonally derived rat MF cell line (BB) were placed in monocol-+

222 TREATMENT WITH URSODEOXYCHOLIC ACID, BUT NOT WITH CHOLIC ACID, INCREASES BILE FLOW INDEPENDENTLY OF CYSTIC FIBROSIS TRANSMEMBRANE REGULATOR IN MICE
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BACKGROUND: Ursodeoxycholic acid (UDCA) treatment is frequently applied for cystic fibrosis-related liver disease (CFLD). It has been hypothesized that UDCA is beneficial through its choleretic activity. The hepatic expression of the CFTR protein is restricted to the cholangiocytes. Here CFTR is involved in the generation of ductular bile flow. It is not known to what extent the choleretic activity of bile salt treatment depends on expression of competent CFTR protein, and whether or not UDCA differs in this respect from other bile salts. We evaluated the role of CFTR in the acute and chronic choleretic effect of bile salt treatment, through comparative studies in Cfr-null, homozygous F508del, and corresponding wild type control (WT) mice. METHODS: Bile flow (BF) was determined after gallbladder cannulation. BF during 30 minutes after acute interruption of the enterohepatic circulation was regarded as basal BF. After 30 min, taurocholic acid (TCA) or tauroursodeoxycholic acid (TUDCA) were i.v. administered in stepwise increasing dosages, up to 1200 nmol/min/100 g BW, to Cfr-null mice and WT littersmates fed a standard chow diet. Other Cfr-null, homozygous F508delΔ/Δ mice and their respective WT littersmates were fed either the standard chow, or the same diet supplemented with cholic acid (CA, 0.5 wt%) for 3 weeks. Finally, Cfr-null mice and WT littersmates were fed the standard chow or the same diet supplemented with UDCA(0.5 wt%) for 3 weeks. RESULTS: Upon a regular chow diet, the basal BF was similar in Cfr-null and F508delΔ/Δ mice, compared to their respective WT littersmates (Cfr-null, 6.3±1.8 vs. 6.2±0.1; and, F508delΔ/Δ, 5.4±2.8 vs. 8.5±10.8 μl/min/100 g BW, resp.; NS). i.v. administration of TCA or TUDCA to Cfr-null mice and WT littersmates increased BF to similar extents. Dietary CA treatment increased basal BF significantly less in Cfr-null and F508delΔ/Δ mice than in their respective WT littersmates (Cfr-null, 13.7±3.0 vs. 17.9±1.7, p<0.05; and, F508delΔ/Δ, 20.9±5.5 vs. 26.2±6.2 μl/min/100 g BW, p=0.06; resp.). Interestingly, dietary UDCA treatment increased basal BF more profoundly than CA treatment, and to similar levels in Cfr-null mice and WT (40.0±5.9 vs. 31.0±4.4 μl/min/100 g BW, resp.; NS). CONCLUSION: Upon chronic treatment, UDCA is highly choleretic in mice, independently of the presence of functional CFTR. The independence of functional CFTR is UDCA specific, since the choleretic activity of chronic CA treatment is diminished in Cfr-null and homozygous F508del mice. We speculate that this specific, Cfr-independent choleretic effect of chronic UDCA treatment could be therapeutically important for cystic fibrosis.

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DEFICIENCY OF TRAIL OR OVEREXPRESSION OF HUMAN MCL-1 ATTENUATES LIVER INJURY IN THE BILE DUCT LIGATED MOUSE
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Hepatocellular apoptosis is a key mechanism of liver injury during cholestasis. Apoptosis during cholestasis is triggered by hepatotoxicity of toxic bile acids. Although we have reported that toxic bile acids increase hepatocyte expression of tumor necrosis factor-related apoptosis inducing ligand (TRAIL) death receptor 5 (DR5), the contribution of TRAIL and its opponent myeloid cell leukemia sequence-1 (Mcl-1) to cholesiatic liver injury have not been explored. Thus, our AIM was to ascertain if genetic deficiency of TRAIL or overexpression of human Mcl-1 (hMcl-1) attenuates liver injury in the bile duct ligated (BDL) mouse. METHODS: C57/Bl6 wild-type (wt), TRAIL knockout (TRAIL-/-) and hMcl-1 transgenic (TG) mice were used for these studies. Real-time polymerase chain reaction (PCR) was used to measure mRNA transcripts for TRAIL. Hepatocyte apoptosis was quantified by the Tunel assay and immunofluorescence for activated caspases 3/7. Liver injury was assessed by histopathology, quantification of bile ducts and serum ALT determinations. Hepatic fibrosis was assessed by Sirius red staining and quantitative morphometry. RESULTS: Following 7 days of BDL, hepatic TRAIL mRNA was 6-fold greater in BDL vs. sham-operated wild-type animals (p < 0.01). As compared to sham-operated wild-type mice, BDL mice displayed a 13-fold increase in Tunel and an 11-fold increase in caspase 3/7 positive hepatocytes (p = 0.01). The number of Tunel and caspase 3/7 positive cells was reduced by > 80% in BDL TRAIL knockout and hMcl-1 TG animals (p < 0.01). Consistent with the apoptosis data, histologic examination of TRAIL knock out and hMcl-1 TG BDL livers also demonstrated a 50% reduction in the number of bile ducts as compared to wild-type BDL mice. Serum ALT values, which were increased 200-fold in wild-type BDL vs. sham-operated animals, were also reduced by > 80% in TRAIL knockout or hMcl-1 TG BDL mice. These differences could not be ascribed to differences in cholestasis as serum total bilirubin concentrations were nearly identical in wt, TRAIL knock out and hMcl-1 TG BDL mice (12.15 mg/dL). Finally, hepatic fibrosis was 3-fold increased in wt as compared to hMcl-1 TG animals following 14 days of BDL. In CONCLUSION: The present study demonstrates that in the BDL mouse, hepatocyte apoptosis, liver injury and hepatic fibrosis are attenuated in the TRAIL-deficient or hMcl-1 TG mouse. In conclusion, these observations support a pivotal role for TRAIL-mediated liver injury that can be inhibited by Mcl-1 and further define conditions under which TRAIL is hepatotoxic.

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STIGMASTEROL INHIBITS BILE ACID – INDUCED ACTIVATION OF HETEROMERIC BILE ACID TRANSPORTER GENES Ostx/β
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Background: The heteromeric transporter Ostx/β (organic solute transporter) is expressed on the basolateral side of hepatocytes and functions to efflux organic solutes including bile acids (BA). It has been previously shown that Ostx/β genes are upregulated in both human and mouse models of cholestasis, principally by FXR (farnesoid X receptor)-mediated pathways. The molecular mechanism of Total Parenteral Nutrition-associated cholestasis (TPNAC) is unknown, but there is evidence to suggest that BA efflux from hepatocytes may be impaired. We hypothesized that Stigmasterol (Stig), a phytosterol that is an obligate component of TPN lipids, interferes with BA-induced Ostx and/or Ostβ expression at the RNA and/or protein level in human and murine hepatocytes. Methods: HepG2 cells and primary mouse hepatocytes isolated from 8-10 week old C57BL/6 male mice (n = 4-6 mice/group) were cultured using standard protocols. Cells were maintained in 0.25% charcoal stripped serum media and treated with 50 µM chenodeoxycholic acid (CDCA) with or without 10 µM Stig or vehicle (DMSO). Cells were harvested 24 hrs post treatment for RNA isolation and protein extraction. qRT-PCR and western blot analysis were performed to determine changes in Ostx/β gene and protein expression. Electromobility shift assay was performed to assess nuclear protein binding to FXR elements in Ostx and Ostβ promoters. Results: In HepG2 cells, CDCA-induced mRNA expression of Ostx and Ostβ was 28 and 19-fold respectively. Stig reduced CDCA-induced mRNA expression of Ostx by 29% (p<0.01) and of Ostβ by 47% (p<0.05) vs CDCA alone. In primary mouse hepatocytes, Ostβ mRNA expression was increased 32-fold by CDCA treatment which was reduced by 34% (p<0.05) in the presence of Stig; no changes were seen in Ostx mRNA with any treatments. Western blot analysis of primary mouse hepatocytes showed no change in Ostx protein levels between all treatment groups; CDCA-induced Ostβ protein levels (4-fold) were reduced by 63% (p<0.05) with Stig vs CDCA alone. Electromobility shift assay revealed CDCA-induced nuclear protein (previously shown to be FXR) binding to the Ostx promoter was reduced by 94% (p<0.01) with Stig vs CDCA alone; Stig had no effect on protein binding to the Ostβ promoter in HepG2 cells. Conclusion: Our data indicates that Stig may contribute to hepatotoxicity seen in infants with TPNAC by interfering with BA transport by antagonizing BA induced expression of FXR target genes Ostx/β. Thus, resulting in accumulation of BA within hepatocytes and ultimately hepatocellular damage. This data furthers our ongoing molecular explanation for the development of TPNAC in infants on long-term TPN.

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FIC1 DEFICIENT RAT HEPATOCYTES REVEAL STRUC-TURAL AND FUNCTIONAL DEFECTS IN THEBILE CANALICULAR MEMBRANE WHEN EXPOSED TO HYDROPHOBIC BILE ACIDS
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Background: FIC1 (ATPB8B) is a P4-type ATPase which is a putative aminophospholipid flippase. FIC1 is broadly expressed and localized at the apical membrane of epithelial cells including hepatocytes and cholangiocytes. Mutations in FIC1 gene result in progressive familial intrahepatic cholestasis type 1 (PFIC1) where reduced expression of the bile acid nuclear receptor FXR (NR1H4) has also been observed. In contrast, Fic1 deficient mice do not develop cholestasis, although bile salt homeostasis and excretion are disturbed when treated with hydrophobic bile salts. Therefore, the pathogenesis of FIC1
induced cholestasis remains unclear. Aim: To assess the effect of Fic1 deficiency on canalicular membrane structure and function and bile acid transporter expression. Methods: Fic1 was knocked down in primary rat hepatocytes maintained in a collagen sandwich culture system using adenoviral siRNAs for 4 days. Gene and protein expression were determined using realtime quantitative PCR (Q-PCR) and Western blot. Bsep and Mrp2 localization and activities were assessed using immunocytochemistry and CGamF and CMFDA excretion. Canalicular membrane structure was examined by electron microscopy (EM). Results: Adenoviral siRNA reduced Fic1 expression by 90% at both mRNA and protein levels. All virally infected preparations made cell contacts with well formed bile canaliculi. No significant differences were seen in the expression of Fxr, Bsep, Ostα, Oatp2, Mrp2, Mrp3, and Mrp4 at mRNA or protein levels. Immunofluorescent staining confirmed that Bsep and Mrp2 were localized to the canalicular membrane and EM images showed intact canalicular membrane structures in these cells when they were maintained in 5% bile acid free charcoal-stripped serum medium. In contrast, canalicular excretion of CGamF (a substrate of Bsep) was significantly reduced in Fic1 knockout cells compared with scrambled siRNA controls whereas CMFDA (an Mrp2 substrate) secretion was not significantly changed. However when hepatocytes were treated with 5µM CDCA for 24 hrs, blinded analysis of electron micrographs revealed that the canalicular membrane was disrupted in many Fic1 knockout cells, but not CDCA treated control cells. Conclusion: Fic1 knockdown hepatocytes maintain expression of bile salt transporters although bile salt excretion is reduced. However our findings indicate that Fic1 deficiency predisposes the canalicular membrane to injury by hydrophobic bile acids, explaining the development of PFIC.

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226 PHENOTYPIC TRANSDIFFERENTIATION OF HEPATOCYTES TO BILIARY EPITHELIAL CELLS IN RAT LIVER AND IN HEPATOCYTE ORGANOID CULTURES: ROLE OF HGF AND EGF SIGNALING

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Transdifferentiation of hepatocytes to biliary epithelial cells (BECs) has been speculated as a mechanism to restore the injured bile ducts in chronic biliary liver disease (CBLD). We have documented the actual occurrence of this event in vivo and in vitro in rodents. The present study is aimed at identifying the mechanisms of hepatobiliary transdifferentiation, using an experimental biliary injury model of F344 rats exposed to methylene dianiline (DAPM, 50 mg/kg). Hepatocyte to BEC transdifferentiation was indicated in this model by the periportal hepatocytic immunostaining of biliary specific cytokeratins AE1/AE3 and CK19. In chimeric rats carrying dipeptidyl peptidase IV (DPPIV)-positive hepatocytes (DPPIV) and treated with DAPM (50 mg/kg, single administration), one treatment did not elicit bile ductules bearing the donor hepatocyte marker DPPIV. Repeated administration, however, of DAPM (50 mg/kg, at 2 days interval, 3x) resulted in DPPIV positive bile ducts (~10%) producing a direct evidence for hepatocyte to BEC transdifferentiation. These findings indicate that hepatocyte to BEC transdifferentiation occurs only when biliary proliferation is impaired. To investigate the mechanisms of this transdifferentiation, an in vitro organoid culture of rat hepatocytes was utilized where hepatocyte-to-BEC transdifferentiation has been previously demonstrated. Growth factors known to be involved in differentiation including HGF, EGF, VEGF, PDGF, SCF, MSP, FGF-a, FGF-b, and FGF-8b were tested for their ability to promote hepatocyte transdifferentiation. Except HGF and EGF, none of the growth factors examined were able to induce formation of BEC despite the presence of their cognate receptors. Among the protein kinases common to HGF and EGF signaling including ERK1/2, p38, JNK, and AKT, Western blot analyses showed activation of only AKT in the HGF and EGF treated cultures showing biliary epithelium formation. Microarray analysis of the HGF- and EGF-treated organoid cultures indicated upregulation of ~500 genes including various ECM proteins, genes regulated by Wnt/beta-catenin, TGFbeta/BMP, and AKT pathways while downregulation of ~200 genes including GSTYb4, CYP1A1, CYP2D6, tumor suppressor protein p53 etc. Taken together, these data suggest that HGF and EGF signaling play a unique role in transdifferentiation of hepatocytes to BECs through specific set of target genes upregulated/downregulated revealed in the microarray analysis. These findings also provide a potential novel therapeutic approach of stimulating the native hepatocytes transdifferentiation to compensate for the lost biliary epithelium in CBLD patients.

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227 TAUROLITHOCHOLIC ACID-INDUCED CHOLESTASIS IN RAT LIVER

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The impact of taurine conjugation for the anticholestatic effect of ursodeoxycholic acid (UDCA) and its taurine conjugate (TUDCA) is unclear (Nature CP Gastro Hepat 2006;3:318). Side chain shortened nor-ursodeoxycholic acid (norUDCA) exerts therapeutic effects superior to UDCA in Mdr2/Abcc4 knockout mice that develop chronic progressive sclerosing cholangitis (Gastroenterology 2006;130:465). In contrast to UDCA, norUDCA is poorly conjugated by rat and human hepatocytes (Hepatology 2005;42:1319). Taurolithocholic acid (TCA)-induced cholestasis represents a well-established experimental model of hepatocellular cholestasis. Preliminary studies suggested that UDCA, but not norUDCA, reverses TCA-induced cholestasis. Aim: To compare the effect of norUDCA and its taurine conjugate (TnorUDCA) on bile formation and liver cell injury in TCA-induced cholestasis. Methods: The effect of norUDCA and TnorUDCA (25µmol/l, each) on bile flow and biliary secretion of the Mrp2/Abcc2 substrate, 1,2-dinitrophenyl-S-glutathione (GS-DNP), was studied in presence or absence of TCA (10µmol/l) in isolated perfused rat livers (JBC 2003;278:17810). Bile acid administration was started after 45 min, and 1-chloro-2,4-dinitrobenzene (30µmol/l), the precursor of GS-DNP, was admin-
228 THE ROLE OF PPARγ IN BILE DUCT LIGATION-INDUCED HEPATIC INJURY IN RATS

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Aims: Peroxisome proliferator-activated receptor (PPARγ) is ligand-activated intracellular transcription factors that have been implicated in important biological processes such as inflammatory response, lipogenesis, glucose uptake and vascular tone regulation. The liver injury in response to bile duct ligation (BDL) is related to enhanced inflammatory response and increased vasoconstrictor production. In this study, we sought to determine the effects of PPARγ modulation on BDL-induced hepatic injury. Materials and Methods: Rats were divided into four groups: sham operation with vehicle treatment; BDL with vehicle treatment; BDL with PPARγ antagonist (GW9662, 1mg/kg i.p.); BDL with PPARγ agonist (15d-PGJ2, 200µg/kg i.p.). Rats were sacrificed 24 hours thereafter. The plasma 15d-PGJ2 levels (PPARγ ligand) were measured by ELISA. Gene expression of inflammatory cytokines (IL-1β, IL-6, IL-12, IFN-γ and TNF-α) and vasoconstrictr-related genes (endothelin-1 and thromboxane A2 synthase) in the liver were determined by real-time RT-PCR. Serum levels of reactive oxygen species (ROS) were also measured. Results: 15d-PGJ2 levels were significantly increased in BDL rats. GW9662 increased, whereas 15d-PGJ2 decreased the gene expression of inflammatory cytokines such as IL-1β, IL-12, IFN-γ, and TNF-α (Figure). The expression of endothelin-1 and thromboxane A2 synthase were also showed a similar trend. Serum ROS levels did not show significant difference among the groups. Conclusion: These results indicated that the activation of PPARγ protect the liver against hepatic injury through the attenuated production of inflammatory cytokines and vasoconstrictors. PPARγ activation with 15d-PGJ2 might be a potent therapeutic option for hepatic injury in BDL rats.

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229 TTR-ABCB11 MICE FED CHOLATE HAVE ATTENUATED INCREASES OF HEPATIC INTERLEUKIN-1β AND SERUM ALT, BUT INCREASED EXPRESSION OF APOPTOTIC PATHWAYS

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Background: TTR-Abcb11 transgenic mice overexpress the liver bile salt export pump (Abcb11) and hypersecrete bile salts into bile. Abcb11 expression increases with cholate feeding, and this may serve as a protective mechanism against cholate injury. The hydrophobic bile salt deoxycholic acid (DCA) is also a potent inducer of the Fas (CD95) and tumor necrosis factor-associated apoptosis-inducing ligand (TRAIL) apoptotic pathways. Aim: To determine if hepatic Abcb11 overexpression can attenuate hepatic cholate-induced injury. Methods: Male 8-12 week old TTR-Abcb11 and FVB/NJ (wt) mice were fed chow or a 0.5% cholate diet (w/w) for 3 days. The bile salt pool was analyzed using HPLC and serum ALT levels were measured spectrophotometrically. Hepatic interleukin 1β (IL-1β) levels were determined utilizing Meso Scale Discovery electrochemiluminescence and hepatic gene expression was determined using quantitative PCR. Data expressed as Mean±SD. Results: Cholate feeding expanded the bile salt pool (p<0.01) in TTR-Abcb11 (52±15 to 116±30 µmol/100g bw) and FVB/NJ (69±11 to 124±11 µmol/100g bw) mice. In contrast, TTR-Abcb11 mice fed cholate had a 2.9-fold greater concentration of taurodeoxycholic acid (TDCA) in their bile salt pool than FVB/NJ (13.6±4.3 vs 4.7±1.3 µmol/100g bw, p<0.01). Cholate feeding increased serum ALT levels in all mice, however, the increase in ALT levels was significantly lower in TTR-Abcb11 mice (p<0.03). Cholate feeding caused an increase in ALT levels from 26±10 to 96±33 U/L vs 28±9 to 187±67 U/L; in TTR-Abcb11 vs FVB/NJ mice, respectively. TTR-Abcb11 mice fed cholate did not have an increase in hepatic levels of the inflammatory cytokine IL-1β, however, hepatic IL-1β levels were markedly increased in cholate-fed FVB/NJ compared to chow fed mice (97±128 vs 37±18 pg/g liver, p<0.01). In contrast, analysis of apoptosis pathway genes demonstrated that TTR-Abcb11 mice fed cholate had a 4-fold greater increase in gene expression of Fas receptor, p<0.01; a 7.5-fold increase in Fas ligand, p<0.01; and a 2-fold increase in TRAIL-R2 (Receptor 2), p<0.05 compared to FVB/NJ mice fed cholate. There were no differences detected in TRAIL ligand, TNF-R1
MEDIATED REDUCTION OF HEPATIC NUCLEAR RXR

pathways are due to the increased levels of the taurine conju-
gated reduction of nuclear RXRα may help reduce cholate-
duced inflammation, while the activation of apoptotic
pathways are due to the increased levels of the taurine conju-
gate of the pro-apoptotic bile salt DCA.

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230 REDUNDANT ROLES FOR JNK1 AND JNK2 IN IL-1β-
MEDIATED REDUCTION OF HEPATIC NUCLEAR RXRα IN VIVO

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Inflammation leads to suppression of many liver functions including bile flow. Previous work suggests that the cytokine Interleukin-1β (IL-1β) plays a central role in mediating inflammation-induced cholestasis. Multiple in vivo and in vitro studies indicate that a major mechanistic contribution to a decrease in hepatic gene expression by IL-1β is a targeted reduction in the functional activity of members of the nuclear receptor (NR) superfamily involved in directing the expression of critical hepatobiliary transporter genes. In HepG2 cells, IL-1β signaling induced nuclear export of Retinoic X Receptor alpha (RXRα), the heterodimeric partner of multiple NRs, which was dependent on JNK-mediated phosphorylation of Ser260. JNK1 and JNK2 isoforms play distinct roles in hepatocyte growth and apoptosis, yet individual roles for either isoform in mediating the effect of IL-1β on RXRα are unknown. In this study we explored the in vivo effects of IL-1β on hepatic NR-dependent gene expression, nuclear RXRα levels and roles for individual JNK isoforms. C57Bl/6 mice were given 0.5, 1, or 5 μg murine IL-1β or saline by i.p. injection. Livers were harvested after 1, 4, 8 or 16 hrs. Jnk1-/- and Jnk2-/- mice were given 5 μg IL-1β or saline 1 hr prior to harvest. Primary hepatocytes were treated with 10 ng/ml IL-1β for 15, 30, 45 and 60 min +/- 30 min pretreat-
mament with 30 μM of the JNK inhibitor SP600125. Gene expression was determined by TaqMan Real-Time PCR. RXRα, P-JNK1 and total JNK levels in nuclear and cytosolic fractions were analyzed by Western blot. In vivo, IL-1β treatment resulted in a dose and time-dependent induction of hepatic TNFα, IL-1β and IL-6 RNA levels while 5 μg IL-1β reduced the expression of the NR-dependent genes Ntcp, Cyp7a1, Cyp8b1, Abcg5, Mrp2, Mrp3, and Cyp3a11 in a time-dependent manner. Nuclear RXRα levels were reduced dose-dependently after 1 hr of IL-1β treatment, whereas equivalent JNK activation was seen at all doses. IL-1β treatment of primary hepatocytes reduced nuclear RXRα levels, which was prevented by pretreatment with a JNK inhibitor demonstrating direct involvement of JNK in IL-1β-mediated reduction of nuclear RXRα in hepatocytes. To determine which of the 2 JNK isoforms expressed in liver is involved, Jnk1-/- and Jnk2-/- mice were treated with 5 μg IL-1β for 1 hr. Absence of either JNK1 nor JNK2 significantly influenced the reduction of hepatic nuclear RXRα after IL-1β treatment. This suggests that for IL-1β mediated alterations in hepatic nuclear RXRα levels, functional redundancy exists for JNK1 and JNK2, stressing the importance of this pathway in mediating the hepatic response to inflammation.

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231 ROLE OF CA2+-DEPENDENT PROTEIN KINASE C ISO-
FORMS IN ESTRADIOL 17β-D-GLUCURONIDE-INDUCED
CHELOSTASIS IN THE RAT

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The endogenous estradiol metabolite, estradiol 17β-D-glu-
curonide (E17G), induces an acute cholestasis in rat liver due in part to retrieval of the canalicular bile salt export pump (Bsep, Abcc11) and the multidrug resistance protein 2 (Mrp2, Abcc2), and the consequent loss of their function. Activation of the classical, Ca2+-dependent isoforms of protein kinase C (cPKC) has been shown to affect bile formation and normal transporter localization (J Biol Chem 279:10323). We evaluated the involvement of cPKC isoforms in E17G-induced changes in canalicular transporter function and localization.

Methods: The non-recirculating isolated perfused liver (IPL) from female rats was used to assess the effect of cPKC inhibition by Gö6976 (500 nM in perfusate) on changes in bile flow, total glutathione (GSH) and 3H-taurocholate (3H-TC) excretion induced by E17G. Bile was collected before and after 5 min after E17G (2 μmol, intraportal bolus dose) for 60 min. Liver samples were taken 20 min after E17G for localization of Bsep and Mrp2 by confocal immunofluorescence laser microscopy. Isolated hepatocytes were cultured for 5 h and exposed to E17G (50 μM) for 5-20 min. After subcellular fractionation, activation of the cPKC isoform, PKCcα, and the novel Ca2+-inde-
dependent PKC isoform, PKCc, were evaluated by western blot-
ing of cytosolic and total membrane fractions. Results: E17G induced an acute, significant (p<0.05 vs control) decrease in bile flow (61%) and biliary excretion of total GSH (62%) and [3H]-TC (79%). The selective cPKC isoform inhibitor Gö6976 partially protected against the decreases in bile flow (15%), total GSH (23%) and [3H]-TC (20%; p<0.05 vs E17G alone). In E17G-treated livers, both Bsep and Mrp2 demonstrated endocytic retrieval from the canalicular membrane; Gö6976 completely protected against E17G-induced retrieval measured by ImageJ (NIH) software analysis. In hepatocytes, E17G induced a 60% increase (p<0.05) in membrane-bound PKCc within 5 min, an indicator of PKC activation. This increase persisted for 15 min and returned to control values by 20 min. The proportion of PKCc associated with the membrane fraction was not modified by E17G. Conclusions: E17G selectively activ-
ates cPKC isoforms within minutes. Selective blockage of cPKC activation with Gö6976 completely prevented retrieval of Mrp2 and Bsep, and markedly protected against their loss of function. These findings support a central role for cPKC isoforms in E17G-induced cholestasis by a mechanism involving mainly transporter retrieval from the canalicular membrane.

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Background & Aim: Expression of hepatic organic anion transporters is decreased during inflammatory cholestasis. Down-regulation of the multispecific conjugate-exporter Mrp2 is mediated by interleukin-1β dependent signals (Geier et al. Hepatology 2003) but the underlying mechanism is still unclear. YB-1 is a predominantly cytoplasmic protein which translocates to the nucleus in response to various insults and has previously been characterized as a transcriptional repressor of rat Mrp2 (Geier et al. BBRC 2003). Consequently, we characterized the role of YB-1 as a suppressor of hepatic negative acute phase genes such as Mrp2. Materials & Methods: Male Sprague-Dawley rats were i.p. injected with 5 mg/kg LPS (1 μg/kg in saline) and livers were harvested after 20 hours. Liver tissue was fixed in 4% formalin and paraffin-embedded. 4 μm-thick sections were immunohistochemically stained with YB-1-specific antibodies. Liver RNA was reverse-transcribed and cDNA used as template in real-time PCR reactions with primers for Mrp2 and YB-1. The rat hepatoma cell line FAO was stimulated with LPS, IL-1β and TNFα. Nuclear and cytoplasmic protein extracts were prepared and YB-1 protein expression was quantified from Western Blotting analysis. A YFP-labelled YB-1 expression vector was transfected into cells, stimulated with IL-1β and visualized by confocal microscopy. Results: Mrp2 mRNA was downregulated to 18±20% in LPS-treated rats compared to untreated controls while YB-1 mRNA expression levels increased to 247±27%. Immunohistochemical staining showed strong up-regulation of YB-1 protein in endotoxemic livers. LPS-stimulation of FAO cells caused a moderate dose-dependent increase in nuclear as well as cytoplasmic protein after 8 to 16 hours. Furthermore, nuclear YB-1 protein transiently increased in a moderate fashion one hour after LPS-application. IL1β-stimulation of FAO cells resulted in a 5-fold upregulation of YB-1 in the nuclear compartment with a dose-dependent peak between 1.5 and 2 hours. Similarly, IL1β stimulation in YB-1-YFP-transfected cells showed an increase in nuclear fluorescence one hour post-treatment. TNFα did not affect YB-1 protein levels. Conclusions: YB-1 is activated during the hepatic acute phase response by two different mechanisms: a rapid nuclear shift within 2 hours mediated by interleukin-1β and a transcriptional induction during endotoxemia. This induction of YB-1 now explains the IL-1β mediated suppression of Mrp2 expression in endotoxemic rats. Moreover, YB-1 may serve as a central mediator of further regulatory events in inflammatory liver disease such as induction of other YB-1 target genes including MMP-2.

Disclosures:
The following people have nothing to disclose: Ina V. Martin, Peter R. Mertens, Sebastian Voigt, Bjoern Frye, Christian Trautwein, Andreas Geier
BACKGROUND & AIDS: Half of patients with HCV-2 and HCV-3 infection attained sustained virologic response (SVR) following Peg-interferon alfa-2a (Peg-IFN) monotherapy. However, guidelines recommend Peg-IFN with ribavirin for 24 weeks in all patients. Efforts to select patients who might benefit from Peg-IFN monotherapy have not been pursued. Methods: In a multicenter trial, 144 HCV-2 and HCV-3 patients were started on Peg-IFN alfa-2a (180 µg/week) and ribavirin (1000-1200 mg/day) for 12 weeks: those with RVR at week 4 were randomized to either discontinue ribavirin or remain on Peg-IFN alfa-2a, (n=59) or to continue combination therapy (n=61). To delineate patients' features that might help identify individuals likely to benefit from ribavirin discontinuation, an SVR prediction model was developed including gender, age, HCV genotype, baseline HCV-RNA levels, BMI, ALT values, and advanced fibrosis. Stepwise logistic regression analysis was used to compare P values and odds ratios for the effect of prognostic factors on either SVR and RVR rates. Results. In the 24 patients with no RVR, 15 (63%) were end-of-treatment (EOT) responders, and 12 (50%) were SVR. Baseline features of RVR patients randomized to ribavirin withdrawn or to standard treatment were not different. All but one RVR patients had EOT response. As expected, SVR rates were lower after discontinuation of ribavirin: 54% versus 82% (p<0.001). Twenty-seven (46%) and 10 (17%) EOT patients, respectively, relapsed during the follow up [difference (29%, CI 27.5-30.6; p<0.001). In the discontinuation group, low body weight (p=0.022), low BMI (p=0.034), low viremia (p<0.01) genotype 3 (p=0.031) and mild liver disease (p=0.01) were associated with SVR; in the multivariate analysis, low viremia and mild liver disease remained significant predictors with respective odds ratios of 56.8 (CI 1.4-3745) and 27.3 (CI 1.4-521). In patients who did or did not discontinue ribavirin, SVR rates were similar in those with <300,000 IU/ml viremia (86% vs. 81%) and in patients with intermediate viremia (70% vs. 71%), but disappointingly low in those with >700,000 IU/ml viremia (37% vs. 88%, p=0.004). Conclusions: in HCV-2 and HCV-3 patients, withdrawn of ribavirin and continuation with Peg-IFN alfa-2a monotherapy may be appropriate to attain SVR, providing viremia is cleared early during therapy and associated with low baseline viral load. Our investigation warrants future prospective testing, since it can give rise to considerable saving in cost and quality of life related to overtreatment.

Disclosures: The following people have nothing to disclose: Angelo Andriulli, Carmela Cursaro, Raffaele Cozzolongo, Angelo Iacobellis, Maria R. Valvano, Alessandra Mangia, Nicola Minerva, Donato Baccà, Maria Stanzione, Alessandra Scuteri, Giuseppe Montalto, Pietro Andreone

Background & Aims: Half of patients with HCV-2 and HCV-3 infection attained sustained virologic response (SVR) following Peg-interferon alfa-2a (Peg-IFN) monotherapy. However, guidelines recommend Peg-IFN with ribavirin for 24 weeks in all patients. Efforts to select patients who might benefit from Peg-IFN monotherapy have not been pursued. Methods: In a multicenter trial, 144 HCV-2 and HCV-3 patients were started on Peg-IFN alfa-2a (180 µg/week) and ribavirin (1000-1200 mg/day) for 12 weeks: those with RVR at week 4 were randomized to either discontinue ribavirin or remain on Peg-IFN alfa-2a, (n=59) or to continue combination therapy (n=61). To delineate patients’ features that might help identify individuals likely to benefit from ribavirin discontinuation, an SVR prediction model was developed including gender, age, HCV genotype, baseline HCV-RNA levels, BMI, ALT values, and advanced fibrosis. Stepwise logistic regression analysis was used to compare P values and odds ratios for the effect of prognostic factors on either SVR and RVR rates. Results. In the 24 patients with no RVR, 15 (63%) were end-of-treatment (EOT) responders, and 12 (50%) were SVR. Baseline features of RVR patients randomized to ribavirin withdrawn or to standard treatment were not different. All but one RVR patients had EOT response. As expected, SVR rates were lower after discontinuation of ribavirin: 54% versus 82% (p<0.001). Twenty-seven (46%) and 10 (17%) EOT patients, respectively, relapsed during the follow up [difference (29%, CI 27.5-30.6; p<0.001). In the discontinuation group, low body weight (p=0.022), low BMI (p=0.034), low viremia (p<0.01) genotype 3 (p=0.031) and mild liver disease (p=0.01) were associated with SVR; in the multivariate analysis, low viremia and mild liver disease remained significant predictors with respective odds ratios of 56.8 (CI 1.4-3745) and 27.3 (CI 1.4-521). In patients who did or did not discontinue ribavirin, SVR rates were similar in those with <300,000 IU/ml viremia (86% vs. 81%) and in patients with intermediate viremia (70% vs. 71%), but disappointingly low in those with >700,000 IU/ml viremia (37% vs. 88%, p=0.004). Conclusions: in HCV-2 and HCV-3 patients, withdrawn of ribavirin and continuation with Peg-IFN alfa-2a monotherapy may be appropriate to attain SVR, providing viremia is cleared early during therapy and associated with low baseline viral load. Our investigation warrants future prospective testing, since it can give rise to considerable saving in cost and quality of life related to overtreatment.

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Background: The impact of amantadine on virologic response of interferon-based treatment of chronic hepatitis C is controversial. Dose-dependent increase in HCV RNA decline was observed for amantadine during first weeks of interferon-based treatment. Objectives: Assessment of virologic response rates in patients with chronic HCV 1-infection treated with 400mg amantadine or placebo in combination with Peginterferon alfa-2a (40kD) and ribavirin for 48 weeks. Patients and Methods: Seven hundred and four previously untreated chronically HCV genotype 1-infected patients (mean age 46 ± 12 yrs.) received amantadine-sulphate (400 mg/day) (n=352) or placebo (n=352) in combination with 180 µg peginterferon alfa-2a once weekly and ribavirin (1000-1200 mg/day) for 48 weeks. End of treatment and sustained virologic response after a 24-week follow-up period were assessed by qualitative RT-PCR (Cobas Amplicor HCV, sensitivity 50 IU/mL). Results: Demographic and baseline virologic parameters were similar in both treatment groups. For 61 patients (9 %) liver cirrhosis or transition to liver cirrhosis were reported. No significant differences were observed between patients receiving amantadine or placebo regarding end of treatment and sustained virologic response, respectively. Intent-to-treat virologic response rates are given in the Table. On-treatment drop-out rate in the amantadine-group was significantly higher than in the placebo-group (32% vs. 23%; p=0.01). However, adverse events and laboratory abnormalities were similar between both groups and per-protocol analysis revealed similar virologic response rates in both treatment groups (52.8% vs. 54.5%). Conclusion: In this large placebo-controlled multicenter study, amantadine even at a dose of 400mg/day did not improve virologic response of antiviral treatment with peginterferon alfa-2a and ribavirin.
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RITUXIMAB COMBINED WITH PEG-INTERFERON-RIBAVIRIN IN REFRACTORY HCV-ASSOCIATED CRYOGLOBULINEMIA VASCULITIS
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Background: Treatment of hepatitis C-related mixed cryoglobulinemia (HVC-MC) remains difficult and one-third of patients continue to have active disease while receiving anti-CD20 monoclonal antibody or antiviral therapy. Objective: To report the results of a prospective open study using rituximab combined with Peg-Interferon (IFN)α2b-ribavirin in HVC-MC vasculitis. Patients: Sixteen consecutive HCV-MC patients were treated with rituximab (intravenously weekly for 4 weeks) combined with Peg-IFNα2b-ribavirin (for 12 months). All patients had severe active disease which was resistant to previous combination antiviral therapy. Results: Fifteen patients (93.7%) showed rapid clinical improvement, 10 of whom (62.5%) were complete responders. Compared with clinical complete responders, the partial or non responders had a 3.6 times longer duration of vasculitis prior to therapy and a lower rate of early virologic response. Complete response was associated with a significant reduction of cryoglobulin, rheumatoid factor activity and HCV RNA and increased C4. Treatment was well tolerated with no infectious complications. Flare-up of psoriasis and worsening of peripheral neuropathy occurred in one patient each. Clinical relapse occurred in two patients, which was associated with the simultaneous reappearance of HCV RNA and cryoglobulin and an increase in the number of peripheral blood B-cells. Conclusion: Rituximab combined with Peg-IFNα2b-ribavirin may act synergistically and represents a safe and effective therapeutic option in severe HVC-MC. This therapeutic schedule should be considered as induction therapy for HVC-MC patients.

Disclosures:
The following people have nothing to disclose: David Saadoun, Mathieu Resche rigon, Damien Sene, Laurent Perard, Jean charles Piette, Patrice Cacoub

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TWICE VS ONCE WEEKLY DOSING OF PEGINTERFERON ALFA 2A IN CHRONIC HCV GENOTYPE 1 INFECTION: ANALYSIS OF EARLY VIRAL KINETICS
Jordan J. Feld1, Glen A. Lutchman2, Rohit Loomba1, Apurva A. Modi1, Yaron Rotman, Pothu Raju Nagabhyru, Marc Ghany, Theo Heller1, Vanessa Haynes-Williams1, T. Jake Liang3, Avidan U. Neumann, Jay H. Hoofnagle
Background: Treatment outcomes in chronic hepatitis C are highly correlated with early viral kinetics. Pegylation of interferon greatly improved treatment responses and allowed for once weekly dosing. However viral kinetic data have shown that many patients receiving peginterferon weekly have a rebound in HCV RNA between doses. This may result in suboptimal viral inhibition and prolong time to clearance of viremia. Aim: To compare early viral kinetics between once and twice weekly dosing of peginterferon in patients with chronic HCV genotype 1. Methods: Consecutive patients with HCV genotype 1 were divided into 2 groups: Group A received peginterferon alfa 2a 180µg once weekly and weight-based ribavirin for 4 weeks and Group B received an initial 180µg dose of peginterferon followed by 90µg twice weekly plus ribavirin for 4 weeks. Both groups were then treated with peginterferon 180µg once weekly and ribavirin for an additional 44 weeks. HCV RNA was measured at 0, 12, 24, 48 and 72 hrs and at days 7, 9, 14, 21 and 28. Early viral kinetics and baseline characteristics were compared. Results: Patients in Group A (n=25) had similar baseline characteristics to those in Group B (n=22): sex (54% vs 62% male), race (71% vs 77% Caucasian) and HCV RNA level (6.2 vs 6.3 log IU/ml). The groups had similar first phase (day 0-3) kinetics (Group A 0.76 log vs Group B -0.10 log), but starting at day 3, HCV RNA levels diverged (Figure). Patients in Group B had a more rapid decline in HCV RNA from day 3 to 28, with a slope of -0.62 compared to -0.39 log/week in Group A (p=0.03). The overall reduction in viral load from day 0 to 28 was greater in Group B (3.1 vs 1.8 log, p=0.029) and this resulted in a lower mean HCV RNA at day 28 for the twice-weekly group (A 4.4 vs B 3.2 log IU/ml, p=0.03). 4 patients in Group B were HCV RNA negative by day 28 compared to 0 in Group A (p=0.049). Conclusions: Twice-weekly dosing of peginterferon alfa 2a improves early viral kinetics in HCV genotype 1 infection.

Disclosures:
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SUSTAINED VIROLOGICAL RESPONSE IS ASSOCIATED WITH ERADICATION OF HEPATITIS C VIRUS AND DECREASE IN ANTI-HCV TITER IN PATIENTS TREATED FOR CHRONIC HEPATITIS C WITH INTERFERON ALPHA 2B OR PEGYLATED INTERFERON ALPHA-2B+RIBAVIRIN
Sarah Maylin1,2, Michelle Martinot-Peignoux1, Nathalie Boyer2, Marie Pierre Ripault2, Ana C. Cardoso2, Nathalie Giuly2, Corinne Castelnau2, Marie-Helene Nicolas-Chanoine3, Patrick Marcellin1,1,1,1, INSERM U773-CRB3 Université paris VII, Hopital Beaujon, Clichy, France; 2Service d’Hépatologie, Hopital Beaujon, Clichy, France; 3Service de Microbiologie, Hopital Beaujon, Clichy, France
It is unclear whether hepatitis C virus (HCV) is eradicated in patients with chronic hepatitis C who achieve a sustained virological response [SVR]. In this study, HCV-RNA was measured in serum, and peripheral blood mononuclear cells (PBMCs) and a follow-up of anti-HCV antibodies were performed in patients with chronic hepatitis C who achieved an SVR. PATIENTS–METHODS: 215 patients with an SVR after a treatment with IFN alpha-2b or PEG-IFN alpha-2b+ribavirin (PEG-IFN 1.5µg/kg/week, plus ribavirin 800-1200 mg /day according to weight), were included in this study. HCV-RNA was tested: (1) in serum for all the 215 patients every year and at the time of PBMCs collection, (2) in PBMCs collected in 71
Preemptive treatment (PT) of HCV after liver transplantation (LT) is controversial due to the high morbidity, high cost and unclear efficacy. Methods and patients: 75 consecutive patients with chronic hepatitis C and SVR, evaluated up to 10 years after treatment cessation, none demonstrated late relapse. HCV-RNA was detectable, by a very sensitive assay (TMA), in PBMCs in 1 patient. HCV antibody titters showed a marked decrease. These results demonstrate a durable response to IFN alpha 2b or PEG-IFN alpha-2b+ribavirin and indicate that SVR is associated with HCV eradication.

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PREEMPTIVE TREATMENT OF HCV AFTER LIVER TRANSPLANTATION IS UNJUSTIFIED EXCEPT FOR GENOTYPE 3A

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Background: Preemptive treatment (PT) of HCV after Liver Transplantation (LT) is controversial due to the high morbidity, high cost and unclear efficacy. Methods and patients: 75 consecutive HCV LT recipients were followed prospectively in a preemptive intent to treat protocol with pegylated interferon (PegIFN) and ribavirin (Rib) for 48 weeks. Viral load and protocol biopsies were followed in all treated patients. Preemptive treatment was defined as starting less than 180 days after LT and before the onset of histologically proven recurrent HCV (Grade 1 Stage 1). Viral genotype, pretreatment viral load, time to initiation of treatment, immunosuppression, rejection episodes and completion of treatment were analyzed for their effect on response. Results: Demographics: Pts. - n = 75. Age - 52±7. Sex - 50 m, 25 f. HCC - 23. Follow up - 27 ± 13 m. Actuarial Survival (patient and graft) - 1 year = 91 & 88%, 3 year = 84 & 81% Of 75 LT patients (pts.) with HCV 29 (39%) qualified for PT. 14 pts. were treated after HCV recurrence was histologically documented. 32 pts. (43%) received no treatment against HCV. Reasons for exclusion were: preoperative viral clearance - 9; early death - 3; psychosocial - 6; re-LT - 3; medical - 15; noncompliance - 5; early HCV recurrence - 3. Of the 29 who started PT only 13 (17 & 45%) completed treatment. Of the 29 on PT 17 had complete response, 11 end of treatment response and 6 sustained viral response (SVR). Of the patients with SVR non were genotype 1a (3-3a, 1-2b, 1-1b, 1- indeterminate). Of 5 pts. with genotype 3a in the PT group all cleared the virus with one recurrence. Of 15 pts. with advanced recurrence (Grade >1 stage > 1) 12 had genotype 1a. No pts. With genotype 1a achieved SVR. 2 pts. And 3 (4%) grafts were lost to recurrent HCV. Of 15 pts. with moderate to severe recurrent HCV (>Grade 1 Stage 1) 12 were 1a, 2 2a and 1 4e. After censuring for early death and early recurrent hepatitis there was no survival advantage in the non treated vs. the PT group. There were no deaths, graft loss or rejection episodes associated with treatment for HCV. 75% of PT patients required growth factor treatment. Intent to treat SVR was 6% for the cohort, 20% for the PT group and 45% for the pts. that completed treatment. Conclusions: 1. PT for HCV after LT is expensive, time consuming and associated with considerable (though not lethal) morbidity. With an SVR of 6% it appears to be unjustified to expose all HCV pts. to treatment. 2. Genotype 3a appears to respond favorably and consistently to treatment and may be an indication for PT.

Disclosures:
The following people have nothing to disclose: Hadar J. Merhav, Luis Mielles, Misty Ottman, Stephen Chris Pappas, Rafael C. Botero

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ASSOCIATION BETWEEN GENE POLYMORPHISMS AND PSYCHIATRIC SYMPTOMS DURING PEGINTERFERON TREATMENT FOR CHRONIC HEPATITIS C

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Background: Psychiatric side effects (PSE), a common occurrence in patients undergoing peginterferon (IFN) therapy, can have adverse effects on patient and treatment outcomes. Risk factors for PSE are not well known. The inflammatory response system (IRS) and proinflammatory cytokines, such as IFN, can induce depression. Therefore, it is plausible that gene variants involved in the IRS and interferon-signaling pathways may be implicated in developing PSE. The current study sought to identify single nucleotide polymorphisms (SNPs) that are associated with the development of IFN-induced PSE. Methods: Participants in the Virahep-C study, a NIH-funded study of antiviral therapy for chronic hepatitis C, were treated with PegIFNα-2a and ribavirin for up to 48 weeks. Four measures of PSE, collected prospectively, were used to create a dichotomous variable. Data were available from 374 of 401 participants. Candidate genes for Virahep-C were selected a priori based on potential involvement in treatment response. SNPs representing the entire region of candidate genes were genotyped. 896 potential involvement in treatment response. SNPs representing the entire region of candidate genes were genotyped. 896 SNPs were analyzed. Statistical analysis: Associations between PSE and SNPs were tested using relative risk (RR) models using a modified Poisson regression approach (Zou 2004). RR mod-
RESULTS: Thirty-four patients were sustained virological response by real-time PCR. G3PDH and HMB were used as internal controls. USP18 (a specific remover of ISG15 from ISGylated protein), mRNA expressions of RIG-I, Cardif, MDA5, LGP2, ISG15, patients with non-viral liver disease. Pretreatment hepatic 1b treated with PEG-IFN alfa-2b plus RBV for 48 weeks and 5 positives in genetic association analyses. Results and Conclusions: Exploratory analyses revealed 7 SNPs significantly associated with IFN-induced PSE in CA. No statistically significant associations were found for AA. Each of the 7 SNPs had a false positive rate (q-value) below 0.50, suggesting that it is unlikely that all of the independent associations were false positives. Replication and a more systematic assessment of the implicated genes are needed to further define their role in the development of IFN-induced PSE.

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Disclosures: The following people have nothing to disclose: Donna Evon, Kirk C. Wilhelmsen, Darmendra Ramcharan, Steven H. Belle, Michael W. Fried

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241 GENE EXPRESSION OF CYTOPLASMIC VIRAL SENSORS AND REGULATORS INVOLVING INNATE IMMUNITY AND RESISTANCE TO PEG-INFERNBERON ALFA-2B PLUS RBAVIRIN TREATMENT IN CHRONIC HEPATITIS C

Yasuhiro Asahina, Namiki Izumi, Itsuko Hirayama, Mitsuaki Sato, Tomohiro Tanaka, Nobutoshi Komatsu, Naoki Umeda, Takanori Hosakawa, Ken Ueda, Hiroyuki Nakamishi, Kaoru Tsuchiya, Jun Itakura, Masayuki Kurosaki, Masakatsu Uchihara, Shozo Miyake; Gastroenterology and Hepatology, Musashino Red Cross Hospital, Tokyo, Japan

BACKGROUND: Mechanisms involving resistance to interferon (IFN) and ribavirin (RBV) have not been fully elucidated, and prediction of treatment responses before initiation of therapy is difficult. RIG-I and the related MDA5 initiate the host antiviral response by detecting intracellular viral dsRNA. Cardif (ISP-I, MAVS, VISA) is an adaptor molecule that connects RIG-I sensing to downstream signaling. On the other hand, LGP2, a helicase belonging to the RIG-I family, and E3 ubiquitin ligase have been shown to negatively regulate RIG-I sensing. Moreover, these molecules are ISGylated by ISG15, a ubiquitin-like protein. However, the clinical significance of these innate immune systems in terms of treatment response is unclear. AIM: To elucidate the mechanisms underlying resistance to PEG-IFN plus RBV, and to determine whether analysis of the innate immune system is useful in predicting treatment responses. METHODS: We studied 74 chronic hepatitis C virus (HCV) patients with genotype 1b treated with PEG-IFN alfa-2b plus RBV for 48 weeks and 5 patients with non-viral liver disease. Pretreatment hepatic mRNA expressions of RIG-I, Cardif, MDA5, LGP2, USP15, and OAS1 (a specific remover of ISG15 from ISGylated protein), and serum HCV dynamics during therapy were measured by real-time PCR. G3PDH and HMB were used as internal controls. RESULTS: Thirty-four patients were sustained virological responders (SVR), 24 were transient responders (TR), and 20 were non-virological responders (NVR). The hepatic levels of all transcripts except Cardif were 2- to 8-fold higher in HCV patients than non-HCV patients. RIG-I, MDA5, and LGP2 expression was significantly up-regulated in NVR compared with TR or SVR. Cardif expression negatively correlated with RIG-I expression and was significantly suppressed in NVR. The difference between NVR and SVR or TR in the RIG-I/Cardif expression ratio was pronounced (NVR/TR/SVR = 1.2/0.6/0.5 copies/control). Like RIG-I and MDA5, ISG15 and USP18 expression was significantly up-regulated in NVR (ISG15, NVR/TR/SVR = 0.8/0.4/0.2 copies/control; USP18, NVR/TR/SVR = 0.9/0.6/0.4 copies/control). The RIG-I/Cardif ratio and the expression of MDA5, ISG15, and USP18 significantly correlated with viral decline rates in the first and second phases of HCV dynamics. Multivariate and ROC analyses revealed that a higher ratio of RIG-I/Cardif and a higher expression of MDA5, ISG15, and USP18 predicted NVR. CONCLUSION: Intracellular sensors and their regulators were variously up-regulated upon HCV infection especially in NVR. Quantifying hepatic gene expression involving an innate immune system is of use in identifying patients who are at a higher risk for NVR.

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242 LIVER GENE EXPRESSION SIGNATURE TO PREDICT RESPONSE TO PEGYLATED INTERFERON PLUS RIBAVIRIN COMBINATION THERAPY IN PATIENTS WITH CHRONIC HEPATITIS C

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Background and Aims The gold standard treatment of chronic hepatitis C is the combination of pegylated interferon plus ribavirin. Considering side effects and treatment cost, prediction of treatment response is important. The aim of our study was to identify a liver gene signature to predict sustained virological response in patients with chronic hepatitis C. Patients and Methods Pretreatment liver biopsies from sixty nine naïve patients with chronic hepatitis C who were subsequently treated (same complete treatment of PEG-IFN alpha-2b with ribavirin) and for whom treatment responses were known were used for the study. Group A (training set): 40 patients with chronic hepatitis C including 14 non responders and 26 sustained responders. Group B (validation set) independent validation set of 29 patients including 9 non responders and 20 sustained responders. We selected 58 genes from the literature, associated with liver gene expression dysregulation during chronic hepatitis C. We used real-time quantitative RTPCR assays to analyse the mRNA expression of these 58 selected genes in these 69 liver specimens obtained before treatment. Results Many of the genes founded differentially expressed between SVR and NVR in both the training and the validation set (IFI6, IFI27, ISG15, OAS2, IFIT1) belong to IFN-inducible genes. Interestingly, all these genes were up-regulated in SVR and NVR samples in comparison with normal liver. From the Group A data, we identified three genes whose expression differed significantly between non responders and sustained responders: IFI6, G1P3, IFI27 and ISG15/G1P2. These three genes also showed significant
differences in their expression profiles between non responders and responders in the independent sample (Group B). The best classification results from group A were obtained using a twogene (IFI27 and CXCL9) signature, which accurately predicted treatment response for 23 of 29 patients (79.3%) of the validation set (Group B). Conclusion Our study has demonstrated that NR and SVR have prior to treatment, different gene expression patterns. The most notable changes in gene expression were mainly observed in the interferon stimulating genes. We used two independent groups of patients (training set and validation set) and we could predict treatment response with a two-gene signature (IFI27 and CXCL9). These genes code molecules secreted in the serum and provide a logical functional approach for the development of serum markers to predict response to treatment. Gene signatures will probably be used in the future for optimised and tailored treatment.

Disclosures:
The following people have nothing to disclose: Ana C. Cardoso, Rami Moucari, Nathalie Boyer, Tarik Asselah, Asma Laatari, Marie-Pierre Ripault, Nathalie Boyer, Michelle Martinot-Peignoux, Dominique Valla, Michel Vidaud, Patrick Marcellin

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POSITIVE IMPACT OF ANTIVIRAL THERAPY ON THE LONG TERM OUTCOME OF CHRONIC HEPATITIS C PATIENTS WITH CIRRHOSIS
Ana C. Cardoso1,2, Rami Moucari1,2, Nathalie Boyer1, Tarik Asselah1, Asma Laatari1, Marie-Pierre Ripault1, Michele Martinot-Peignoux2, Sarah Maylin1,4, Pierre Bedossa1, Patrick Marcellin1; 1Hepatologie, Hopital Beaujon, Clichy La Garenne, France; 2Microbiologie, Hopital Beaujon, Clichy La Garenne, France; 3Inserm CRB3, Hopital Beaujon, Clichy La Garenne, France; 4Anatomie Pathologique, Hopital Beaujon, Clichy La Garenne, France; 5Medecine, Hopital Beaujon, Clichy La Garenne, France; 6Pharmacologie, Hopital Beaujon, Clichy La Garenne, France; 7Pharmacologie, Hopital Beaujon, Clichy La Garenne, France; 8Microbiologie, Hopital Beaujon, Clichy La Garenne, France

Background and Aim The major consequence of chronic hepatitis C (CHC) is the progression to cirrhosis and its potential complications: haemorrhage, ascites and hepatocellular carcinoma (HCC). The influence of antiviral therapy on the long-term outcome of CHC has not been determined. The aim of this study was to evaluate the influence of antiviral therapy on the long-term outcome of CHC patients with bridging fibrosis or cirrhosis. Patients and Methods 244 consecutive patients with CHC and bridging fibrosis or cirrhosis (METAIVIR F3-F4) were retrospectively evaluated. They had all received at least one treatment course with interferon (conventional or pegylated) with or without ribavirin for at least 12 weeks. Sustained virological response (SVR) was defined as undetectable HCV RNA in serum 24 weeks after treatment discontinuation. Cumulative incidence of haemorrhage, ascites, and HCC were compared between patients who developed or not SVR. The influence of treatment response on histology was also assessed on paired-liver biopsies in 64 patients. Results The baseline characteristics of the study population were: male gender (68%), mean age (55±10 years), mean BMI (26±5 kg/m²). Mean serum HCV RNA level was 1.08±1.34 10^6 IU/ml. Genotype distribution was: 1 (60%), 2 (19%), 3 (16%), 4 (14%). Median follow-up period was 5 years (1-18) after the first biopsy, and 2 years after the last treatment (1-15). SVR developed in 34% of patients. SVR was more frequent in patients with bridging fibrosis (F3) than in those with cirrhosis (F4) (44% vs. 21%, p<0.001), and in patients with genotypes 2 or 3 than in those with genotypes 1 or 4 (56% vs. 23%, p<0.001). SVR was not associated with age, BMI, serum HCV RNA level, liver necroinflammation or steatosis. Patients without SVR developed more frequently complications of cirrhosis than those who achieved SVR: haemorrhage (7% vs. 1%, p=0.05), ascites (21% vs. 5%, p=0.005). HCC developed in 22 patients; among them HCC developed in four patients 1 to 4 years after therapy despite SVR. Cumulative incidence of HCC was higher in patients who failed treatment (p=0.06). 64 patients had paired-liver biopsies. The second biopsy was performed after a median period of 2 years (1-15) following treatment discontinuation. Histological analysis showed a decrease of at least one point of the fibrosis score (METAIVIR) in 41% of patients as compared with 11% of those without SVR (p=0.005). Conclusion In CHC patients with bridging fibrosis (F3) or cirrhosis (F4), antiviral therapy is associated with significant regression of fibrosis and long-term improved outcome with lower rates of complications of cirrhosis and HCC.

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LONG-TERM PROGNOSIS OF ELDERLY PATIENTS WITH HEPATITIS C VIRUS-RELATED CHRONIC LIVER DISEASE — A COHORT STUDY OF 2,379 JAPANESE PATIENTS
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Background: Interferon therapy is effective in reducing hepatocarcinogenesis and in improving survival rate of patients with HCV-related chronic hepatitis, but the clinical influence of interferon is considered less advantageous in elderly patients because of short longevity. In order to elucidate the prognosis of elderly patients of 60 years or older, and to evaluate the advantage of interferon treatment in the elderly, we analyzed a large cohort of patients with chronic hepatitis type C from viewpoints of hepatocellular carcinogenesis and survival period. Patients and Method: Among 7,235 patients with hepatitis C virus related chronic liver disease in our hospital, prognosis of 2,379 elderly patients was analyzed. A total of 459 elderly patients began interferon therapy before development of liver cancer. The elderly cohort was observed for a median of 6.3 years (25% 2.6 years, 75% 10.8 years). Results: (1) Cumulative survival rates in untreated elderly patients without overt cirrhosis were 94% at the end of 10th year and 79% at the 15th year in high platelet (≥150,000/mm³) group, 87% and 73% in intermediate (100,000-149,000/mm³) group, and 71% and 36% in low platelet group (<100,000/mm³), respectively. (2) Fifth- and 10th-year hepatocarcinogenesis rates in the intermediate and low platelet group (<150,000/mm³) were 12% and 22% in interferon therapy group (N=85) and 19% and 43% in untreated group (N=474), respectively (P=0.028). Multivariate analysis disclosed that interferon independently decreased carcinogenesis risk with a hazard ratio of 0.53 (P=0.012) in the subgroups. In the high platelet group (≥150,000/mm³), on the contrary, 5th and 10th-year carcinogenesis rates were 3% and 9% in interferon-treated group (N=96), and 5% and 13% in untreated group (N=598), respectively (P=0.88). (3) Interferon treatment significantly increased cumulative survival rates in the subgroup of lower platelet group (P=0.0028), but did not affect in the subgroups of higher platelet (P=0.20). Multivariate analysis also showed interferon was significantly associated with a longer survival in the lower platelet subgroup (hazard ratio 2.44, P=0.011). Conclusion: Initial platelet count was significantly associated the survival time of the elderly patients with chronic hepatitis C.

HEPATITIS C VIRUS-RELATED CHRONIC LIVER DISEASE
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Interferon for a subgroup of intermediate and low platelet count had significant advantages from the viewpoints of hepatocarcinogenesis and survival.

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EFFICACY AND SAFETY OF ESCITALOPRAM FOR THE PREVENTION OF DEPRESSIVE EPISODES INDUCED BY PEG-INTERFERON ALPHa2A AND RIBAVIRIN IN CHRONIC HEPATITIS C PATIENTS. A DOUBLE-BLIND, RANDOMIZED, PLACEBO-CONTROLLED TRIAL

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Interferon is associated with a high prevalence of major depression, which is one of the main reasons for treatment withdrawal and failure. The aim of this study was to determine the efficacy and safety of an antidepressant for preventing depression induced by pegylated interferon ( PegIFN) alpa2a in chronic hepatitis C (CHC). Method: Multicenter double-blind study of CHC patients who were eligible for PegIFN-alpha2a and ribavirin (RBV) therapy. Patients with mental disorders at baseline were excluded. They were randomly assigned to receive the antidepressant escitalopram (15 mg/day) or placebo. Treatment was begun 2 weeks before PegIFN therapy and continued for the following 12 weeks. Main variables studied were the presence of a major depressive episode, according to DSM-IV diagnostic criteria, and scores on the Montgomery-Asberg Depression Rating Scale (MADRS) and the Hospital Anxiety and Depression Scale (HADS). The study protocol conformed to the guidelines of the Declaration of Helsinki and was approved by the Ethics's Committees of all centers involved and the Spanish Agency of Medicines. All patients signed a written informed consent before entering the study. Results: A total of 133 patients were included (74% genotype 1), 67 treated with escitalopram and 66 with placebo. Eighty-three (62%) patients were male, mean age 45.5 years. Seventeen patients (13%) had a past history of major depression and 22 (17%) of substance abuse. Placebo and escitalopram groups did not differ significantly in any measure at baseline. During the first 12 weeks of treatment, only 1 patient (2%) in the placebo group and 78% in the escitalopram group. Figures for the depression subscale of the HADS were 0.7 and 1.0 respectively. Differences between both groups were not statistically significant. Biochemical response at week 12 (normalization of transaminases) was achieved in 67% of patients in the placebo group and 78% in the escitalopram group. Virological response at week 12 (negativization of RNA-HCV) was obtained in 80% and 66% respectively. Conclusions: In CHC patients, pretreatment with an antidepressant is not an effective strategy for reducing depression induced by PegIFN and RBV, at least in a population of patients with low psychiatric risk. On the other hand, the use of escitalopram in CHC patients is safe regarding biochemical and virological response to treatment at week 12. This study has been supported by a grant from Roche Farma.

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Maria Dolores Gimenez - Grant/Research Support: Roche
Helena Masnou - Grant/Research Support: Roche
Ricard Solà - Grant/Research Support: Roche
Pilar Giner - Grant/Research Support: Roche; Grant/Research Support: Other
Rocío Martín-Santos - Grant/Research Support: Roche
The following people have nothing to disclose: Pere Castellvi

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RESPONSE TO PEGINTERFERON ALFA-2B + RIBAVIRIN COMBINATION THERAPY IN GENOTYPE 2 AND 3 PATIENTS WITH POOR BASELINE PROGNOSTIC FACTORS: RESULTS OF THE CANADIAN POWER PROGRAM

Robert J. Bailey1, David K. Wong2, Curtis Cooper2, Nir Hilzenrat3, Kevork Pellekian2, Jeff Daiter2, Nabil Abadir4, Paul Marotta5,1Royal Alexandra Hospital, Edmonton, AB, Canada; 2Toronto Western Hospital, Toronto, ON, Canada; 3Ottawa Hospital General Campus, Ottawa, ON, Canada; 4Jewish General Hospital, Montreal, QC, Canada; 5Queens Health Sciences Centre, Halifax, NS, Canada; 6Ontario Addiction Treatment Centers, Richmond Hill, ON, Canada; 7Schering-Plough Canada Inc, Pointe Claire, QC, Canada; 8London Health Sciences Centre, London, ON, Canada

Background: This subanalysis of the POWer study evaluated the effect of fibrosis and baseline viral load on sustained virologic response (SVR) rates in treatment-naive genotype 2 (G2) and G3 patients with chronic hepatitis C treated with weight-based peginterferon (PEG-IFN) alfa-2b and weight-based ribavirin (RBV). Methods: POWer is an open-label observational trial conducted in community and academic clinics across Canada between 2002 and 2006. G2/G3 patients received PEG-IFN alfa-2b (1.5µg/kg/wk) plus RBV (800–1200mg/d) for 24 weeks. SVR was undetectable HCV RNA levels 24 weeks posttreatment. Results: 276 G2 and 389 G3 patients enrolled in POWer (38% of all patients). Baseline viral load and fibrosis scores were available in 72% and 37% of G2 and G3 patients, respectively. Numerically, more G3 than G2 patients had high viral load (HVL; 49% vs 44%) and advanced fibrosis/cirrhosis (F3-F4) (40% vs 33%). G3 patients had lower SVR rates than G2 patients (72% vs 79%, P=0.04) due to a lower end-of-treatment response (77% vs 86%, P=0.01); relapse rates were identical (7%). G2 patients responded well regardless of baseline viral load and fibrosis score (Table). G3 patients with low viral load (LVL) attained SVR more often than those with HVL (76% vs 65%, P=0.03). An inverse relationship between SVR and fibrosis score was observed in G3 patients; <50% of G3 cirrhotic patients attained SVR. Conclusions: G2/G3 patients respond very differently to combination PEG-IFN alfa-2b plus RBV therapy. G2 patients respond well regardless of baseline characteristics. Conversely, lower response rates are observed in G3 patients who have poor prognostic factors. The lower response rate in the G3 population after 24 weeks’ treatment may not be detected in smaller studies that combine G2/3 patients for analysis purposes. New treatment strategies such as increased dose or duration of treatment are needed for G3 cirrhotic patients. Future studies should avoid combining G2/3 patient populations when reporting results.
SVR Rates Stratified by Baseline Viral Load and Fibrosis Score

<table>
<thead>
<tr>
<th>G2 (n=276)</th>
<th>G3 (n=389)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HVL&lt;sup&gt;+&lt;/sup&gt;</td>
<td>83</td>
</tr>
<tr>
<td>LVL</td>
<td>79</td>
</tr>
<tr>
<td>F0-F1</td>
<td>80</td>
</tr>
<tr>
<td>F2</td>
<td>81</td>
</tr>
<tr>
<td>F3</td>
<td>80</td>
</tr>
<tr>
<td>F4</td>
<td>77</td>
</tr>
</tbody>
</table>

*113/276 G2 and 136/389 G3 with fibrosis score, 201/276 G2 and 281/389 G3 with baseline viral load data.

HVL=baseline HCV RNA levels >600,000 IU/mL or >2×10<sup>6</sup> copies/mL.

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Nir Hilzenrat - Grant/Research Support: Schering-Plough
Kework Pellekian - Grant/Research Support: Schering-Plough
Jeff Daiter - Grant/Research Support: Schering-Plough
Nabil Abadir - Employee: Schering-Plough
Paul Marotta - Speakers Bureau: Schering-Plough; Grant/Research Support: Roche; Grant/Research Support: Astellas; Grant/Research Support: Novartis; Grant/Research Support: Schering-Plough

247 CHANGES IN ANTRODUODENAL MOTILITY DURING INTERFERON TREATMENT FOR CHRONIC HEPATITIS C

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Objective: In the majority of the patients treated with interferon (IFN) for chronic hepatitis C, weight loss accompanying loss of appetite is observed which greatly affects their quality of life (QOL) during the treatment. However, the mechanism of appetite loss due to IFN treatment is still unknown. The subjects of this study were patients with chronic hepatitis C who were undergoing IFN treatment. We quantitatively evaluated the antroduodenal motility in these patients using ultrasound examination and the relationship between the IFN treatment and appetite loss. Method: The subjects were 13 patients who were receiving a combination treatment of PEGIFN-α2b and ribavirin. During IFN treatment, we evaluated gastric emptying, antral motility and duodenogastric reflux by ultrasonography after liquid test meals. Digestive symptom scores and the changes in body weights were recorded. Results: The gastric emptying rate was significantly decreased posttreatment compared with pretreatment [percentage change 12 weeks after initiation of IFN therapy: -29.60±21.26%]. The frequency and intensity of antral contractions were decreased posttreatment. Duodenogastric reflux was seen significantly more frequently posttreatment. The digestive symptom score was significantly increased posttreatment, and the body weight was decreased. There was a positive correlation between the percentage change of the gastric emptying rate and weight loss (r=0.749, p=0.021). We examined 9 chronic hepatitis C patients undergoing IFN treatment, matching their ages and IFN/ribavirin doses. To these patients, we administered the gastric prokinetic agent nizatidine 150 mg BID (nizatidine group) in addition to the IFN treatment, and we compared this group with the group without nizatidine (non-nizatidine group). The gastric emptying rate 12 weeks after the IFN treatment was significantly higher for the nizatidine group compared with the non-nizatidine group (percentage change: nizatidine group:+10.49±10.63% and non-nizatidine group: -29.60±21.26%, p<0.005). The posttreatment rate of weight loss was less in the nizatidine group compared with the non-nizatidine group. The posttreatment increase in the digestive symptom score was significantly less in the nizatidine group compared with the non-nizatidine group. Conclusion: The loss of appetite and weight loss due to IFN treatment is not simply from subjective symptoms. Rather, it was confirmed that such symptoms are caused by the decline in antroduodenal motility. This study suggested that combining a gastric prokinetic agent with IFN treatment can result in weight loss suppression and improvement in QOL during treatment.

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248 VACCINATION WITH HCV MRNA TRANSECTED DENDRITIC CELLS IN HCV TRIMERA MICE

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Introduction: Spontaneous resolution from hepatitis C is associated with broad HCV specific CTL and Th cell responses. In contrast, viral persistence occurs in the presence of dysfunctional immune responses. Thus, stimulation of sufficient HCV-specific T cell reactivities might represent a new therapeutic strategy. We have shown previously effective T cell stimulation in the humanized HCV trimera mouse model by peptide or virus like particle vaccination (AASLD 2005). We now aimed to enhance CTL and Th cell stimulation by vaccination with autologous dendritic cells (DC). Methods: DC were generated from separated blood monocytes by incubation in IL-4 and GM-CSF. After 2 days, DC were transfected with mRNA encoding for the HCV derived proteins NS3, NS5b or EGFP. Lethally irradiated Balb/c mice, reconstituted with ncd.scid mouse bone marrow, were transplanted with human PBMC from HLA A2 positive recovered or chronic HCV carriers. Vaccination of such trimera was performed i.p. with HCV- or mock-transfected DC or synthetic nonapeptides representing well known HLA A2 restricted NS3 or NS5b derived CTL epitopes; vaccination with tetanus toxoid and EBV peptide 280-288 served as controls. HCV-, TT- and EBV-specific human T cell frequencies were analysed 10 days after vaccination from peritoneal lavage by tetramer analysis or IFNg ELISPOT with autologous APC pulsed with recombinant antigens, overlapping 15-mer peptides or nonapeptides. Results: Strong NS3 specific CTL responses were stimulated by vaccination with NS3- but not with NS5b- or mock-transfected DC in trimera mice implanted with PBMC from two different recovered chronic HCV carriers (after successfull treatment). In contrast, NS5b transfected DC were rather inefficient in T cell stimulation. Results in chronic HCV carriers and testing different RNA constructs will be presented at the meeting. Conclusions: Our studies demonstrate that HCV specific CTL, that are barely detected in donor PBMC, can effectively be expanded in vivo by vaccination with mRNA transfected DC. This approach might lead to new therapeutic vaccination strategies.

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PRETREATMENT INSULIN SENSITIVITY CONTRIBUTES TO THE TREATMENT RESPONSE TO PEGINTERFERON PLUS RIBAVIRIN COMBINATION THERAPY FOR PATIENTS WITH CHRONIC HEPATITIS C

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Insulin resistance plays a key role in the entire suite of glucose abnormalities. The impact of insulin resistance on the treatment response of pegylated interferon-alpha plus ribavirin has not been fully clarified. This study aimed to evaluate the role of insulin sensitivity and the related viral factors in the treatment response in patients with HCV infection in Taiwan. Methods: A total of 430 patients (243 males) were included. All patients were treated with 24-week course of peginterferon alpha-2a (180 µg/week) and weight-based ribavirin 1000-1200 mg/day, with a 24-week follow-up period. We assessed insulin sensitivity and beta-cell function of these patients in a fasting state (homeostasis model assessment of insulin resistance [HOMA-IR] and homeostasis model assessment of beta-cell function [HOMA-beta]) before treatment and 24 weeks after treatment. Results: Sustained virological response (SVR) were observed in 336/430 (78.1%) patients. The rates of SVR in genotype-1 and non-1 patients was 64.5% (118/183) and 88.3% (218/247), respectively. The baseline HOMA-IR of those patients achieving an SVR was significantly lower than that of those without [2.28 vs. 3.40, P=0.002]. Multivariate logistic regression analysis demonstrated that high baseline HOMA-IR (>2.5) was a significant negative factor regarding SVR in all patients (OR=2.91, 95% CI= 1.38-6.58, P=0.006). For 172 non-diabetic patients with genotype-1 HCV infection, the SVR rate (49.0%, 25/51) of those with high baseline HOMA-IR was significantly lower than that of those without (<2.5, 87/121, P=0.004). Meanwhile, multivariate logistic regression analysis demonstrated that high baseline HOMA-IR (>2.5) was a significant negative factor regarding SVR in genotype-1 patients (OR=2.91, 95% CI= 1.38-6.58, P=0.006), followed by viral load and age. Conclusions: Pretreatment insulin sensitivity contributes to treatment response in chronic hepatitis C patients, especially for those with genotype-1 infection.

Correlation between pretreatment HOMA-IR and SVR in non-diabetic chronic hepatitis C patients receiving peginterferon plus ribavirin

<table>
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<th>Total, n=389</th>
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<th>G-non-1, n=217</th>
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<tr>
<td>SVR+</td>
<td>SVR-</td>
<td>P</td>
</tr>
<tr>
<td>HOMA-IR &gt;2.5</td>
<td></td>
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</tr>
<tr>
<td>83 (69.1)</td>
<td>38 (30.9)</td>
<td>0.002</td>
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<tr>
<td>&lt;2.5</td>
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<tr>
<td>220 (82.7)</td>
<td>46 (17.3)</td>
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</table>

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RESPONSE TO HIGH RIBAVIRIN DOSE IN COMBINATION WITH PEG-INF ALFA-2A FOR TREATMENT OF HCV GENOTYPE 1 PREVIOUS NON-RESPONDERS

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BACKGROUND: Patients with hepatitis C (HCV) genotype 1 and previous non-responders to treatment with pegylated interferon (peg-INF) and ribavirin (RBV) are a difficult-to-cure group. We have previously shown that high doses of RBV (mean 2550 mg/d) offered high sustained virological response (SVR) in treatment-naive patients with hepatitis C genotype 1. The aim of this controlled study was to evaluate the efficacy, safety and tolerability of high doses of RBV in combination with standard-dosed peg-INF in previous non-responders. METHODS: 10 patients with HCV genotype 1 and previous non-responders to peg-INF alfa-2a and RBV therapy received treatment with individualized high dose of RBV in combination with peg-INF alfa-2a 180mg/week for 48 weeks. Non-responders were defined as not achieving HCV-RNA <50 IU/mL at any time during previous treatment. The initial RBV dose was individualized and calculated from a pharmacokinetic formula based mainly on renal function aiming at a high steady state concentration of RBV of >15 mmol/L. Plasma RBV concentrations were measured during treatment by HPLC and if necessary the RBV dose was adjusted to reach the target concentration. All patients received erythropoietin (epo) at doses 10,000-60,000 IU, once weekly, starting 2 weeks prior to initiation of antiviral treatment. RESULTS: We enrolled 10 patients, mean age 52 years and 7 of 10 with fibrosis stage 3 on a 4-grade scale. The mean initial RBV dose was 2400 mg/d (range 1600-2800). To reach the target concentration within 6 weeks the dose was raised to a mean RBV dose of 3000 mg/d (range 2400-4000). The mean baseline haemoglobin level was 15.3 g/dL, at treatment week 6 mean haemoglobin level was 13.4 g/dL and at week twelve 10.9 g/dL. Two patients have required blood transfusions. Mean baseline viral load was 7.4 ± 106 IU/mL, at treatment week 12 median viral load dropped to 1400 IU/mL (mean drop 3.6 log). Five patients have reached treatment week 24 of whom 4 have HCV-RNA <15 IU/mL (COBAS TaqMan). Of the remaining 5 patients, 4 have reached treatment week 16 whereof 2 have HCV-RNA <15 IU/mL. There are no therapy discontinuations. CONCLUSION: High-dosed RBV in combination with peg-INF alfa-2a seems to offer a mean 3.6 log HCV-RNA decline within 12 weeks in previous non-responders to standard-dosed combination therapy with the same drugs. High-dosed RBV treatment is feasible and seems to be safe, but requires strict attention regarding anaemia. Epo probably contributes to tolerability, especially during the first 12 weeks. The initial virological response is encouraging and the results from the completed trial, including SVR will show if the response is sustained.

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Tony Carlsson - Speaker’s Bureau: Roche
Karin Lindahl - Grant/Research Support: Roche; Speaker’s Bureau: Schering-Plough

The following people have nothing to disclose: Erika Hornfeldt, Lars Stahle, Robert Schwarz, Tony Carlsson, Tony Carlsson, Tony Carlsson, Tony Carlsson, Anna Hollander

SEQUENCE VARIATION OF INTERFERON/RIBAVIRIN RESISTANCE-DETERMINING REGION (IRRDR) OF HCV NSSA IS A PREDICTIVE MARKER FOR SUSTAINED VIROLOGICAL RESPONSE UPON COMBINATION THERAPY WITH PEGYLATED INTERFERON AND RIBAVIRIN

Hak Hotta, Ahmed El-Shamy, Mikiko Sasayama, Motoko Nagano-Fujii, Susumu Imoto, Noriko Sasase, Soo-Ryang Kim, Division of Microbiology, Kobe University Graduate School of Medicine, Kobe, Japan; Department of Gastroenterology, Kobe Asahi Hospital, Kobe, Japan.

Background and Aim: The combination therapy using pegylated interferon and ribavirin (Peg-IFN/RBV) has been considered to be most effective in treating patients chronically infected with hepatitis C virus (HCV). However, a substantial proportion of HCV-1b-infected patients still do not respond to Peg-IFN/RBV. The aim of this study is to explore a predictive marker(s) for sustained virological responders (SVR) and/or Non-SVR (non-responders) who never cleared the virus at any time point during the observation period, and the relapers who once cleared the virus but relapsed later upon Peg-IFN/RBV combination therapy. Methods: A total of 45 patients chronically infected with HCV-1b were treated with Peg-IFN-α-2b (1.5 µg per kg body weight, once weekly, sc) and RBV (600–800 mg daily, per os), according to a standard treatment protocol for Japanese patients established by a hepatitis study group of the Ministry of Health, Labour and Welfare, Japan. NSSA sequences of HCV-1b in the pre-treated sera were determined and compared with a consensus sequence. HCV RNA and core antigen titers in the sera were quantitatively measured before, during and six months after the treatment. Results: The mean number of mutations in the V3 region plus its upstream flanking region, which we refer to as IFN/RBV resistance-determining region (IRRDR) (aa.2334–2379), was significantly larger for SVR (6.1 ± 2.1) than Non-SVR (3.9 ± 1.4) (P<0.001) or NR (3.7 ± 0.9) (P<0.001). The criterion of ≥6 mutations in IRRDR (IRRDR≥6) was closely associated with IFN/RBV responsiveness. Only 2 (8%) of 24 Non-SVR had HCV with IRRDR≥6 whereas 16 (76%) of 21 SVR did (P<0.00001). Among the 24 Non-SVR, 13 patients were relapers. IRRDR≥6 could differentiate even between SVR and the relapers since only 2 (15%) of the 13 relapers had IRRDR≥6 (P<0.001). Moreover, all of 16 patients with IRRDR≥6 showed a significant (≥1 log) reduction of HCV core antigen titers within 24 h after the initiation of Peg-IFN/RBV whereas 10 of 17 patients with IRRDR<6 did (P<0.0001), with the result suggesting that IRRDR≥6 is closely associated with rapid reduction of HCV titers after the initiation of the therapy. The positive predictive value of IRRDR≥6 for SVR was 89% (16/18) (P<0.001) while the negative predictive value was 82% (22/27) (P<0.001). On the other hand, the number of mutations in the IFN sensitivity-determining region (ISDR) of NSSA was not a good marker to predict SVR, Non-SVR or NR in our study. Conclusion: A high degree (≥6) of sequence variation in IRRDR of HCV NSSA would be a useful predictive marker for SVR upon Peg-IFN/RBV combination therapy.

Disclosures:
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OPIOID MAINTENANCE THERAPY IS NOT ASSOCIATED WITH TREATMENT FAILURE TO HEPATITIS C THERAPY IN A LARGE GERMAN MULTICENTRE COHORT

Stefan Mauss1, Dietrich Hüpke2, Elmar Zehnter3, Michael P. Manns4, Gerlinde Teuber2, Tarek Dahban6, Ulrike Meyer2, Bernd Möller2, Nektarios Dikopoulos8, Thomas Wittthé6, Jochen Brack10, Marek Stern11, Stephan Kaiser12, Renate Prinzing13. The bng hepatitis study group

Objective: The largest group of newly infected individuals with chronic hepatitis C in the Western world are intravenous drug users. Emerging data support treating individuals with peginterferon and ribavirin for chronic hepatitis C after stabilisation on opioid maintenance therapy (methadone or buprenorphine). However these data are based on small cohorts or substudies from trials with small patient numbers. Here we report data from a cohort of 2422 patients including 333 patients on opioid maintenance therapy. Methods: A total of 3547 patients treated with at least one dose of peginterferon alfa-2b and weight based ribavirin are currently included in a German multicentre cohort. Only patients included in this cohort beyond 72 weeks of baseline were included in this analysis (n=2422). Patients with missing data at week 72 were counted as treatment failures. Univariate analysis was performed for comparison of demographics in patients on opioid maintenance vs. remaining patients (age, sex, ALT, BMI, HCV-RNA, genotype, ribavirin dose, peginterferon dose). For logistic regression analysis sex, age, baseline HCV-RNA, HCV-genotype, BMI and opioid maintenance were used as independent variables. The dependent variable was being HCV-RNA negative (<400 IU/mL) or positive at week 72 (SVR). Results: Patients on opioid maintenance were younger (35.0±9 vs. 42.2±12 years, p<0.001), and more had genotype 3 (46.0% vs. 31.4%, p<0.001). HCV-RNA levels were lower (45.8% vs. 61.2% <400,000 IU/mL, p<0.001). SVR in all patients on opioid maintenance (n=333) was 64.1% vs. 56.0% in the remaining patients (n=2089) (p<0.05, univariate). In logistic regression analysis, variables positively associated with SVR were younger age, HCV-genotype 2/3 and baseline HCV-RNA <400,000 IU/mL (all p<0.001). Female sex showed a trend for SVR (p=0.055). Opioid maintenance therapy was not associated with treatment outcomes in the logistic regression analysis. Conclusion: Efficacy of peginterferon and ribavirin was not different for patients on opioid maintenance therapy. Due to favourable factors for SVR such as HCV-genotype 3, younger age and lower HCV-RNA, patients on opioid maintenance therapy showed a better adjusted SVR compared to patients not on this therapy. Treatment of patients on opioid maintenance therapy in daily practice is feasible and success rates are not inferior to results from prospective, controlled studies.

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FINAL RESULTS OF THE CANADIAN POWER (PEGINTERFERON ALFA-2B PROSPECTIVE OPTIMAL WEIGHT-BASED DOSING RESPONSE) PROGRAM. SUSTAINED VIROLOGIC RESPONSE (SVR) TO WEIGHT-BASED PEGINTERFERON ALFA-2B + RIBAVIRIN IN A LARGE, MIXED COMMUNITY AND ACADEMIC OBSERVATIONAL STUDY

Paul Marotta1, Saya V. Feinman2, Cameron Ghent3, Linda Scully4, Michael Varenbu5, Jeff Daiter5, Helga B. Witt-Sullivan5, Jean Robert5, Barbara Romanowski5, John Farley9, Nabil Abadir10, Robert J. Bailey11, 1London Health Sciences Centre, London, ON, Canada; 2Mount Sinai Hospital, Toronto, ON, Canada; 3University of Western Ontario, London, ON, Canada; 4Ottawa Hospital - Civic Campus, Ottawa, ON, Canada; 5Ontario Addiction Treatment Centers, Richmond Hill, ON, Canada; 6Hamilton Health Sciences General Site, Hamilton, ON, Canada; 7Centre Sida Amitié des Laurentides, St. Jerome, QC, Canada; 8University of Alberta, Edmonton, AB, Canada; 9Private Practice, Vancouver, BC, Canada; 10Schering-Plough Canada Inc, Pointe Claire, QC, Canada; 11Royal Alexandra Hospital, Edmonton, AB, Canada

Background: To determine the impact of hepatitis C virus (HCV) genotype (G), baseline viral load, weight, and fibrosis stage on SVR rates in treatment-naive patients with chronic hepatitis C who were treated with weight-based peginterferon (PEG-IFN) alfa-2b and weight-based ribavirin (RBV) in a “real-life” observational setting. We report final SVR results from the POWer program. Methods: POWer was an open-label observational trial conducted in academic and community clinics across Canada between 2002 and 2006. All patients received PEG-IFN alfa-2b (1.5µg/kg/wk) plus RBV (800–1200mg/day) for either 24 [G2 and G3] or 48 weeks [G1]. SVR (defined as undetectable HCV RNA 24 weeks post-treatment) was stratified by genotype, baseline viral load, and fibrosis score. Results: 1977 patients initiated treatment. Patients were excluded if they had undetectable HCV RNA at end of treatment but no 6-month follow up, had no treatment data available, or had HCV/HCV coinfection. This analysis was based on 1800 patients, including those who discontinued because of side effects, lack of response, or personal reasons. Most patients were infected with G1 (60%), followed by G3 (22%) and G2 (15%). Three percent of patients had G4/G5/G6 or no specified genotype. Baseline viral load was available in 1477 patients; 52% had high viral load (HVL; >600,000 IU/mL or 2×106 copies/mL). Liver biopsy specimens were available in 946 patients (53%), revealing F0-F2 fibrosis in 60% and F3-F4 fibrosis/cirrhosis in 40%. SVR rates were higher in patients with minimal (F0-F2) fibrosis than in those with advanced (F3-F4) fibrosis/cirrhosis (60% vs 35% P<0.001) and in patients with low viral load than in those with HVL (57% vs 50% P=0.009). Baseline viral load and fibrosis score were negative predictive factors for SVR in G1 and G3 patients but not G2 patients. Conclusions: Despite poor predictive factors (advanced fibrosis and HVL), excellent SVR rates and low relapse rates with PEG-IFN alfa-2b plus RBV may be attained in a real-life observational setting. Observational trials include a more heterogeneous patient population than those observed in controlled trials and provide useful information to practitioners and regulators on postapproval drug use.
EOT, SVR, and Relapse Rates Stratified by Genotype

<table>
<thead>
<tr>
<th>Genotype</th>
<th>EOT, %</th>
<th>SVR, %</th>
<th>Relapsr, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>All (n=1800)</td>
<td>16.7</td>
<td>54.3</td>
<td>12.0</td>
</tr>
<tr>
<td>G1 (n=1078)</td>
<td>50.2</td>
<td>41.6</td>
<td>17.0</td>
</tr>
<tr>
<td>G2 (n=276)</td>
<td>85.5</td>
<td>79.0</td>
<td>7.6</td>
</tr>
<tr>
<td>G3 (n=389)</td>
<td>76.9</td>
<td>72.0</td>
<td>6.4</td>
</tr>
<tr>
<td>G4/G5/G6 (n=411)</td>
<td>70.7</td>
<td>65.9</td>
<td>6.8</td>
</tr>
</tbody>
</table>

*16 patients no genotype data. P<.001. EOT=end of treatment.

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Jean Robert - Grant/Research Support: Schering-Plough
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255 INFLUENCE OF NK CELL RECEPTOR AND HLA-C LIGANDS GENES ON THE RESPONSE TO ANTIVIRAL THERAPY OF CHRONIC HEPATITIS C INFECTED PATIENTS

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Virologic and host factors are associated with response to antiviral therapy in patients with chronic hepatitis C. The activation of the natural killer cells is important to limit viral replication in the liver. Therapy with interferon appears to be more effective in individuals with HCV infection in which NK cells are activated by this drug. The function of NK cells is regulated by a balance between the signals generated by its cellular receptors (KIR) of activation and the inhibition through the interaction with HLA class I molecules in the target cells. Khakoo et al (Science, 2004) found that the presence of the receptor-ligand pair KIR2DL3/HLA-C1 has a role in the clearance of acute HCV infection. Aims: to evaluate the role of the KIR and HLA-C ligands genes on the response to antiviral therapy of chronic HCV infected patients. Methods: We compared the frequency of KIR genes and their receptor-ligands pairs between 45 patients with chronic HCV infection who had a sustained virologic response and 41 subjects who were non-responders to combination therapy with standard or pegylated interferon and ribavirin. A hundred and two blood donors served as controls. Genotyping of the KIR was carried out using PCR-SSO kit (One Lambda, Canoga Park, CA, USA). Results: There was no difference between responders and non-responders in terms of ethnic origin, however there was a female predominance among non-responders. No difference related to ethnic background was found between the HCV patients and healthy controls but the latter group had a higher frequency of males. We observed a lower frequency of the genes KIR2DL5 (38% vs 64%) and KIR2DS3 (24% vs 46%) (p=0.03) among responders compared to non-responders. On the contrary, there was higher frequency of the receptor-ligand pair KIR2DL3/HLA-C1 among patients who responded to therapy compared to non-responders (64% vs 39%) (p=0.018). The Mantel-Hazel test ruled out any influence of the HCV genotype on the differences detected. When all patients with chronic hepatitis C were compared to the healthy blood donors, a higher frequency of the genes KIR2DL5 (OR=1.79; CI=1.01-3.19), 2DS5 (OR=1.96; CI=0.98-4.05) and 3DL3 (OR=2.39; CI=1.05-5.83) was found in the former group. Conclusions: Our data suggest that host factors such as the NK cellular receptors genes KIR2DL5 and KIR2DS3 are associated with non-response to antiviral therapy. On the other hand the receptor-ligand pair KIR2DL3/HLA-C1 may favor the response to antiviral treatment among patients with chronic hepatitis C. In addition, the genes KIR2DL5, 2DS5 and 3DL3 may play a role in the predisposition to the development of chronic HCV infection.

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The following people have nothing to disclose: Valdirene L. Carneiro, Denise C. Lemaire, Maria Teresita Bendichio, Sabrina L. Souza, Lourianne N. Cavalcante, Patricia S. Brandao, Luiz G. Lyra, Andre C. Lyra

256 HEPATIC EXPRESSION OF INNATE IMMUNE MOLECULES WITH RESPECT TO INTRAHEPATIC HCV REPLICATION AND OUTCOMES OF PEGINTERFERON PLUS RIBAVIRIN COMBINATION TREATMENT

Nobukazu Yuki, Shintaro Matsumoto, Michio Kato, Toshikazu Yamaguchi

Background: Recognition of dHCV RNA by TLR3 and RIG-I results in IFN-β production in the liver, which in turn upregulates PAMP receptor expression. HCV proteins in infected cells interrupt this amplification loop. The clinical relevance of overall hepatic expression of these molecules remains unclear. Methods: From ten liver tissues were obtained from 49 patients with HCV genotype 1b. Positive- and negative-strand HCV RNA was quantified by real-time PCR and expressed as copies/100 ng RNA (J Hepatol 2006; 44: 302-9). Relative expression of TLR3, RIG-I and IFN-β mRNA was determined by real-time PCR using the comparative Ct method, where the target amount was normalized to GAPDH mRNA and relative to a normal liver reference. All patients were treated with weight-based PEG-IFNα2b (60-120µg/kg/wk) plus ribavirin (600-1000 mg/day) for 48 wk. Treatment was extended to 72 wk in SVR (HCV RNA-positive at wk 12 but negative at wk 24). Virologic response was analyzed on a per protocol basis. Results: TLR3, RIG-I and IFN-β mRNA were expressed with significant correlations (r =0.474 to 0.497, P<0.001). TLR3 and IFN-β mRNA levels bore no relation to HCV replication, whereas RIG-I mRNA was directly correlated with replication assessed by liver positive- and negative-strand HCV RNA (r = 0.420, P = 0.003 and r = 0.487, P<0.001, respectively) but not with serum HCV RNA (r = 0.078, P = 0.594). TLR3 mRNA levels were higher in 18 women than in 31 men (median 1.9, range 0.6-3 vs. 1.3, 0-3.6; P = 0.043). Otherwise, expression of the innate immune molecules had no relation to baseline patient characteristics. The medians (range) of TLR3 mRNA expression were 1.2 (0-4.9) for EVR at wk 12 (>2 log drop or undetectable HCV RNA)(n = 34) vs. 2.4 (0.1-6.3) for no EVR (n = 11)(P = 0.008), 1.0 (0-3.4) for VR at wk 24 (undetectable HCV RNA)(n = 26) vs. 2.2 (1.1-4.9) for no VR (n = 14)(P<0.001), and 0.9 (0.4-2.4) for EVR at wk 48 (n = 15)(P = 0.001)
for SVR \( (n = 22) \) vs. 1.9 \( (0.4-4.9) \) for no SVR \( (n = 16) \) (\( P = 0.004 \)). Positive and negative predictive values of TLR3 mRNA expression of <1.5 were 90 \( (19/21) \) and 38% \( (9/24) \), respectively, for EVR, 95 \( (18/19) \) and 62% \( (13/21) \) for VR at wk 24, and 89 \( (16/18) \) and 70% \( (14/20) \) for SVR. In contrast, RIG-I and IFN-\( \beta \) mRNA expression were not associated with the treatment outcomes. Conclusions: TLR3, RIG-I and IFN-\( \alpha \) are expressed in the liver with a shared regulatory mechanism but bear distinctive relevance. Only RIG-I is upregulated in parallel with HCV replication, raising a possibility that RIG-I serves as a major viral sensor. In the context of antiviral therapy, favorable treatment outcomes can be efficiently predicted by low levels of TLR3 expression at baseline.

Disclosures:
The following people have nothing to disclose: Nobukazu Yuki, Shinji Matsumoto, Michio Kato, Toshikazu Yamaguchi

257

EARY HEPATITIS C VIRUS DECAY WITH WEIGHT-BASED RIBAVIRIN PLUS EITHER PEGIFN-\( \alpha \)-2A OR PEGIFN-\( \alpha \)-2B IN HIV/HCV COINFECTED PATIENTS

Eugenia Vispo, Pablo Barreiro, Sonia Rodríguez-Novoa, Pablo Labarga, Luz Martín-Carbonero, Vicente Soriano; Department of Infectious Diseases, Hospital Carlos III, Madrid, Spain

Background: PegIFN plus RBV is the recommended therapy for chronic hepatitis C in HIV+ patients. Pharmacokinetic differences between the two available pegIFN molecules might impact on their respective antiviral activities. Methods: All consecutive HCV/HIV patients who had initiated first-line treatment with pegIFN-\( \alpha \)-2a or -2b plus RBV \( (1,000-1,200 \text{ mg/day}) \) at our institution were identified. Rapid and early virological responses (RVR and EVR), and undetectable HCV-RNA (<10 IU/mL) at week 24 were assessed by ITT analysis. Baseline liver fibrosis was estimated by transient elastometry. RBV plasma trough levels were measured by HPLC at week 12. Results: A total of 207 patients were identified, of whom 138 received -2a and 69 -2b. Their respective main features were: mean age, 45 and 44 years; male gender, 73 and 75%; mean BMI, 23 and 22 kg/m2; mean CD4 count, 547 and 523 cells/µL; HCV-RNA <50 cop/µL, 68 and 79%; mean HCV-RNA, 6.07 and 6.15 log IU/mL; G1/4, 67 and 73%; and mean hepatic stiffness, 10.9 and 9.9 KPa, respectively. Mean RBV plasma levels at week 12 were 2.4 and 2.3 ug/mL (\( p=ns \) for all variables). Comparing pegIFN-\( \alpha \)-2a and -2b, mean HCV-RNA decay (log IU/mL) was 2.7 and 2.2 at week 4 \( (p=0.09) \); 4.5 and 4.3 at week 12 \( (p=0.5) \); and 3.7 and 3.2 at week 24 \( (p=0.1) \). Virological responses were: RVR, 43 and 30\% \( (p=0.08) \); EVR, 71 and 70\% \( (p=0.8) \); undetectability at week 24, 64 and 58\% \( (p=0.4) \). The rate of RVR was better with pegIFN-\( \alpha \)-2a versus -2b in patients with baseline HCV-RNA >500,000 IU/mL (37 vs 15\%, \( p=0.01) \), and in patients carrying G2/3 (85 vs 56\%, \( p=0.02) \). In multivariate model (OR [95\% CI]), greater RBV plasma concentrations predicted RVR \( (1.39 [0.96-2.03] \) 0.07), EVR \( (1.83 [1.13-2.97] \) 0.01) and week 24 response \( (1.94 [1.23-3.05] \) 0.004), independently of the pegIFN used. The positive predictive value of RBV levels >2.1 ug/mL for week 24 response was 82\%. Conclusions: The intrinsic antiviral activity of pegIFN-\( \alpha \)-2a and -2b seems to be comparable, except for the very early phase of viral decay. However, in this study higher RBV plasma concentrations rather than the pegIFN modality were the main determinant of virological response at any time point.

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The following people have nothing to disclose: Eugenia Vispo, Pablo Barreiro, Sonia Rodríguez-Novoa, Pablo Labarga, Luz Martín-Carbonero, Vicente Soriano

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ASSESSMENT OF THE IMPACT OF PSYCHIATRIC DISORDERS ON SAFETY, COMPLIANCE, AND SUSTAINED VIROLOGICAL RESPONSE AFTER HEPATITIS C TREATMENT (CHEOBS)

Jean-Philippe Lang\(^1\), Pascal Melin\(^2\), Denis Ouzan\(^3\), Michel Rotily\(^4\), Thierry Fontanges\(^5\), Patrick Marcellin\(^6\), Michel Chousterman\(^7\), Patrice Cacoub\(^8\); \(^1\)Centre Hospitalier Erstein, Erstein, France; \(^2\)Hôpital Général, Saint Dizier, France; \(^3\)Institut Arnaud Tzanck, Saint Laurent du Var, France; \(^4\)INSERM, Bagneux, France; \(^5\)Centre de l’Appareil Digestif, Bourgoin Jallieu, France; \(^6\)Hôpital Beaujon, Clichy, France; \(^7\)Hôpital de Créteil, Créteil, France; \(^8\)Hôpital Pitié Salpêtrière, Paris, France

Purpose: The CHEOBS study is a French multicenter, prospective, observational study designed to analyze the factors related to compliance with the combination treatment with Peginterferon-\( \alpha \)-2b and Ribavirin in patients (pts) with chronic hepatitis C virus (HCV) infection. This analysis evaluates mental safety, quality of life, compliance to treatment and sustained virological response (SVR) in pts presenting psychiatric disorders (ppd) before the start of HCV treatment and compares them with those of pts not presenting psychiatric disorders (ppd). Methods: From Jan 2003 to Dec 2004, 1,972 pts with chronic HCV infection were included in CHEOBS and began antiviral therapy: 444 ppd pts and 1,528 ppd pts. Among ppd pts, 232 (53\%) had depressive disorders, 179 (40\%) anxiety disorders, 21 (5\%) schizophrenia and 7 (2\%) bipolar disorders. Baseline characteristics and the impact of ppd on compliance, virological response and quality of life (QoL, SF-36) were analysed. Results: At baseline (Day 0), ppd and ppd populations were different as the ppd population was younger (45 years vs 47 years), with a lower educational level (68\% vs 53\%), less well paid jobs (48\% vs 61\%), higher debts (13\% vs 5\%), more chronic diseases (32\% vs 26\%), higher alcohol intake (31\% vs 23\%), higher tobacco (66\% vs 42\%) and drug consumption (9\% vs 2\%), and higher rate of genotype 3 infection (30\% vs 23\% \( p<0.05) \). At the end of HCV treatment, there was no significant difference between ppd and ppd populations for compliance, duration of combination antiviral therapy (35 ± 17 vs 36 ± 17 weeks), premature antiviral therapy discontinuation due to adverse events or the pts request (53\% vs 52\%). The results of SVR, mental adverse events and QoL are shown in the Table. Conclusion: In a real life study, pts infected by HCV and starting HCV treatment frequently present with psychiatric disorders. The ppd profile did not have a negative impact on compliance, duration, premature treatment discontinuation or SVR. The QoL was impaired by HCV treatment and its adverse effects that was even more pronounced in ppd pts.

Disclosures:
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TABLE 26

DANAZOL INCREASES THE PLATELETS COUNT IN THROMBO-CYTOPENIC PATIENTS WITH CHRONIC HEPATITIS C AND LIVER CIRRHOSIS TREATED WITH PEG-INTERFERON ALFA 2A AND RIBAVIRIN

Guillermo Cabrera-Alvarez1, Luis Cañedo-Dorantes2, Jorge Reyes-Esparrza3, Lourdes Rodriguez-Fragoso3, Nahum Mendez-Sanchez5, Ana Burguet4, Vicente Madrid-Marinad,1 Gastroenterology, Universidad Autónoma del Estado de Morelos, Cuernavaca, Mexico; 2Faculty of Medicine, Postgraduate Division, Universidad Autónoma del Estado de Morelos, Cuernavaca, Morelos, Mexico; 3Faculty of Pharmacology, Universidad Autónoma del Estado de Morelos, Cuernavaca, Morelos, Mexico; 4Chronic Infections and Cancer Division, Instituto Nacional de Salud Publica, Cuernavaca, Morelos, Mexico; 5Liver Unit, Medica Sur Clinic & Foundation, Mexico city, Mexico

Background and Aim: Chronic hepatitis C (HCV) infection has been associated with the development of several extrahepatic alterations, including thrombocytopenia. Currently it remains unresolved. Danazol, an attenuated androgen has been successfully used in patients with autoimmune thrombocytopenia. The aim of the present study was to investigate the effects of Danazol treatment for thrombocytopenia associated with peginterferon alfa-2a and ribavirin therapy in naïve HCV patients. Methods: A prospective study carried out in patients with chronic hepatitis C or liver cirrhosis patients who were under antiviral therapy. The protocol was approved by the Review Board/Ethics committee of the Hospital. The inclusion criteria including both gender, age (20 to 70 yr), without co-infection with hepatitis B virus or human immunodeficiency virus (HIV-1/2), thrombocytopenia during peginterferon alfa-2a and ribavirin therapy was defined when the count was ≤ 90,000 platelets/mL in the last month. Danazol 300-600 mg/day was administered until the end of therapy. We considere as a control patients those on antiviral therapy who did not receive adjuvant danazol due to only mild thrombocytopenia on antiviral therapy, matched for baseline platelet count, presence of cirrhosis, age, sex and HCV genotype. Efficacy was evaluated as the capacity to increase in platelet counts until the end of the treatment period. Results: A total of 41 patients with HCV-associated thrombocytopenia with PEG IFN/ribavirin treatment were studied: 26 patients (20 females, 6 males), mean age of 54.57 ± 8.40 yr who received danazol and 15 controls (9 females, 6 males), mean age of 55.8 ± 13 yr. Ninety percent of 41 patients had cirrhosis and the HCV genotypes were similar between groups. The platelet count increases in the Danazol group from baseline (75300 ± 11502) after treatment (123,900 ± 30411 p<0.0063). Whereas in the control group the mean count range from (238953.3 ± 141962.9 to 1742020 ± 91643, p=0.9246) respectively. No association between genotypes and thrombocytopenia was observed (P>0.05). Danazol safety was assessed by the absence of collateral negative effects, except colestasis reversible in two patients. Conclusions: Adjuvant use of Danazol is associated with increased platelets counts in patients on antiviral therapy with interferon and ribavirin for HCV infection and cirrhosis. This is new therapeutic option to treat thrombocytopenia and maximize the sustained viremic response.
261 RELAPSE RATES AMONG HCV GENOTYPE 1 EARLY VIROLOGICAL RESPONDERS IN A RETROSPECTIVE COMMUNITY-BASED COHORT OF PATIENTS TREATED WITH PEGETRON® IN BRITISH COLUMBIA, CANADA

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PURPOSE: Response profiles from a community-based cohort of HCV genotype 1 infected patients undergoing a 48 wk course of Peginterferon alpha-2b plus Ribavirin (PEGETRON®) in British Columbia were assessed for the impact of residual viremia at wk 12 during Early Virological Response (EVR) determination. End of Treatment (EOT) response, Sustained Virological Response (SVR) and relapse rates were categorized with respect to whether a partial virological response or early virological clearance was achieved at wk 12. METHODS: Databases maintained by BC PharmaCare and the British Columbia Centre for Disease Control were used to calculate EOT, SVR and relapse rates among genotype 1 patients whose wk 12 EVR result was reported between June 12, 2003 and February 6, 2005. Patients were required to have a HCV RNA baseline viral load of > 61,500 IU/mL (Versant HCV 3.0 assay) and achieved an EVR. EVR was defined as either a > 2-log10 drop in viral load at wk 12 with residual viremia (wk 12 > 615 IU/mL or a positive qualitative HCV RNA (Roche, Cobas AMPLICOR), i.e. partial virological response); or a > 2-log10 drop in viral load at wk 12 with aviremia (wk 12 < 615 IU/mL or a negative qualitative HCV RNA, i.e., early virological clearance). Viral relapse was defined as a negative qualitative HCV RNA at EOT and detectable HCV RNA 24 wks after EOT. Relapse rates were reported as ranges to include or exclude missing SVR data. Lower range estimate was defined as EOT responder and SVR non-responder, and higher range estimate as EOT responder and SVR missing and/or non-responder.

RESULTS: A total of 696 HCV genotype 1 patients underwent wk 12 testing during PEGETRON® treatment between June 12, 2003 and February 6, 2005. Of these, 507/696 (73%) had baseline viral loads > 61,500 IU/mL and achieved an EVR. 380/507 (75%) demonstrated early virological clearance and 127/507 (25%) had a partial virological response at wk 12. EOT results were available for 248/507 (49%) of whom 204/248 (82%) were aviremic at wk 12 and 44/248 (18%) were viremic at wk 12. 197/248 (79%) were EOT responders; EOT response rate was 183/204 (90%) for aviremic at wk 12 and 14/44 (32%) for viremic at wk 12. Relapse rates for aviremic patients were 10% to 32% (low and high ranges) whereas for viremic patients at wk 12, the relapse rates were 50% to 79%. CONCLUSION: Among patients treated with PEGETRON® for 48 wks, EVR aviremic patients had an EOT response rate of 90% and relapse rates of 10% to 32%. In contrast, EVR viremic patients had an EOT response rate of 32% and relapse rates of 50% to 79%. The presence of residual viremia at wk 12 has an unfavourable outcome with fixed duration therapy.

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The following people have nothing to disclose: Amanda Yu, Warren D. Hill, Helen Mah, Annie Mak

262 VIRAL AND STAT KINETICS IN PATIENTS WITH CHRONIC HEPATITIS C TREATED WITH PEGEYLATED INTERFERON A-2B PLUS RIBAVIRIN

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Background: Treatment with pegylated interferon-α (PEG-IFN-α) and ribavirin (RBV) induces sustained virologic response (SVR) in about 55% of patients with chronic hepatitis C (HCV). Among several factors, HCV interference with IFN-α signal transduction Jak-STAT pathway has been proposed as a possible determinant of treatment failure. Viral kinetic studies have shown that early viral response predicts SVR. Aims: To evaluate kinetics of STAT proteins in peripheral blood mononuclear cells (PBMC) in comparison to viral kinetics and to assess whether STAT kinetics may predict the outcome in HCV patients with low probability of response. Methods: 15 patients with HCV genotype 1 (n=12) or 4 (n=3) treated with PEG-IFN-α 2b (1.5 mg/kg once weekly) plus RBV (1,000-1,200 mg daily) were prospectively evaluated one hour before treatment (T0) and at days 1 (T1), 2 (T2), 7 (T7) and 14 (T14) for quantitative serum HCV-RNA and PBMC cytoplasmic and nuclear STAT1 and STAT2. PBMC protein extracts were analyzed by Western blot and EMSA. Results: Six patients (40%) achieved SVR, two (13.3%) were relapers and seven (46.7%) were virologic non responders (NR). HCV-RNA levels fell ≥ 1 log in many patients, mostly responders, at T1 and T2, with an intermediate increase at T7. At T14, only seven patients showed a renewed ≥ 1 log HCV-RNA decline (5 SVR, 1 NR, 1 relaper). PBMC analysis showed that STAT1-STAT2 nuclear translocation was restricted to the SVR patients and one relaper with a proportion that progressively increased from T1 to T14. Among the seven patients showing STAT1-STAT2 in the nucleus of PBMC at T14, EMSA analysis showed both STAT2 and STAT1 DNA binding only in the six SVR. Comparing early viral and STAT kinetics during the first two weeks, we found that whereas the occurrence of a ≥ 1 log fall of HCV-RNA at T1 was in most cases not associated to STAT1-STAT2 nuclear localization in PBMC, the rate of this association increased through time, being highest at T14 (5 SVR, 1 relaper). When we compared STAT kinetics at T14 and virologic response at week 24, we found that among the eight HCV-RNA negative patients (six SVR and both relapers), only the six SVR showed also STAT1-STAT2 nuclear import and DNA binding, whereas none of the seven HCV-RNA positive patients showed nuclear STAT1-STAT2. Conclusions: Our data suggest that the variability of virological response to treatment with PEG-IFN-α and RBV is associated with intrinsic differences in the pattern of IFN signaling pathway and that STAT1-STAT2 nuclear translocation and DNA binding in PBMC may predict SVR as early as two weeks after treatment start.

Disclosure:

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MORPHOLOGICAL ANALYSIS AND ASSESSMENT OF HCV-RNA AND RIBAVIRIN CONCENTRATION IN SEMINAL FLUID OF CHRONIC HEPATITIS C PATIENTS UNDERGOING ANTIVIRAL COMBINATION THERAPY

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Background and Aim: At present, combination therapy with pegylated interferon-alpha and ribavirin is the treatment of choice for patients with chronic hepatitis C (CHC). Due to the possible teratogenic effect of ribavirin effective contraception is mandatory during antiviral therapy. Aim of the study was to evaluate seminal parameters, ribavirin and HCV-RNA concentration in seminal fluid and serum prior to and during antiviral treatment. Patients and Methods: So far, 10 male patients (mean age: 43±9 [years±SD]) were treated with combination therapy (Copegus®, Roche, Austria) in combination with ribavirin (Copegus®, Roche, Austria) were investigated. All patients were negative for HIV or HBV co-infection. The HCV genotype distribution was HCV-1 (n=5), HCV-2 (n=1), HCV-3 (n=1), HCV-4 (n=3). Seminal fluid (sperm concentration, motility and morphology) was analyzed morphologically. HCV-RNA and ribavirin concentration (serum and seminal fluid [diluted 1:10]) were determined by quantitative PCR (TaqMan®, Roche Austria); LOD: 10 IU/ml and HPLC, respectively. Examinations were carried out at baseline, at week 4 and at week 12 of antiviral therapy. Results: HCV-RNA was detectable in the seminal fluid of only one patient prior to antiviral therapy (with a serum viral load of 4.04 MU/ml) and was undetectable in all patients after 4 and 12 weeks of combination therapy. Ribavirin concentration was substantially higher in the seminal fluid (week 4: 4.7±1.9 µg/ml, [mean±SD]; week 12: 4.3±0.4) than in serum (week 4: 2.2±0.3 [p=0.01]; week 12: 1.9±0.3 [p=0.02]). Morphological semen abnormalities were common at baseline (asthenoteratozoospermia: n=4, asthenozoospermia: n=1, teratozoospermia: n=3). Sperm density (BL: 70±31x10^6/ml, Week 4: 50±32, week 8: 59±43 [n.s.]), percentage of sperms with progressive motility (BL: 45±25%, Week 4: 30±28, week 8: 30±23 [n.s.]), and percentage of sperms with normal morphology (BL: 21±14%, Week 4: 19±11, week 8: 11±6 [n.s.]) tended to further decrease during antiviral therapy. Conclusion: HCV-RNA is detectable in seminal fluid only in a low proportion of CHC patients. In contrast, pre-treatment semen abnormalities with reduced percentage of spermatozoa with normal progressive motility and normal morphology are common in patients with chronic HCV infection with further impairment during antiviral therapy. Ribavirin concentration is twofold elevated in seminal fluid compared to serum levels, which reinforces the need of contraception during antiviral combination therapy.

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FOSAMPRENAVIR IN HAART SCHEDULE INDUCES A RAPID VIROLOGICAL AND BIOCHEMICAL RESPONSE TO HCV COUPLED TO TH1 BOOSTING IN HIV/HCV COINFECTED PATIENTS

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Objectives: Pegylated recombinant Interferon plus ribavirin after HAART induced immune system restoration is the treatment schedule in HIV/HCV patients (pts). We aimed to evaluate the effect of Fosamprenavir on immunological function in naïve-drug coinfected patients before to start treatment with Interferon plus ribavirin. Methods: We studied 10 naïve pts (4 F and 6 M) with HIV/HCV infection with at least one year history of HCV persistent infection and treated with: [AFC 300mg + 3TC 300mg Twice] + (Fosamprenavir 700mg twice) + (RTV 100mg)], assayin CD4+/CD4+, INF-γ and IL-4 ELISPOT specific response (HCV-core peptides; Pro-Immune,Oxford,UK), HIV and HCV Viral Load (Amplificor Roche system) and Transaminasis before to start HAART treatment (T0) and every month. Sign Test was used for statistic analysis. Results: At Time 0: CD4+ =186 ± 23 (mean ± s.d.); ALT= 121 ± 44; AST =93 ±31; HCV-RNA = 569x10^3 ± 23x10^3 IU/ml; HIV-RNA = 90x10^3 ± 19x10^3 IU/ml, while ELispot was IFN-γ 62 ± 10 SFC and IL-4 93 ±12 Spot Forming Colonies (SFCs). At T1: CD4+ = 414 ± 63; HIV-RNA = 209 ± 427 IU/ml but more surprisingly we had ALT = 22 ± 9; AST = 25 ± 8; HCV-RNA = 15x10^3 ± 30x10^3 IU/ml (two out of ten pts had become negative at Viral load). Concerning ELISPOT we had IFN-γ =112 ± 14 SFC and IL-4 = 52 ± 16 SFCs. At T3: CD4+ ≥ 486 ± 48, with negative HIV and HCV viral load and normal transaminasis serum levels. Differences were statistically significant (p <.01). Conclusion: Fosamprenavir treatment in HAART schedule induces a decrease of HIV-RNA with CD4+ increasing within the first month, but more interestingly also a rapid HCV virological and biochemical response with a boost of Th1 immune network. Fosamprenavir treatment may be an important strategy in the therapy of HIV/HCV naïve coinfected patients.

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IMPROVED RESPONSE RATES WITH TREATMENT EXTENSION TO 72 WEEKS IN SLOW RESPONDERS TO PegIFINTERFERON AND WEIGHT-BASED RIBAVIRIN IN CHRONIC HEPATITIS C VIRUS (HCV) INFECTION

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Background: For therapy-naive, chronic hepatitis C genotype 1-infected patients, treatment with pegylated interferon and ribavirin for 48 weeks has become the standard of care. For slow responders to treatment, there has been interest in extending therapy duration in hopes of improving rates of sustained virologic response (SVR). Two recent studies suggested that slow responders to treatment enjoy improved SVR rates with 72 weeks of therapy compared to 48 weeks, because of a diminution in rates of relapse; however, both studies used suboptimal doses of ribavirin (800 mg daily) (Gastroenterol 130:1086, 2006; Gastroenterol 131:431, 2006). It is unclear if therapy prolongation in slow responders would be beneficial, if weight-
based ribavirin were utilized. Methods: We analyzed data from two studies in which slow responders received either a customary treatment duration of 48 weeks or treatment extension to 72 weeks. One study was from the U.S. (Hepatology, In-press, 2007), and the other was from Europe (AASLD 2006, abs #390; EASL 2007, abs #641). The U.S. cohort was 48% African American and 52% Caucasian; the European cohort was 90% Caucasian. Slow response was defined by at least a 2-log decrement in baseline serum HCV RNA yet detectable viremia at 12 weeks of therapy with undetectable serum HCV RNA at 24 weeks. Slow responders in the European study also had detectable viremia at 4 weeks. The lower limits of RNA detection for the U.S. and European studies were 10 and 50 IU, respectively. Results: Rates of SVR were significantly superior in slow responders when treated for 72 weeks compared to 48 weeks, largely because of an improvement in relapse rate (Table). Conclusions: Treatment extension to 72 weeks relative to 48 improved SVR rates in slow-responders to peginterferon and weight-based ribavirin, in two disparate patient populations. SVR was improved because of a decrement in relapse rate. Results should be confirmed in larger prospective trials.

<table>
<thead>
<tr>
<th>Interferon type</th>
<th>Study One 1</th>
<th>Study Two 2</th>
</tr>
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<tbody>
<tr>
<td>Peg alpha 2-b</td>
<td>Peg alpha 2-a</td>
<td></td>
</tr>
<tr>
<td>800-1,200 mg/day</td>
<td>1,000-1,200 mg/day</td>
<td></td>
</tr>
</tbody>
</table>
| Study population | U.S. >100%
| genotype 1      | Europe >90%
| Total sample size | 205 |
| Slow responders analyzed (percentage total sample) | 101(28) |
| SVR (48 weeks)  | 18% (16/91) |
| SVR (72 weeks)  | 28% (25/92) |
| Relapse rate (48 weeks) | 32% |
| Relapse rate (72 weeks) | 20% |

*48% African American; 1Hepatology, In-press, 2007; 2AASLD 2006, abs #390;
3EASL 2007, abs #641.

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Brian Pearlman - Speaker's Bureau: Schering-Plough
The following people have nothing to disclose: Carole Ehleben

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**GENE EXPRESSION BIOMARKERS PREDICTING RESPONSE TO PEGYLATED INTERFERON ALPHA (PEG-INFN) AND RIBAVIRIN (RBV) IN TREATMENT-NAIVE PATIENTS WITH CHRONIC HEPATITIS C (CH-C)**

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Responsiveness to HCV therapy depends on viral and host factors. AIM: Develop a biomarker profile predicting sustained virologic response (SVR) after antiviral therapy. METHODS: 30 CH-C patients (naive) were started on standard doses of PEG-INFN alpha 2a or 2b and RBV. Blood samples were collected prior to treatment, 1 day, 1 week, 4 and 8 weeks after initiation of treatment. Total RNA was extracted, quantified and used for one step RT-PCR to profile 317 mRNAs (160 interferon-inducible, interferon pathway, immune response, and housekeeping related genes). Expression levels of mRNAs were normalized with 6 “housekeeping” genes and a reference RNA. Multiple regression analysis and stepwise selection were performed to assess differences in gene expression at different time points and predictive performance was evaluated for each model. RESULTS: Demographics: 47 ± 7 years, 64% Male, 61% Caucasian & 64% genotype 1 (G1). Prior to treatment, SVR was predicted by expression of STAT6 and CCL3 genes (Model p-value = 0.0063, AUC = 0.826, Sensitivity = 0.923, Specificity = 0.600) for all patients. In G1, SVR was predicted by expression of EP300 and SOC56 (Model p-value = 0.001, AUC = 0.940, Sensitivity = 0.857, Specificity = 0.917). At day 1, SVR for entire cohort was predicted by expression of IFN-dependent genes (IFIS3, IRF8, IL15RA, GTPBP2, BCL2) (Model p-value = 0.0094, AUC = 0.901, Sensitivity = 0.846, Specificity = 0.929) and by IL18 and ADAM9 in G1 (Model p-value = 0.0091, AUC = 0.909, Sensitivity = 0.857, Specificity = 0.909). At day 7, SVR for entire cohort was predicted by expression of IL10, IF8 and HIF1A (Model p-value = 0.0002, AUC = 0.928, Sensitivity = 0.769, Specificity = 0.867), while for G1, SVR was predicted by PRKIR (Model p-value = 0.009, AUC = 0.845, Sensitivity = 1.000, Specificity = 0.750). At Day 28, SVR was predicted by expression of AIM2, IRF2, YARS, IFNAR1, IRF8, HIF1A, CREB1 and CD58 (Model p-value = 4.482e-006, AUC = 1.000, Sensitivity = 1.000, Specificity = 1.000) for all patients. For G1, SVR was predicted by expression of AIM2, PLAUR, CCL3, IRF8 and CD58 (Model p-value = 0.00045, AUC = 1.000, Sensitivity = 1.000, Specificity = 1.000). At day 56, SVR was predicted by expression of PRKIR, IF5 and PSM2E (Model p-value = 0.000065, AUC = 0.959, Sensitivity = 0.846, Specificity = 1.000) for all patients and IRF4, IF5, TRAF6, TAP1, IFNAR1 and PSM89 in G1 (Model p-value = 0.000015, AUC = 1.000, Sensitivity = 1.000, Specificity = 1.000). CONCLUSIONS: A panel of non-invasive gene expression biomarkers is developed to predict SVR in CH-C patients (naive). If validated, this biomarker panel can become useful in the management of HCV.

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**SUCCESSFUL ANTI-HCV THERAPY IS ASSOCIATED WITH SUSTAINED DOWNREGULATION OF PD-1 ON VIRAL-SPECIFIC CD8+ CYTOTOXIC T CELLS (CTLs) AND NATURAL KILLER CELLS (NKS)**

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HCV Infection is characterized by impaired proliferative and cytotoxic function of immune cells. The effect of antiviral therapy on the immune response in HCV patients remains controversial. AIM: To characterize expression of PD-1 (an important marker of exhaustion) on immune cells in HCV-infected patients before and after antiviral therapy. METHODS: 72 HCV genotype 1 patients from the Virahep-C study cohort were selected (30 African Americans, 42 Caucasian Americans) based upon
expression of relevant HLA alleles. We used multi-parametric FACS to characterize PD-1 on T, NK, and natural T (NT) cells before and after therapy with combination pegylated interferon/ribavirin. A panel of 10 Class I pentamers incorporating frequently targeted HCV epitopes was used. Results: Compared to normal subjects, HCV-infected subjects had significantly higher PD-1 on both CD4+ and CD8+ T cells (p<0.0001) as well as higher PD-1 expression on CD56bright NK cells (considered immature NK cells with low natural cytotoxicity) (p<0.0001) and NT cells (p<0.0001). Highest PD-1 expression was found on HCV-specific CTLs (p<0.0001 vs total CD8). The effects of antiviral therapy on PD-1 levels on immune cells comparing patients before (SO02) and after (FU24) with and without a sustained virological response (SVR) by race. Achievement of an SVR was associated with a decrease in PD-1 expression on total CD4+ T cells as well as HCV-specific pentamer+ CTLs (Figure); whereas patients without an SVR had no change in PD-1 expression on total CD4+ T cells or HCV specific CTLs. Successful antiviral therapy also led to sustained down-regulation of PD-1 on NK cells, in particular the CD56bright subset (Median decrease=1.94%, p = 0.051; figure). In non-responder patients, levels of PD-1 on NK cells increased after treatment (Median=+2.1%, p=0.02). When adjusted for race, greater decline in PD-1 expressions on NK cells was significantly associated with SVR in AAAs only. Conclusions: Collectively, these data indicate that PD-1 is plays a critical role in the lack of vigorous immune responses to HCV during persistent infection and is upregulated globally on numerous cell types. Successful antiviral therapy results in decreases in PD-1 expression and restoration of immunity.

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268 TREATMENT OF RECENTLY ACQUIRED HEPATITIS C INFECTION IN INJECTING DRUG USERS: PRELIMINARY RESULTS FROM THE AUSTRALIAN TRIAL IN ACUTE HEPATITIS C (ATAHC)

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Short duration treatment for acute hepatitis C virus (HCV) infection with pegylated interferon monotherapy has been shown to be highly effective, but has been investigated to a very limited extent in injecting drug users (IDUs), even though they are the population most at risk of infection in many high-income countries. The Australian Trial in Acute Hepatitis C (ATAHC) was funded by the US National Institutes of Health to examine the natural history and treatment of recently acquired HCV in IDUs. A longitudinal cohort was defined by having a first positive anti-HCV antibody test within 6 months of screening, plus either a negative antibody test within the preceding 24 month period or acute clinical hepatitis within the prior 12 months. Those in the cohort with detectable HCV RNA at screening were assessed for treatment with PEG-IFN α-2a (180 mcg weekly for 24 weeks), and follow up was undertaken in parallel protocols for both treated and untreated participants. Treatment outcomes among the initial 50 HCV monoinfected subjects commenced on treatment are presented on all subjects, and separately on only those subjects with available HCV RNA assessment at week 24. Since August 2004, 132 participants have been enrolled, with 85 (64%) commencing treatment, including 87 with HCV infection and 26 with HIV/HCV co-infection. Of the initial 50 participants with HCV only who received treatment (mean age 30 years, 66% male), 41 (82%) became infected via injecting drug use. Half had HCV genotype 1, the median HCV RNA was 380,000 copies/ml, and 23 (46%) had symptomatic acute infection. HCV treatment commenced a median 35 weeks (range, 18 – 81 weeks) after the estimated date of infection. Two thirds received 80% or more of the 24 planned PEG-IFN injections and 5 subjects (10%) received less than 50%. End-of-treatment response (ETR) as defined by undetectable HCV RNA at 24 weeks was 60% overall and 75% in those with available HCV RNA results. ETR was not related to prior duration of infection: <35 weeks (56%) vs >35 weeks (64%). At a median 26 weeks after treatment, 23 out of 24 assessable participants who were HCV RNA negative at week 24 still had undetectable HCV RNA. In this cohort of people who had newly acquired hepatitis C infection predominantly acquired through injecting drug use, the outcome of PEG-IFN α-2a monotherapy was very encouraging. Strategies to improve HCV treatment adherence in this population may further enhance these outcomes.

Disclosures: Gregory J. Dore - Grant/Research Support: Roche; Consultant/Adviser: Roche; Speaker: Roche
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Geoff W. McCaughan - Grant/Research Support: Roche; Consultant/Adviser: Roche; Consultant/Adviser: Schering-Plough; Consultant/Adviser: Novartis; Consultant/Adviser: Astellas
William D. Rawlinson - Consultant/Adviser: Novartis; Grant/Research Support: Roche; Consultant/Adviser: Human Genome Sciences
John M. Kaldor - Grant/Research Support: Roche.

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269 SHORT-TERM PROLONGATION OF PEGINTERFERON PLUS RIBAVIRIN COMBINATION THERAPY IS A SAFE AND EFFECTIVE TREATMENT STRATEGY FOR GENOTYPE 1B CHRONIC HEPATITIS C PATIENTS

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Introduction: It has been shown that 72 weeks (wks) of peginterferon plus ribavirin combination therapy (PEG/R) for genotype 1 chronic hepatitis C (CHC) patients, whose serum HCV RNA was positive at 4 wks of treatment increases sustained
viral response (SVR) rate. However, the prolongation of treatment duration increases therapy discontinuation because of the decrease of medication tolerability and the increase of medical expense. This result suggests 72 wks of PEG/R is difficult for some patients to complete. In this study, we focused on the patients with early viral response. Since they represent about a half of patient population, an increase of SVR rate of these patients should greatly affect the overall SVR rate. We set short-term prolonged treatment durations of PEG/R for genotype 1b CHC patients, depending on the time point when serum HCV RNA turned negative. And its efficacy was evaluated. Methods: Total of 52 genotype 1b CHC patients (30 male, 22 female, mean age 55±1 years) were enrolled in this study from December 2004 to September 2005. They were treated with peginterferon alpha 2b (1.0-1.5 μg/kg/wk) and ribavirin (600-1000mg/day) and serum HCV RNA levels were estimated every 4 wks. The viral response was defined and the treatment durations were determined by the time point when serum HCV RNA turned negative. 1) 4 wks; rapid viral response (RVR); 48wks duration, 2) 8 wks; early viral response (EVR); 52wks duration, 3) 12 wks; EVR; 56 to 60 wks duration, 4) 16 to 24 wks; late viral response (LVR); 72 wks duration. Patients with positive serum HCV RNA at 24 wks finished treatment without prolongation of PEG/R. And we prospectively investigated SVR rates of these groups. Results: Numbers of the patients achieved RVR, EVR and LVR were 4 (7.6%), 28 (53.2%) and 6 (11.5%), respectively. Only two patients (3.8%) could not complete this treatment protocol. SVR rates of the RVR, EVR and LVR patients were 100% (4/4), 78.6% (22/28) and 66.7% (4/6), respectively. The SVR rate of these 3 groups was 78.9% (30/38). While 86.4% (19/22) of male and 85.7% (6/7) of female under age of 55 achieved SVR, only 44.4% (4/9) of female aged 55 or over achieved SVR. Conclusion: Our results suggest that the short-term prolonged treatment duration of PEG/R determined by the time when serum HCV RNA turned negative is a safe and effective treatment strategy for genotype 1b CHC patients. However, this treatment is insufficient for female age 55 or over.

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270 ELEVATED EXPRESSION AND POLYMORPHISMS OF SOCS3 INFLUENCE PATIENT RESPONSE TO ANTIVIRAL THERAPY IN CHRONIC HEPATITIS C

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Background: The response to antiviral therapy of chronic hepatitis C virus (HCV) infection is determined by virological, environmental and genetic factors. We have tested the hypothesis that the expression of specific genes and their haplotype frequencies can differentiate between non-responders (NRs) and sustained virological responders (SVRs) to antiviral treatment. Methods: We used a methodological approach based on molecular marker-discovery and validation to study the genes influencing the antiviral treatment in lymphoblastoid cell lines from 74 genotype 1b hepatitis C virus patients (44 from Southern Italy and 30 from Northern Italy) treated with pegylated interferon-α and ribavirin. Furthermore, we performed an association study, testing three single nucleotide polymorphisms of SOCS3 in 162 NR and 184 SVR subjects [SOCS3 -8464 A/C (rs12952093), -4874 A/G (rs4969170), and 1383 A/G (rs4969168)]. Findings: SOCS3 basal expression levels were significantly increased in two independent sets of NR groups (P<0.05). We found a highly significant association between NRs and both the positively associated haplotype (OR=2.01, 95% CI: 1.15-3.50, P=0.0002) and the negatively associated haplotype (OR=0.56, 95% CI: 0.42-0.76, P=0.0014). In particular, the SOCS3 -4874 AA genotype was strongly associated with failure of antiviral therapy (OR=4.00, 95% CI: 2.09-7.66, P=0.0003) and the AA genotype carriers had significantly higher SOCS3 mRNA and protein levels (P<0.05). Interpretation: Basal levels of SOCS3, an inhibitor of the interferon-α-induced Janus kinase-signal transducer and an activator of transcription pathways, and its genetic polymorphisms influence the outcome of antiviral treatment. SOCS3 thus represents a new blood biomarker for the a priori prediction of treatment response.

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The following people have nothing to disclose: Marcello Persico, Mario Capasso, Roberta Russo, Eliana Persico, Vincenzo La Mura, Claudio Tiribelli, Achille Iolascon

271 PREDICTORS OF RESPONSE TO PEGYLATED INTERFERON-α2A AND RIBAVIRIN IN A COHORT OF PATIENTS INFECTED WITH THE SAME STRAIN OF HCV: THE O’BRIEN PROJECT

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O’Brien is a small rural town of Argentina (2200 inhabitants) with a high prevalence of HCV infection (102/1832 (5.6%), 12.6% in >40 years, 23.4% in >60 years). All viremic patients (n=83) were infected with genotype 1b and showed 90.4-97.5% homology in NS5b nucleotide sequencing (Hepatology, 2002: 1660A). The presumed common source of infection, present in all patients, was the administration of unsafe injections a mean of 35 years before diagnosis. Goals: to analyze results of combined pegylated interferon-α2A (PEG) and ribavirin (RIB) therapy for 48 weeks and to identify predictors of sustained virological response (SVR). Methods: the study included 32 patients (50% males) aged 51±10 years. Viral load was investigated by Amplicor Monitor 2.0 and HCV RNA by RT-PCR. At baseline 21 patients (75%) had high VL (>80000 IU, mean 6.4±0.8 log IU) and only 9 (28%) elevated ALT. Liver biopsy showed stage 0 in 2, 1 in 5, II in 12, III in 6 and IV (cirrhosis) in 5 patients by METAVIR score. Patients received 180 μg/week of PEG and 1000-1200 mg/day of RIB according to body weight (> or <75 Kg). Results: Two patients discontinued therapy (weeks 2 and 8) due to adverse effects and the remaining 30 completed 48 weeks. Dose reductions of PEG were required in 5/30 (17%) patients and of RIB in 8/30 (27%). However, all fulfilled the 80%/80%/80% rule at all time points of treatment. Thirteen patients (43%) received G-CSF and 6 (20%) EPO. On an intention-to-treat basis, virological responses were: 91% (29/32) at week 12 (EVR), 91% at week 24, 91% at week 48 (ETR) and 59% (19/32) at week 72 (SVR). Predictors of SVR by univariate analysis were age (48±2 vs. 56±2 years, p=0.039), significant alcohol (>80 grams in males and >50 grams in females) consumption (10.5% vs. 45.5%, p=0.029), low fibrosis stages (p=0.008) and cirrhosis (0% vs. 45.5%, p=0.001). Female gender (63% vs. 27%, p=0.058), lower BMI (25±1.5 vs. 29±1, p=0.08) and low VL...
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TIME TO HCV RNA NEGATIVATION IN HEPATITIS C VIRUS (HCV) TYPE 1-INFECTION DURING PEG-INTERFERON-ALPHA-2B PLUS RIBAVIRIN THERAPY: DIFFERENCES IN RELATION TO THE ASSAY SENSITIVITY (INDIV-1 STUDY GROUP)

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The following people have nothing to disclose: Valeria I. Descalzi, Silvina E. Yantorno, Sonia M. Soria, Fernando M. Cairo, Nancy Massenzio, Jorge E. González, Maria S. Munne, Gaston Picchio, Federico G. Villamil

showing frequencies of around 3.5-6.7% per week. Conclusion: The most important discrepancies between the two test systems (bDNA vs TMA) with respect to frequency and rate of virologic response can be observed within the first 12 weeks of therapy. At the end of treatment, however, frequencies of undetectable HCV RNA levels did not differ greatly and reached around 70% independently whether bDNA or TMA was used. From all these data it emerges that there is a risk to overestimate virologic response rates when HCV RNA levels are only assessed by the quantitative assay.

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IMPACT OF ANTIVIRAL THERAPY AND RESPONSE TO TREATMENT ON LONG-TERM OUTCOME OF CHRONIC HEPATITIS C (CHC): A PROPENSITY SCORE ANALYSIS IN A POPULATION-BASED COHORT OF 1159 PATIENTS

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Background: The effect of antiviral therapy and sustained virologic response (SVR) on the long-term outcome of CHC has never been assessed through population-based cohort study reflecting usual medical practice using clinical endpoints. Concerns can be raised for clinical trials and observational studies which suggested the clinical benefit of antiviral therapy regarding representativeness of the studied population, follow-up duration, or selection biases associated with treatment allocation. The aim of this study was to assess treatment and SVR effects on the outcome of 1159 HCV mono-infected viremic patients recruited in a well-defined population of 1,005,817 inhabitants, using the propensity score technique to reduce bias in the comparison of non-randomized treatment groups. Methods: All HCV mono-infected viremic patients diagnosed in the study area between 1994 and 2001 were included and followed for 4.9±2.6 years. 409 patients (35.2%) received antiviral therapy ([peg]-interferon-α±ribavirin), of whom 142 (34.7%) achieved SVR. The effects of antiviral therapy and SVR on the risk of decompensated cirrhosis, hepatocellular carcinoma and death (liver-related or not) were estimated separately by time-dependent Cox regression analyses including a propensity score to adjust for observable differences between treated and untreated patients and considering all the demographic variables known to influence the natural history of CHC. Results: Treated and untreated patients were significantly different for age, gender, place of residence, route of infection, self-reported excessive alcohol consumption, and liver damage according to the Metavir score. Using Kaplan-Meier estimates, the 5-year rates of decompensated cirrhosis and of all-causes and liver-related deaths were 6.0%, 18.5% and 6.5% in untreated patients, 2.6%, 4.9% and 2% in treated patients who did not achieve SVR, and 0.7%, 0% and 0% in treated patients who achieved SVR, respectively (p=0.019 and p<0.0001 by log-rank tests). Propensity-adjusted multivariable Cox regression failed to demonstrate a better outcome in patients who received antiviral therapy, despite lower RR of cirrhosis decomposition (0.45) and hepatocellular (0.29) and the 0% rate of death in patients who achieved SVR. Interestingly, the risk of all-causes
DEATH: 0.48 for treated patients without SVR (p=0.06). Conclusion: This population-based study suggests a benefit of antiviral therapy on the long-term outcome of CHC. Using propensity-adjusted Cox regression analysis, the complete demonstration should be given with a larger studied population and/or a longer follow-up duration to increase the number of events.

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274 ARTIFICIAL INTELLIGENCE PLATFORM FOR CHRONIC HEPATITIS C (CHC): PREDICTION OF CLINICAL OUTCOME AND MORE EFFICIENT TREATMENT WITH PEGYLATED INTERFERON PLUS RIBAVIRIN

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INTRODUCTION: no bioinformatics platform dedicated to study and to predict the clinical outcome as well as therapeutic rentability and efficiency of CHC exist. The accuracy of the prediction of the prognosis in CHC is estimated at less than 50% among physicians. AIMS: To develop an easy bioinformatic platform based on algorithm decisions (Bayesian network) for a better and more efficient prediction of treatment response, to identify key points in the progression of liver disease and to establish new factors involved in prognosis and treatment response. PATIENTS AND METHODS: 385 consecutive CHC patients (mean age 45±9 years; 45% females) treated were included. More than 40 variables (epidemiological, biochemical, virological, histological, type of treatment, type of response, adverse events, withdrawals...etc) were analysed and included in a database. DLife platform has been used in this database for constructing the bioinformatic model of CHC based on bayesian networks and novel algorithms that are very effective in modeling and simulating situations where there are some unknown data and permits the knowledge automatization. The prediction accuracy of the bioinformatic network was compared to the true data collected in a clinical retrospective study. The model was then validated twice with external data from CHC patients treated in other hospitals.

RESULTS: The accuracy of this bioinformatic network for treatment response in this cohort of 385 patients is 83.3%, which is higher than accuracy obtained by physicians based on the study of clinical data and their own experience (50-65%). Some variables were detected as ‘key points’ regarding treatment outcome: age at treatment, gender, weight, comorbidity, type of treatment, genotype and basal viraemia, presence of autoantibodies, basal T4 level, basal aminotransferase levels, haematological variables, and viraemia during the treatment and follow-up. The ROC curve areas after validation with another cohort of patients were: 0.907 for SVR, 1 for non-response and 0.81 for relapse after treatment and follow-up. CONCLUSIONS: This bioinformatic platform is an easy comfortable and a very intuitive tool for applying to CHC patients by clinicians. It is efficient for the prediction of treatment response in CHC patients with a higher accuracy than clinicians.

Disclosures: The following people have nothing to disclose: Maria Trapero-Marugan, Manuel Marin, Oscar Nuñez, Xamila SAlcedo-Mora, Jose Manuel del Rio, Juan Pablo Pivel, Gerardo Clemente, Ricardo Moreno-Otero

275 INSULIN RESISTANCE (IR) DEFINED BY THE HOMEOSTASIS MODEL OF ASSESSMENT INSULIN RESISTANCE (HOMA-IR) INDEX HAS A DIRECT EFFECT ON EARLY VIRAL KINETICS DURING PEGYLATED-INTERFERON THERAPY FOR CHRONIC HEPATITIS C

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Background: Insulin resistance (IR), a central feature of the metabolic syndrome, has emerged as a key factor reducing the response to PEGylated-Interferon (PEG-IFN) based therapy in chronic hepatitis C. The pathogenic mechanisms underlying this association are still unclear. Aim: To examine the relationship of baseline serum insulin and of homeostasis model of assessment insulin resistance (HOMA-IR) index with the early virological response to PEG-IFN, taken as a measurement of the intracellular response to Interferon (IFN) signaling. Methods: In 30 patients treated with weight-based doses of PEG-IFN plus Ribavirin, baseline serum, insulin and HOMA-IR were measured the same day of the first IFN injection. HCV-RNA levels were measured by RealTime PCR (Abbott m2000, LoD 12 IU/mL) in all patients at baseline, as well as 24 hours and at week 1, 4, 12 after treatment initiation to define the individual kinetics of response. Results: Mean baseline insulin level was 13.38±7.21 mIU/L (range: 1.5-28) while, mean baseline HOMA-IR was 3.11±1.64 (range: 0.36-6.29). No statistically significant association was found between baseline insulin levels and baseline viremia or HCV-RNA decay at the different time-points during therapy. On the other hand, when patients were stratified by baseline HOMA-IR, those with high insulin resistance index showed a significant reduction in virus decay already at 24 hours and thereafter compared to cases with lower HOMA-IR (see Table). By Kaplan-Meier analysis, patients with HOMA-IR>4 had 0% rate of HCV-RNA negativity during the first 12 weeks of treatment while, the percentage of HCV-RNA negative cases among those with HOMA-IR<4 was 20% at week 1, 40% at week 4 and 50% at week 12. Baseline body weight and body mass index did not show a significant association with virus decay. Conclusions: We have demonstrated a direct effect of HOMA-IR on the early response during PEG-IFN based therapy for chronic hepatitis C, independently of insulin levels and BMI, suggesting that insulin resistance is the key factor in reducing the cellular response to IFN in hepatitis C infected patients.

<table>
<thead>
<tr>
<th>HOMA-IR</th>
<th>24 Hours</th>
<th>Week 1</th>
<th>Week 4</th>
<th>Week 12</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;3</td>
<td>1.11±0.52</td>
<td>0.44±0.64</td>
<td>1.63±1.84</td>
<td>3.29±2.46</td>
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<tr>
<td>p=0.04</td>
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<tr>
<td>&gt;3</td>
<td>0.55±0.71</td>
<td>0.14±0.43</td>
<td>0.71±0.70</td>
<td>2.34±2.36</td>
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<tr>
<td>p=0.05</td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>&lt;4</td>
<td>1.08±0.58</td>
<td>0.44±0.57</td>
<td>1.61±1.57</td>
<td>3.54±2.31</td>
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<td>p=0.02</td>
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<tr>
<td>&gt;4</td>
<td>-0.05±0.35</td>
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<td>0.50±0.20</td>
<td>0.74±0.36</td>
</tr>
<tr>
<td>p=0.006</td>
<td></td>
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</table>

Disclosures: The following people have nothing to disclose: Gladis Bortoletto, Stefano Realdon, Francesca Dal Pero, Martina Gerotto, Laura Scribano, Sara Bonisegna, Diego Marines, Alfredo Alberti
TREATMENT OF CHRONIC HEPATITIS C WITH PEGINTERFERON ALFA-2A (40KD) (PEG) AND RIBAVIRIN (RBV) IN NAIVE PATIENTS WITH HIV-HCV CO-INFEC-TION IN THE REAL-LIFE SETTING IN GERMANY

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Background: HCV co-infection and related concomitant diseases, especially of the liver, have become more important since antiretroviral therapy has reduced morbidity and mortality associated with HIV. Clinical trials have demonstrated that treatment with PEG and RBV can achieve high rates of SVR in co-infected patients, but less is known about what happens in real life. Methods: The Association of German independent Gastroenterologists (bng) in cooperation with Roche conducted an observational study. From March 2003 to May 2007 data from >20,000 patients were collected from >500 centers. Efficacy, safety and compliance data were recorded. An analysis for naive patients with HCV-mono (MONO) - or HCV/HIV coinfection (CO) who have been treated with PEG and RBV, was performed. Results: 737/15636 patients were HIV/HCV co-infected. Treatment rate for CO was 34.1% [251/737] and 44.7% [6661/14899] for MONO. Reasons reported for not treating HIV in CO patients were patient desire 35.3%, com- mitant disease 17.5%, current drug use 16.6%, non-compliance 6.4%, lacking activity of disease 5.2% and decompensated liver disease 4.1%. Demographic data of analysed patients are presented in the table. 82.1% [87/106] CO and 87.9% [2599/2957] MONO reached an Early Virological Response (EVR) at week 12 [≥2-log10 drop in HCV RNA or HCV RNA undetectable], with 52.8% [82/156] CO and 65.3% MONO [2548/3904] presenting an EoT response. SVR was achieved by 39.1% [61/156] CO and 53.8% [2104/3906] MONO patients. A total of 42.3% [66/156] CO and 27.2% [1062/3906] MONO patients dis- continued therapy: 37.9/39.5% due to non-response, 27.3/26.4% for poor tolerability, 13.6/16.4% lost to follow-up, 15.2/10.8% for personal reasons and 12.1/9.4% for lack of compliance. The mean duration of treatment for G1 was 34.3 weeks in CO and 38.3 weeks in MONO. In G2/3, CO/MONO patients were treated for 31.4/24.0 weeks, resp. Conclusion: Although HIV co-infection is a serious and hepatis-accelerating disease only a third of CO patients had been treated with PEG and RBV. In this population, combination ther- apy was found to be reasonable safe and sufficiently effective even under real-life conditions. A challenge will be to convince co-infected patients of the need for therapy, whilst minimizing the discontinuation rate and hence the extension of treatment duration.

Characteristics of Tx patients

<table>
<thead>
<tr>
<th>Age (mean yrs)</th>
<th>Male (%)</th>
<th>BMI (kg/m2)</th>
<th>Duration of HCV (mean yrs)</th>
<th>Genotype (%)</th>
<th>GI</th>
<th>G2/3</th>
</tr>
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<tbody>
<tr>
<td>Mean</td>
<td>59.0</td>
<td>72.3</td>
<td>8.0</td>
<td>42.2</td>
<td>66.9</td>
<td>25.0</td>
</tr>
<tr>
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<td>Co-infected (N=156)</td>
<td>Mono-infected (N=3907)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SVR (%)</td>
<td>58.3</td>
<td>38.6</td>
<td>6.1</td>
<td>59.0</td>
<td>34.6</td>
<td>6.4</td>
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</tbody>
</table>


DOES PROLONGED THERAPY IN HCV-GENOTYPE 3 PATIENTS WITH HIGH VIRAL LOAD IMPROVE SUS-TAINED VIRAL RESPONSE RATES?

Stefan Mauss1, Dietrich Hüppe2, Elmar Zehnter3, Michael P. Manns4, Gerlinde Teuber5, Tarek Dahhan6, Ulrike Meyer7, Bernd Möller1, Nektarios Dikopoulos8, Thomas Witthöft9, Jochen Brack10, Marek Stern11, Stephan Kaiser12, Renate Prinz13, ...

Objective: The sustained viral response (SVR) in HCV-genotype 3 patients differs substantially depending on baseline viral load and viral response to therapy. Whereas patients with rapid viral response and low viral load show SVR rates >80%, patients with high viral load and no rapid viral response may reach SVR <50% after 24 weeks of therapy. These patients may be candidates for longer treatment durations. Here we report SVR data from a cohort of 3547 patients including 1148 patients with HCV-genotype 3 treated for 24 vs. 48 weeks with peginterferon and ribavirin. Methods: All patients were treated with at least one dose of peginterferon alfa-2b and weight based ribavirin as part of a German multicentre cohort. Only patients who were beyond 24 weeks of follow up after planned end of therapy (24 or 48 weeks) were included in this analysis (n=571). Patients were stratified according to HCV-RNA below or above/equal to 600,000 IU/mL. Patients with missing data at end of follow up were counted as treatment failures. Statistical analysis was performed using chi-square test. Results:
157/207 (76%) of patients with genotype 3 and HCV-RNA <600,000 IU/mL reached SVR after 24 weeks of therapy versus 82/115 (72%) after 48 weeks of therapy (p=0.05, n.s.). 123/160 (77%) of patients with genotype 3 and HCV-RNA >600,000 IU/mL reached SVR after 24 weeks of therapy versus 66/89 (74%) after 48 weeks of therapy (p=0.05, n.s.). Conclusion: Extending therapy from 24 weeks to 48 weeks for HCV-genotype 3 did not improve SVR rates regardless of baseline viral load. These results confirm findings from the initial prospective, controlled study by Hadziyannis et al. Ann Intern Med. 2004; 140(5):346-55. In addition SVR was comparable for patients with low and high viral load. However it can not be excluded from this study that a more targeted approach based on viral kinetics, i.e. slow viral response, may be beneficial for a smaller subgroup of patients.

Disclosures:
The following people have nothing to disclose: Stefan Mauss, Dietrich Höppe, Elmar Zehnter, Michael P. Manns, Gerlinde Teuber, Tarek Dahlan, Ulrike Meyer, Bernd Möller, Nektarios Dikopoulos, Thomas Wirtholt, Jochen Brack, Marek Stern, Stephan Kaiser, Renate Prinzing.

The bng hepatitis study group

278 Silymarin down-regulates HCV Core and up-regulates Heme Oxygenase-1 Gene Expression in the CNS3 Replicon Line of Human Liver Cells

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Background: Hepatitis C virus (HCV) infection is a global medical problem. The current standard of care for chronic hepatitis C (CHC) is pegylated interferon plus ribavirin therapy, but this treatment is expensive, has significant side effects and, at best, is only 50% effective. Silymarin, the main active ingredient of milk thistle, is a natural antioxidant that is used by patients with CHC [Seeff et al, Gastroent, 2007], although its efficacy for decreasing HCV levels or ameliorating CHC remains uncertain. HCV infection is associated with increased hepatic oxidative stress, and one of the antioxidant enzymes which protects cells against this stress is heme oxygenase-1 (HO-1). Methods: We investigated the effects of silymarin on HCV and HO-1 gene expression in wild type Huh-7 cells, as well as two HCV replicon cell lines, CNS3 and 9-13 cells. Results: Silymarin (100 and 200µM) down-regulated HCV core mRNA (by 20% - 36%) and protein (by 30%-60%) in CNS3 cells. In contrast, silymarin did not decrease HCV N55A mRNA or protein expression in treated 9-13 cells; in fact, it increased N55A mRNA levels by two fold. HO-1 mRNA was up-regulated (60%-400%) by silymarin in Huh-7, CNS3 and 9-13 cells. To explore the mechanism by which silymarin up-regulates HO-1 mRNA, we measured the levels of Bach1 and Nrf2 transcription factors. Bach1 and Nrf2 mRNA levels were not affected by silymarin treatment in Huh-7 cells. In CNS3 and 9-13 cells, there was no clear relationship between silymarin-induced changes in Bach1 and Nrf2 and the induction of HO-1 mRNA. Conclusions: Silymarin significantly down-regulates HCV core mRNA and protein in CNS3 cells. The levels of the antioxidant enzyme HO-1 are up-regulated by silymarin, but the precise mechanism by which silymarin up-regulates HO-1 mRNA levels in these cell lines remains unknown. These and other recent results [Polyak et al Gastroent, 2007] suggest that silymarin is of benefit in CHC, although prospective, randomized clinical trials are needed to be certain.

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The following people have nothing to disclose: Vania Bonifaz, Ying Shan, Richard Lambrecht, Susan E. Donohue, Darcy Moschenross, Herbert Bonkovsky

279 Treatment of Hemodialysis (HD) Patients with Chronic Hepatitis C (CHC) Using an Escalating Dose Regimen of Pegylated Interferon (PEG-IFN) Alfa-2b

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Background: CHC is a prevalent condition among patients receiving HD, and post-renal transplant CHC patients have poorer treatment-related outcomes. Additionally, treatment with IFN therapy has been associated with graft rejection. In this study, we evaluated the efficacy and safety of PEG-IFN alfa-2b in CHC patients receiving HD. Methods: This study included treatment-naive CHC patients aged 18-70 years with compensated liver cirrhosis and adequate hematologic parameters. Patients with coinfection, significant cardiovascular dysfunction, uncontrolled diabetes, and any contraindications to IFN therapy were excluded. Of the 46 CHC patients undergoing HD who were screened, 34 enrolled in the study. All patients received PEG-IFN alfa-2b 0.5µg/kg/wk; doses were increased by 0.25µg/kg/wk every 4 wks if well tolerated. Maximum dose was defined as 1µg/kg/wk at 48 wks in patients with genotype (G) 1 and 24 wks in those with G2/3. Patients unable to tolerate study medication were discontinued from the trial. Results: Baseline patient characteristics included mean age of 41.4±11.9 years [61.8% ≥40 years]; males, 44.1%; G1, 70.6%; G3, 29.4%; mean body mass index, 22.6±4.4 kg/m²; median HD duration, 5.8 years [range, 3.8-8.2 years]; normal alanine aminotransferase (ALT) levels, 55.9%; HCV RNA ≥700,000 IU/mL, 50%; and fibrosis ≥2 (modified Histology Activity Index score), 26.5%. In total, 97% (33/34) of patients reached maximum dose; however, 21% were unable to maintain that dose. Early virologic response (EVR; undetectable HCV RNA [<50 IU/mL] at wk 12) was attained in 1.5 of 20 G1 patients; end-of-treatment (EOT) and sustained virologic responses (SVRs) were attained in 10 of 15 and 9 of 18 G1 patients, respectively. Conversely, 8 of 10 G3 patients attained EOT and SVR; and EVR and SVRs were attained in 8/9 and 8/10 G3 patients, respectively. Almost 30% [11/34] of patients discontinued treatment early because of adverse events (AE). The most commonly reported AE was anemia; 64.7% of patients required blood transfusions. Low-dose epoetin was administered to 85.3% of patients during the trial. One patient died during the study of acute coronary syndrome after the 41st dose. Conclusions: In total, SVRs were attained in 37.5% (9/24) of G1 patients undergoing HD who were treated for 48 wks with an escalating dose regimen of PEG-IFN alfa-2b monotherapy. In contrast, 80% (8/10) of G3 patients, who received only 24 wks of this therapy, attained SVR. These results indicate that G3 patients undergoing HD can be successfully treated with short-term PEG-IFN alfa-2b monotherapy. Because PEG-IFN alfa-2b can be associated with AEs, supportive therapy may be necessary.

Disclosures:
Thomas Korompis - Employee: Schering-Plough
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IFN SENSITIVITY DETERMINING REGION IN NS5A OF HEPATITIS C VIRUS CORRELATES WITH THE RESPONSE TO PEG-IFN ALFA-2B PLUS RIBAVIRIN TREATMENT
Michihito Murao, Kyoko Kobayashi, Narumi Komura, Hiroaki Shimazaki, Takuji Nakano, Yoshifumi Nitta, Masao Harata, Naoto Kawabe, Senju Hashimoto, Kentaro Yoshioka; Division of Liver, Biliary Tract and Pancreas Diseases, Fujita Health University, Toyoake, Japan

Background: Many studies have linked the response to IFN mono-therapy to IFN sensitivity determining region (ISDR) in NS5A, while this remains controversial. PEG-IFN alfa-2b plus ribavirin (RBV) has improved sustained virological response (SVR) rate from 5% of IFN mono-therapy to 60%. Few reports have studied whether ISDR is also an important predictor for the response in PEG-IFN plus RBV treatment as well as in IFN mono-therapy. Aim: To evaluate the correlation of ISDR with the response to PEG-IFN plus RBV treatment. Methods: The sequence of ISDR of 65 patients treated with PEG-IFN plus RBV was determined by PCR and direct sequencing. Results: Eleven patients had 4 or more mutations in ISDR, 25 had more than one to less than 4 mutations, and 29 had one or less mutations. Nine patients became negative for HCV RNA by week 4, and obtained rapid virological response (RVR). RVR rate was significantly higher in the patients with 4 or more mutations (55%) than in the other patients (6%)(p=0.0004). Multivariate analysis revealed that ISDR was the only independent factor predicting RVR (OR=0.04, 95%CI=0.003-0.485, p=0.012). Thirty two patients became negative for HCV RNA by week 12, and obtained early virological response (EVR). EVR rate was significantly higher in the patients with 4 or more mutations (91%) than in the other patients (41%)(p=0.0024). Multivariate analysis revealed that male gender was an only independent factor predicting EVR (OR=0.31, 95%CI=0.11-0.923, p=0.035). Among 36 patients in whom 6 months had passed after the end of treatment, 20 obtained SVR. Of 20 patients with SVR, 13 had more than one mutations. SVR rate was significantly higher in the patients with more than one mutations (72%) than in the other patients (39%)(p=0.0442). Multivariate analysis revealed that ISDR was an only independent factor predicting SVR (OR=3.81, 95%CI=0.84-17.26, p=0.082). Conclusions: The number of mutations in ISDR was demonstrated to be an important predictor for EVR, EVR and SVR, especially for RVR and SVR in PEG-IFN plus RBV treatment as well as in IFN mono-therapy.

Disclosures: The following people have nothing to disclose: Michihito Murao, Kyoko Kobayashi, Narumi Komura, Hiroaki Shimazaki, Takuji Nakano, Yoshifumi Nitta, Masao Harata, Naoto Kawabe, Senju Hashimoto, Kentaro Yoshioka

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REAL-LIFE RATES OF TREATMENT COMPLETION FOR HCV
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Background: Rates and factors predicting treatment completion for HCV infection in real-life settings are unknown. Methods: We assembled a national cohort of HCV-infected veterans from 1998-2003, using the VA National Patient Care Database for demographic and clinical information, Pharmacy Benefits Management database for pharmacy records and the Decision Support Systems database for laboratory data. We studied the rates and factors predicting treatment completion for HCV. Results: For the 134,934 veterans with at least 1 inpatient or 2 outpatient codes, 16,043(11.9%) were prescribed treatment. Among the 10,641 veterans with > 1 year of follow-up, 22.5% completed a 48 week course of treatment. Non-completers were more likely to be black, have pre-treatment anemia, corona artery disease, depression and more aggregate comorbidities. In multivariable analyses, non-completion was associated with baseline anemia (OR 0.66, 95%CI 0.56-0.78 for hemoglobin 10-14mg/dl) and depression (OR 0.78, 95%CI 0.69-0.89). Pegylated interferon was associated with higher rates of treatment completion. HIV-coinfection did not affect completion rates. Conclusions: A minority of HCV-infected persons were prescribed treatment and less than one-quarter complete a 48-week course. Anemia and depression are potentially modifiable factors that must be addressed at a population level before universal use of pharmacotherapy is advocated.

Baseline characteristics of HCV infected persons who completed a 48 course of treatment for HCV, and a flow sheet of analysis for the current study

<table>
<thead>
<tr>
<th>Variable</th>
<th>Completed %</th>
<th>Non-completed %</th>
<th>Bonferroni</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age + Age (SD)</td>
<td>64.4±9.2B</td>
<td>68.5±6.0</td>
<td><em>p&lt;0.001</em></td>
</tr>
<tr>
<td>Male Sex</td>
<td>59.5</td>
<td>56.1±6.4</td>
<td><em>p&lt;0.05</em></td>
</tr>
<tr>
<td>BMI (kg/m2)</td>
<td>30.5±7.3</td>
<td>30.8±7.3</td>
<td><em>p&lt;0.05</em></td>
</tr>
<tr>
<td>Diabetes</td>
<td>19.3</td>
<td>19.2±6.2</td>
<td><em>p&lt;0.05</em></td>
</tr>
<tr>
<td>Hypertension</td>
<td>47.1</td>
<td>47.2±5.6</td>
<td><em>p&lt;0.05</em></td>
</tr>
<tr>
<td>Anemia</td>
<td>33.9</td>
<td>32.3±9.2</td>
<td><em>p&lt;0.05</em></td>
</tr>
<tr>
<td>Depression</td>
<td>21.8</td>
<td>20.3±9.2</td>
<td><em>p&lt;0.05</em></td>
</tr>
<tr>
<td>Type of antiviral therapy</td>
<td>38.8</td>
<td>37.9±0.00</td>
<td><em>p&lt;0.05</em></td>
</tr>
<tr>
<td>M: completed</td>
<td>46.2</td>
<td>45.1±0.00</td>
<td><em>p&lt;0.05</em></td>
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<tr>
<td>F: completed</td>
<td>49.2±2.2</td>
<td>50.3±2.2</td>
<td><em>p&lt;0.05</em></td>
</tr>
<tr>
<td>M: non-completed</td>
<td>38.8</td>
<td>37.9±0.00</td>
<td><em>p&lt;0.05</em></td>
</tr>
<tr>
<td>F: non-completed</td>
<td>21.8±2.2</td>
<td>20.3±9.2</td>
<td><em>p&lt;0.05</em></td>
</tr>
</tbody>
</table>

Disclosures: The following people have nothing to disclose: Adeel A. Butt, Melissa Skanderson, Kathleen A. McGinnis, C. K. Kwoh, Amy C. Justice

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CLINICAL RELEVANCE OF RAPID VIROLOGICAL RESPONSE (RVR) IN DECOMPENSATED HCV-RELATED CIRRHOSIS TREATED WITH PEG-INTERFERON AND RIBAVIRIN
Angelo Iacobelli1, Brigidia E. Annicchiarico2, Massimo Siciliano2, Grazia Niro1, Laura Accademia1, Nazario Caruso2, Giuseppe Bambardieri2, Angelo Andriulli1, 1Gastroenterology, Hospital Casa Sollievo della Sofferenza, San Giovanni Rotondo, Italy; 2Institute of Pathology and Semeiotica Medica, Catholic University, Rome, Italy

Background: Recent studies provided positive data on the impact of HCV clearance by antiviral therapy in improving hepatic function in decompensated cirrhosis. However, low tolerability of therapy and the risk for severe infections render useful to single out those patients with a higher likelihood of achieving a sustained virological response (SVR). Aim: to determine whether in decompensated cirrhosis naïve to previous combined antiviral therapy RVR could predict SVR. Methods: 104 cirrhotics underwent treatment with peginterferon alfa-2b (1.5 mcg/kg/wk) and ribavirin (800 or 1000 mg) for 24 weeks. Patients: mean age was 62 +/- 7 yrs; 63% of patients

Abu Hassan, Asmarani Abdullah, Boon Phoe Ooi, Sanjay Rampal, Mohd Ismail Merican
were infected by geno 1/4; and 96% were staged in Child-Pugh class B. Results: SVR was achieved in 29 patients (28%), overall; 10 of them (15%) with genotype 1 or 4, and 19 (48.7%) with genotype 2 and 3 (P < 0.01). On-treatment viral clearances, according to HCV genotypes, are given in the table. At treatment week 4, 36 patients cleared HCV (RVR), and 23 of them achieved SVR (63.8%) at a significant difference between genotypes: 9 of 18 genotype 1 and 4 patients (50%), and 14 of 18 genotypes 2 and 3 (78%; P < 0.01). All RVR patients who achieved SVR had a pre-treatment viral load < 350,000 copies/mL. Conclusion: Decompensated patients with HCV-related liver cirrhosis achieve on-treatment viral clearance at different time. Achieving the RVR status may guide tailoring length of treatment. Two-thirds of patients infected by genotypes 2 and 3 may achieve SVR with a treatment length as short as 24 weeks, provided they clear HCV at treatment week 4. In non-RVR patients with the latter genotypes and in those infected by genotypes 1 and 4, the adopted duration of therapy appears insufficient to attain optimal SVR rates. The present study further supports the feasibility of antiviral treatment with Peg-interferon alfa-2b and ribavirin in Child-Pugh class B cirrhotics.

<table>
<thead>
<tr>
<th>Geno 1 e 4</th>
<th>Geno 2 e 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>RVR (4 weeks)</td>
<td>RVR (4 weeks)</td>
</tr>
<tr>
<td>EOT (24 weeks)</td>
<td>EOT (24 weeks)</td>
</tr>
<tr>
<td>SVR</td>
<td>SVR</td>
</tr>
<tr>
<td>1 pt</td>
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<td>1 pt</td>
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Disclosures:
The following people have nothing to disclose: Angelo Iacobellis, Brigida E. Annunciation Morris, Massimo Siciliano, Grazia Niro, Laura Accadia, Nazario Caruso, Giuseppe Bombardieri, Angelo Andriulli

283 ANALYSIS OF GENE EXPRESSION DURING THE FIRST 10 WEEKS FOLLOWING PEG-INFERNOR-ALFA2B/RIBAVIRIN TREATMENT OF A GROUP OF HEPATITIS C PATIENTS

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Our AIM was to analyze interferon gene expression following peginteron and ribavirin therapy in genotype I patients. METH-OIDS: RNA was isolated from PBMC from 20 treatment naive hepatitis C patients treated with Peg–intron /ribavirin, at time points 3, 6, 10, 13, 27, 42 and 70 days after initiation of treatment. All patients were treated with PEG interferon alfa-2b 1.5 mcg/kg QW for 10 weeks. Ribavirin was taken twice daily calculated at 13+ 2mg/kg/day. Affymetrix DNA microarrays (U133) were used to analyze gene expression of over 22,000 genes at each time point. Microarray data was analyzed with MAS 5.0, and arrays for each patient were scaled to a target intensity of 1000. Subsequent to normalization, a list of up and down regulated genes (p<0.001 and >1.5 fold) at each time point compared to baseline (Day 0) was generated.

Changes in gene expression for each patient were assessed using EDGE (http://www.biolstat.washington.edu/software/storey/edge/about.php) and was used to identify significance in genes expression in a temporal fashion. RESULTS: Approximately 300 genes were either up or down regulated on day 3 and day 10, with the number dropping to half at day 6 and day 13, prior to the next administration of interferon/ribavirin. Thus the interferon effect on gene expression attenuated with time. However a large number of genes continued to be differentially regulated up to week 10. The chemokine gene CXCL10 was induced early but not at later times. A group of genes previously identified as playing an important role in anti-viral response including OAS2, MX1, IFI44, IFIH1 were up-regulated throughout this period. Other genes such as Carbonic anhydrase-1 (CA1) and GYPA were upregulated only from days 42 and 70. Functional analysis of differentially expressed genes using Ingenuity Pathways software (Ingenuity Systems v5.0) showed that the most highly significant [-log(p-value) >11] networks and pathways modified by therapy involved genes classified as important in immune response. The intensity of the significance of gene network association with immune response attenuated from Day 3 through Day 70. Interestingly some of the genes of the JAK-STAT pathway were no longer upregulated at late times. Genes down regulated were predominantly related to ribosomal proteins and eukaryotic translation factors. By week 10, 50% of the patients demonstrated a positive response to therapy by having no detectable virus in plasma samples. Viral titers negatively correlated with the number of genes modified at specific time points. This research was approved by the Indiana University IRB, and supported by a grant from Integrated Genetics.

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Paul Kwo - Grant/Research Support: Schering-Plough
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284 IRON DEPLETION AND RESPONSE TO INTERFERON IN CHRONIC HEPATITIS C (HCV) : A META-ANALYSIS

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Background and Aim: Hepatic iron content is known to influence the response to interferon (IFN) in chronic Hepatitis C (HCV) patients. Several studies have reported effect of Iron depletion on response to IFN in HCV. These studies are heterogeneous in design and outcome measures. We performed a meta-analysis of available studies (1995 through May 2007). Methods .Electronic database Medline, CINHAL and Science Citation index were searched. RCTS comparing Interferon treatment (IFN) with Interferon and Phlebotomy (IFN and Phlebotomy) were chosen for the meta-analysis. Sustained Virologic Response (SVR) was the primary outcome. Sustained biochemical response (biochemical SR) and end of treatment Virologic response (ETR) were also analyzed. Data was pooled using Fixed effects (Peto odds Ratio) and Random effects (DerSimonian-Laird) method. Results: A total of 231 studies were identified among which 52 studies were selected and reviewed. The studies with known causes of iron overload (Hemochromatosis, alcohol abuse, Porphyria cutanea tarda or transfusion related overload) were excluded. There were fifteen published studies evaluating effect of IFN with phlebotomy as an adjuvant therapy. The data was extracted from 5 studies [N=334] which met the inclusion criteria (RCTS). IFN with phlebotomy compared with IFN alone increased the probability of achieving SVR (Peto OR= 2.43 95 % CI 1.41-4.16)Virologic
Efficacy of Peginterferon Alfa-2a and Ribavirin in 2101 Patients with HCV Infection in Real-Life Clinical Practice: Results of the French Hepatitis Study

Marc Bourliere, Denis Ouzan, Michel Rosenheim, Michel Doffoel, Patrick Marcellin, Jean-Michel Pawlotsky, Laurent Salomon, Francis Fagnani, Cécile Hayem, Isabelle Lonjon-Domanec, Muriel Vray, Hépato-gastroentérologie, Hôpital Saint Joseph, Marseille, France; 2Arnault Tzanck Institute, Saint Laurent du Var, France; 3Hôpital Pitié Salpêtrière, Paris, France; 4CHU, Strasbourg, France; 5Hôpital Beaujon, Clichy, France; 6Hôpital Henri Mondor, Creteil, France; 7 Louis Mourier, Colombes, France; 8Cemka-Eval, Bourg la Reine, France; 9Roche, Neuilly, France; 10Institut Pasteur, Paris, France.

Objectives: Previous studies conducted in Europe and in the US have shown similar efficacy of peginterferon alfa-2a plus ribavirin in real-world setting compared to randomized controlled trials. A nationwide observational study was also conducted in France to determine the characteristics of hepatitis C patients under treatment, histology assessment and rates of sustained virological response (SVR).

Methods: Between November 2003 and December 2004, 324 physicians recruited chronic hepatitis C patients treated with peginterferon alfa-2a (40KD). Patient demography and liver histology assessment were recorded at baseline and treatment and compliance were recorded every 3 months. Efficacy outcome was evaluated with qualitative PCR more than 3 months after the end of treatment.

Results: Among the 2101 patients analyzed, 62% were male, the mean age was 47±12 years, the mean body weight was 71±14 kg and 17% had cirrhosis. Distribution of genotypes: GT1 53%, GT2 12%, GT3 25%, GT4 8%, GT5 2%. The majority of the patients in the cohort were HCV treatment naive (70%). Liver biopsy and fibrosis markers were performed in 69% and 35% of the patients respectively but the relative proportions changed during the conduct of the study in favour of evaluating fibrosis markers. GT1 patients were biopsied more often than GT2 and 3 patients (77% and 56% respectively). The overall SVR rate was 57% (783/1377) in all patients and 63% (596/949) in naive patients. Among naive patients, SVR rate was 52% in GT1 (227/438), 80% in GT2 (115/144), 74% in GT3 (194/262), 55% in GT4 (42/76) and 59% in GT5 (10/17). Age below 40 years (p<0.001; odds ratio [OR] [95% confidence interval [CI]] = 2.4 [1.7-3.2], genotype 2 and 3 (p<0.001; OR [95% CI] = 2.7 [2.0-3.5], fibrosis less than F2 (p<0.001; OR [95% CI] = 1.7 [1.3-2.2], and naïve status (p<0.001; OR [95% CI] = 3.2 [2.2-4.6], were found to be independently associated with a higher response rate in multivariate analysis. Conclusions: This large study confirmed the efficacy of peginterferon alfa-2a plus ribavirin in the French clinical practice setting compared to randomized controlled clinical trials*. The increasing role of fibrosis markers rather than liver biopsy in the management of hepatitis C patient in France is confirmed. *Fried NEJM 2002; Hadziyannis Annals 2004.
significantly in the sustained virologic responders (16.2 to 12.6 kPa, p=0.019), but not in the nonsustained virologic responders, nor in the untreated controls. CONCLUSION: Liver stiffness measured by the Fibroscan is significantly, but modestly reduced on average at the end of follow-up in patients with chronic hepatitis C who achieve a sustained virological response to peginterferon alpha and ribavirin therapy. Longer follow-up will be necessary to assess whether the improvement subsequently continues.

<table>
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<tr>
<th>Date</th>
<th>Virologic response (n=69)</th>
<th>Non-response (n=11)</th>
<th>Control group (n=10)</th>
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<tr>
<td>Day 0 (mean±SEM)</td>
<td>14.0 (1.5)</td>
<td>21.8 (5.6)</td>
<td>18.7 (3.8)</td>
</tr>
<tr>
<td>Week 4 (mean±SEM)</td>
<td>12.8 (1.2)</td>
<td>18.0 (3.7)</td>
<td>19.5 (3.8)</td>
</tr>
<tr>
<td>Week 12 (mean±SEM)</td>
<td>12.3 (1.2)</td>
<td>20.1 (5.7)</td>
<td>18.6 (3.4)</td>
</tr>
<tr>
<td>Week 24 (mean±SEM)</td>
<td>12.8 (1.4)</td>
<td>17.8 (4.0)</td>
<td>18.5 (3.3)</td>
</tr>
</tbody>
</table>

Disclosures: The following people have nothing to disclose: Christophe Hezode, Laurent Castéra, Isabelle Rosa, Dominique Roulot, Vincent Leroy, Magali Bouvier-alias, Françoise Roudot-Thoraval, Catherine Douvin, Ariane Mallat, Jean-Michel Pawlotsky

**287 INHIBITION OF REPLICATION AND EXPRESSION OF HCV NSSA PROTEIN BY SMALL INTERFERING RNA**

Xiufen Su, Yanfang Jiang, Feng Wang, Junqi Niu; Department of Infectious Disease, First Hospital, Jilin University, Changchun, China

Objective: We design small interfering RNA (siRNA) targeted HCV NSSA in three different sites, in order to detect the inhibitory effect of siRNA on expression of hepatitis C virus (HCV) NSSA protein. Methods: HCV NSSA gene was amplified by polymerase chain reaction (PCR) method from pCDNA 3.1 (+) HCV NS3 45 plasmid, and cloned into pGEM-T vector through TA cloning technology, then inserted into the eukaryotic expression vector pcI-neo, construct HCV NSSA-pcI-neo eukaryotic expression vector. Three different loci pSilencerTM3.1-H1neoRNAi vectors were constructed. Conclusion: HCV NSSA-pcI-neo expression plasmids were constructed and cotransfected the vector with pcI-neo/HCVNSSA into HL-7702 cells by LipofectamineTM 2000. Inhibitory effects of three siRNA on expression of HCV NSSA protein were measured by western blots. Results: Western blots showed that expression of HCV NSSA protein in HL-7702 cells transfected by pcI-neo/HCV NSSA vector was very strong and that there had obviously inhibitory effects on expression of HCV NSSA protein in HL-7702 cells followed by cotransfecting this vector with three pSilencerTM3.1-H1neoRNAi vectors. Conclusion: The first phase viral decline in genotype 5 patients was significantly (P<0.03) more pronounced (mean 2.0 log IU/ml) than that of genotype 1 (1.2 log), and similar to that observed for genotypes 2-3 (2.3 log). Viral decline pattern in all genotype 5 patients was bi-phasic, like for genotype 2-3 patients, and did not show a transient rebound in HCV-RNA towards the end of the week before the next Peg-IFN injection, as seen in some genotype 1 patients treated with Peg-IFNα2a. The second phase decline slope was significantly (P<0.01) faster for genotype 5 (mean 1.6 log/week) than genotype 1 (0.7 log/week) and similar to that of genotype 2-3 patients (1.5 log/week). RVR (<50 IU/ml at week 4) was observed in 75% of genotype 5 patients, significantly (P<0.003) more than genotype 1 (14%) and similar to genotypes 2-3 (86%). Conclusions: HCV genotype 5 early viral kinetics are significantly more rapid than HCV genotype 1 kinetics and similar to those seen for HCV genotypes 2-3. These results may warrant clinical trials to test shorter treatment for HCV genotype 5 patients.

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**289 HEPATITIS C VIRUS GENOTYPE 5: EPIDEMIOLOGICAL DATA AND RESPONSE TO TREATMENT**

Nabil Antaki1, Adnanne Hermes2, Milad Hadad2, Muad Tayeh3, Fadi Antaki4, Naaman Abdo1, Kamel Kebbewar1; 1Gastroenterology and Hepatology, St Louis Hospital, Aleppo, Syria; 2Gastroenterology, Aleksro Military Hospital, Aleppo, Syria; 3Gastroenterology, Ibn Nathif Hospital, Damascus, Syria; 4Gastroenterology, Erasme Hospital, Brussels, Belgium

Background: In Syria and the Middle-East, the most common Hepatitis C Virus (HCV) genotypes are 4 then 1. While no cases of genotype 5 have been reported from neighboring countries, we have recently reported that 10% of HCV cases in Syria are of genotype 5. The response to treatment in genotype 5 has only been described in two small and one larger case series. The presence and treatment response of HCV genotype 5 in the Middle-East have never been described yet. Aims and Methods: We present the epidemiological data and treatment outcome for all HCV genotype 5 patients evaluated at 3 medical centers in Syria between 2004 and 2006. The medical
records were reviewed retrospectively. Genotyping was performed by INNO-LIPA (Bayer Diagnostics). Treatment consisted of Ribavirin 1000-1200 mg daily plus Interferon α2a 3 MU x 3/week or Peg-interferon α2a 180µg/week. The treatment response results for the treatment-naïve patients who have completed the treatment course and the 6-month follow-up period are presented. Results: During the study period, 81 patients were diagnosed with HCV genotype 5. Mean age was 53 ± 12 years. Female to male ratio was 2:1. 91% of cases originated from one district in the northern part of Syria of which 40% are from a small city of 30,000 inhabitants. The mode of transmission (transfusion, tattooing, and IV drug) was determined in 20% only. One family (mother, 2 sons and 2 daughters) is reported confirming that intrafamilial transmission may exist. So far, 26 patients have completed a course of anti-HCV therapy. In these patients, an early viral response was achieved in 88%, an end of treatment response in 88% and a sustained viral response (SVR) in 54%. SVR was higher with Peg (66.6%) v/s IFN (47%), in patients with low fibrosis (75%) v/s advanced fibrosis (37.5%) and in patients with low viremia (69%) v/s high viremia (38%). SVR was similar in patients treated for 24 or 48 weeks. Discussion: There is no obvious cause for the high prevalence rate of HCV genotype 5 in northern Syria. Compared to genotype 4, the SVR of genotype 5 appears to be better for patients treated with IFN (47%/v/s 28%) and comparable with Peg-IFN (66%/v/s 61%). The only three published studies on the treatment outcome of HCV genotype 5 reported SVR rates of 67%, 48% and 60% in series of 12, 21 and 87 patients. Our study confirms these results. Conclusion: HCV genotype 5 does exist in the Middle East. Similar to genotype 4, the response to treatment is, intermediate between genotypes 2/3 and 1. More epidemiological and clinical studies are needed to determine the reason for the high prevalence rate in a small area and the optimal duration of therapy.

Disclosures:
The following people have nothing to disclose: Nabil Antaki, Adnane Hermes, Milad Hadad, Muad Frayeh, Fadi Antaki, Naaman Abdo, Kamel Kebbwar

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PREDICTORS OF SUSTAINED VIROLOGICAL RESPONSE TO INTERFERON-BASED TREATMENT IN HEMODIALYSIS PATIENTS WITH CHRONIC HEPATITIS C VIRUS INFECTION - A PATIENT LEVEL META-ANALYSIS

Craig E. Gordon1, Katrin Uhlig1, Joseph Lau2, Christopher H. Schmidt2, Andrew S. Levey1,2, John B. Wong2; 1Nephrology, Tufts-New England Medical Center, Boston, MA; 2Clinical Care Research, Tufts-New England Medical Center, Boston, MA

Background Hepatitis C virus (HCV) infection has a prevalence of 13% in hemodialysis (HD) patients and increases mortality. Interferon (IFN) and pegylated-interferon (PEG-IFN) may eradicate HCV infection. We studied predictors of sustained virological response rate (SVR) to IFN in HD patients with HCV. Methods After performing a systematic review of IFN and PEG-IFN treatment in HCV-infected HD patients published between 1966 and December 2006, we extracted individual patient data (IPD) from included articles and acquired additional data by contacting study authors. We used univariate and multivariate logistic regression to identify predictors of SVR. Results Twenty studies of IFN monotherapy with 461 patients met inclusion criteria and provided IPD for 428 patients. Three studies of PEG-IFN involved 38 patients but did not supply IPD. The overall SVR was 41% (95% CI, 33-49%) with IFN and 37% (977%) with pegylated-IFN (PEG-IFN). Factors associated with statistically significantly higher SVR with IFN using IPD included: 3 MU or higher dose three times weekly (OR 3.3, 1.2-9.1), intended treatment duration of at least six months (2.0, 1.1-3.9), treatment completion (4.1, 2.4-6.8), lower HCV RNA at baseline (3.6, 1.9-6.7 per log10 lower RNA), and female gender (2.1, 1.3-3.4). Early virological response (EVR), measured as HCV RNA negativity 1-3 months into treatment, was also associated with significantly higher SVR (5.1, 2.6-10.0). HCV genotype 1 and cirrhosis were not associated with SVR. Despite limitations involving missing and clustered data, multivariate analysis documented independent associations of SVR with dose, duration, treatment completion, HCV RNA, and female gender. Conclusions HCV treatment in HD patients resulted in an overall SVR of 41% with IFN and 37% with PEG-IFN. SVR is higher in women, with low baseline HCV RNA, or with early virological response, and with IFN antiviral therapy at 3 MU for at least 6 months and completion of intended treatment duration.

Disclosures:
The following people have nothing to disclose: Craig E. Gordon, Katrin Uhlig, Joseph Lau, Christopher H. Schmidt, Andrew S. Levey, John B. Wong

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BEZAFIBRATE TREATMENT FOR CHRONIC HEPATITIS C AFTER FAILURE OF PREVIOUS COMBINATION THERAPY WITH INTERFERON AND RIBAVIRIN

Viola Weich, Beate Schlosser, Juliane Halangk, Alexandra Bergk, Florian van Bömmel, Thomas Berg; Charite, Campus Virchow Klinikum, Berlin, Germany

Lipoproteins have been reported to be involved in the infection cycle of hepatitis C virus. Former studies from Japan could demonstrate a significant reduction of liver enzymes and viral load after treatment with the lipid lowering agent bezafibrate. Bezafibrate exerts multiple effects on lipid metabolism by activating the peroxisome proliferator-activated receptor-alpha (PPAR-α) which modulates the expression of key genes of lipid transport, hepatic fatty acid and lipoprotein metabolism as well as inflammation. The aim of the present observational study was to assess the efficacy and safety of bezafibrate monotherapy in patients with advanced chronic hepatitis C who failed previous combination therapy with peg-interferon alpha and ribavirin. Patients and Methods: 34 patients from our university hospital [mean age 61 years, HCV type 1 [n=33], HCV type 4 [n=1], compensated cirrhosis [n=16], advanced fibrosis [n=18]] received daily oral bezafibrate treatment (400 mg per day) on the basis of a prospective observational open-label study design. Clinical, biochemical and virological data were evaluated during a mean treatment duration of 12 months (range 2-49 months). Results: Three patients dropped out of the study complaining about vertigo or palpitations within the first days of treatment. In the remaining 31 patients no significant adverse events were observed. During the treatment course, a significant improvement in gamma-glutamyl transpeptidase levels (GGT) as well as alanine aminotransferase levels (ALT) could be demonstrated in all patients. In 3 patients liver enzymes normalized completely. The mean GGT levels decreased from 171±119 to 107±100 IU/l (p<0.001), and the mean ALT levels from 108±70 to 80±47 IU/l (p<0.025).The mean AST levels at baseline and at the end of observation were 91 ± 44 IU/l and 88 ± 33 IU/l (p= n.s.). No significant effect on viral load was observed (mean HCV RNA level at baseline was 5.81 log10 IU/mL ± 0.46 and at the end of observation 5.9 log10 IU/mL ± 0.48, p=n.s.). Only one patient showed viral decline of 1 log after 11 months of treatment. Conclusion: This observational study provides evidence that bezafibrate is effective for patients with chronic hepatitis C by reducing significantly ALT.
and GGT levels and could be therefore a therapeutic option, especially for those, in whom peg-interferon combination treatment was previously unsuccessful. Further larger and randomized clinical trials including also histological endpoints are required to confirm these findings.

Disclosures:
The following people have nothing to disclose: Viola Weich, Beate Schlosser, Juliane Halangk, Alexandra Bergk, Florian van Bommel, Thomas Berg

### Table 1

<table>
<thead>
<tr>
<th>Marker</th>
<th>Virological response</th>
<th>Baseline (mean, range)</th>
<th>Post-treatment (mean, range)</th>
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<tr>
<td>ELF Scheuer</td>
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<td>SVR (n=75)</td>
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<td>0.33 (-1.4-2.5)</td>
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<td>4 Marker</td>
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<tr>
<td>SVR (n=75)</td>
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<td>0.48 (-2.6-3.7)</td>
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### 292 IMPACT OF ANTIVIRAL TREATMENT ON NON-INVASIVE PREDICTORS OF LIVER FIBROSIS IN PATIENTS WITH CHRONIC HEPATITIS C

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Background: Histologic improvement following interferon-based antiviral treatment has been demonstrated in patients with chronic hepatitis C (CHC) achieving viral clearance. The aim of this study was to evaluate the changes in non-invasive markers of liver fibrosis in a large cohort of patients with CHC treated with pegylated interferon and ribavirin. Methods: The study included 130 patients who underwent pegylated interferon and ribavirin treatment. All patients had a baseline liver biopsy (fibrosis scored by the Scheuer classification) and the following fibrosis markers were evaluated at baseline and 6 months after treatment completion: hyaluronic acid (HA), collagen IV (Col4), amino-terminal propeptide of type III collagen (PIIINP), tissue inhibitor of matrix metalloproteinase 1 (TIMP-1), and 4 Marker algorithm (PIIINP, TIMP-1, Col4 and HA) (Rosenberg et al. Gastroenterol 2004). The diagnostic performance of both algorithms for diagnosis of significant liver fibrosis (F2) and cirrhosis (F4) was evaluated at baseline by the area under the ROC curve (AUROC). Results: A total of 55 patients (42.3%) were nonresponders (NR) whereas sustained virological response (SVR) was achieved in 75 patients (57.7%). The AUROC values for F2 were 0.807 and 0.806, and for F4 were 0.810 and 0.804, with ELF Scheuer and 4 Marker algorithms, respectively. Baseline and post-treatment algorithm values in relation to SVR are shown in table 1. In patients who achieved SVR the values of both algorithms decreased significantly from baseline, whereas in non responders there was no change. Conclusions: Serum fibrosis markers can identify the presence of significant fibrosis and cirrhosis accurately in a significant proportion of patients. Moreover, virological clearance is associated with a significant reduction in fibrosis serological markers, which most likely reflect the histological improvement associated with sustained virological response. If confirmed, the latter would support the use of non-invasive markers to follow-up patients with CHC.

**Table 1**

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### 293 EARLY REDUCTION OF RIBAVIRIN LEADS TO RETARDATION OF VIRAL CLEARANCE AND AFFECTS SVR

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Background: It is said a reduction of ribavirin (RBV) will not impact the SVR rate, but no examination has included the effect of the timing of the reduction of RBV while it is known that the timing of HCV RNA loss strongly relates to the SVR rate. Aim: To review the antiviral activity of RBV from the effect of the timing of reduction of RBV on the timing of HCV RNA loss. Methods: One hundred and twenty-four patients with genotype 1b and high viral load (62 males, 62 females; mean age 55.9 years old) who received PEG IFNα-2b plus RBV combination therapy for 48 weeks were included in analysis. All patients received RBV by a total clearance of RBV (CL/F)-based dose (we set 2250ng/ml as target blood concentration and calculated the dose of RBV from CL/F, 2005AASLD). The dose of PEG IFNα-2b of 1.5µg/kg/week was not reduced for the duration of administration. We determined the factors affecting the timing of HCV RNA loss in patients for whom RBV was not reduced (group A) by multiple regression analysis and prepared of a predictive formula. Applying this predictive formula to patients in whom RBV was reduced, we examined whether the timing of RBV reduction affects the timing of HCV RNA loss. Results: Predictive formula based on multiple regression analysis was: Estimated time of HCV RNA loss (week) = 1.8960 x Log HCV RNA (2W) + 8.5722, where HCV RNA (2W) is HCV RNA level (KIU/ml) 2 weeks after the start of treatment. This predictive formula allowed the good prediction of the actual timing of HCV loss. When applied to patients with RBV reduced after 8 weeks of treatment (group B) and in the first 8 weeks of treatment (group C), the fit was 100% (group B) and 69% (group C) that of the actual timing of HCV RNA loss with less than +/-4 weeks of predicted. With patients showing greater than +/-4 weeks difference between predicted and actual timing of HCV RNA loss (group C), the actual timing was delayed from the predicted time. In other words, delay in HCV RNA loss was statistically significant compared to without RBV reduction in patients with early RBV reduction. SVR rate with group A, B, and C was 60%, 62.5%, and 38.5%, respectively, and reflected the difference in the timing of HCV RNA loss. Conclusions: We reviewed patients with genotype 1 and high viral load with and without RBV dose reduction and correlation with the timing of HCV loss. Because HCV RNA loss was delayed in patients for whom RBV was reduced early in treatment, it was thought that antiviral activity of RBV was expressed from a relatively early stage in IFN + RBV combination therapy. A method of administration to avoid reducing RBV is important to increase the SVR rate.

Disclosures:
The following people have nothing to disclose: Yoshiyasu Karino, Joujii Toyota, Tomohiro Arakawa, Yasuaki Kuwata, Jun Akaike, Katsu Yamazaki, Takahiro Sato, Takumi Omura, Shiro Iino
294 INTRACELLULAR TARGETING OF HEPATITIS C VIRUS CORE PROTEIN WITH A SINGLE CHAIN ANTIBODY
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Background and Aims: The hepatitis C virus (HCV) core protein is not only essential for viral genome encapsidation but also implicated in pathogenesis such as steatosis and cell growth regulation. We previously found that expression of HCV core protein increased cell proliferation, DNA synthesis, and cell cycle progression. The acceleration of cell growth was mainly attributable to the activation of HGF (hepatocyte growth factor) and Wnt-1 signaling, pathways that are frequently activated in human HCC samples. In the present study, we evaluated the effect of an intracellular single chain antibody on core protein level and cell growth. Methods: The human single chain Fv antibody fragments were cloned from bone marrow of patients with chronic hepatitis C. The antibody targeting the HCV core protein was selected by affinity binding/purification, and subcloned into the expression vector. The epitope recognized by this antibody is conserved among different HCV genotypes. The effect of the antibody on core protein expression was evaluated by co-transfecting the antibody construct with the core expression construct or the replicon RNA into Huh-7 cells. Results: Co-transfection of the antibody construct with the core protein cDNA markedly reduced core protein level, and inhibited cell proliferation. In contrast, proliferation of cells transfected with the core-null mutant or empty vector was not modulated. Moreover, the antibody construct could also reduce core protein expression from the full-length HCV replicon (JFH1), as evidenced by immunofluorescence staining and Western blot, indicating the ability of the antibody to recognize core protein translated as part of HCV polyprotein. Conclusion: The single chain antibody can efficiently target HCV core in the context of HCV replication and reverse core protein mediated cell proliferation. Further studies will elucidate whether the single chain antibody down regulates core protein abundance/function through degradation and/or steric inhibition. It may represent a novel agent to interrupt the HCV life cycle and to blunt pathogenesis mediated by the core protein.

Disclosures: The following people have nothing to disclose: Raiki Machida, Tobias Heintges, Shuping Tong, Jisu Li

295 TREATMENT UPTAKE AND OUTCOMES AMONG CURRENT AND FORMER INJECTION DRUG USERS (IDUS) RECEIVING DIRECTLY OBSERVED THERAPY WITHIN A MULTIDISCIPLINARY GROUP MODEL FOR THE TREATMENT OF HEPATITIS C VIRUS (HCV) INFECTION
Krista Genoway1,3, Jason Grebely1,3, Fiona Duncan2, Mark Vlijmen3, Lesley Gallagher1,3, Doug Elliott3, Milan Khara2, Jesse D. Raffa2, Stanley deVlaming3, Brian Conway1,3; 1Anesthesiology, Pharmacology and Therapeutics, University of British Columbia, Vancouver, BC, Canada; 2Statistics and Actuarial Science, University of Waterloo, Waterloo, ON, Canada; 3Pender Community Health Centre, Vancouver Coastal Health, Vancouver, BC, Canada

Purpose: We evaluated HCV treatment uptake and outcomes among current and former IDUs attending a weekly peer-support group and receiving directly observed HCV therapy (DOT). Methods: Beginning in March 2005, patients interested in receiving treatment for HCV infection were referred to a weekly peer-support group and evaluated for treatment. Utilizing the existing infrastructure for addiction disease management, we have developed a model whereby the treatment of addiction, HCV and other medical conditions are integrated under the DOT model of care. Patients received directly observed therapy with pegylated interferon alpha 2a or alpha 2b (PEG-IFN alpha 2a or alpha 2b), both in combination with self-administered ribavirin (RBV). Results: Overall, 129 subjects were referred to the support group over a period of 108 weeks, with the mean attendance being 15 subjects per week (range 3-32). Overall, 10 (8%) did not medically qualify for treatment, 39 (30%) were lost to follow-up and 8 (6%) had completed or initiated treatment for HCV infection prior to attending the group. We observed a high uptake of HCV treatment among attendees, with 30% of subjects (39/129) currently under evaluation and 26% (33/129) having initiated or completed treatment for HCV infection. In a comparison of subjects that had initiated or completed treatment for HCV infection (n=33) and those lost to follow up (n=39), those having received treatment for HCV infection had a higher median attendance (34 meetings [Interquartile range, IQR = 11-33] vs. 2 meetings [IQR=1-3, P<0.001]) and were more likely to attend >3 clinic visits (97% vs. 23%, P<0.001) than those lost to follow up. To date, 31 patients (PEG-IFN alpha 2a/RBV = 15; PEG-IFN alpha 2b/RBV = 4) have initiated treatment for HCV infection at our site and 18 have completed therapy, with 67% (12/18) of subjects achieving an end of treatment response (Genotype 1 – 40%, Genotypes 2-100%, Genotype 3 – 67%), despite ongoing drug use in 72% of patients during treatment. Conclusion: These data demonstrate that with the appropriate programs in place, a high uptake of HCV treatment can be achieved among IDUs referred to a peer-support group. Moreover, the treatment of HCV in current and former IDUs within a multidisciplinary DOT program can be successfully undertaken, resulting in end of treatment responses similar to those reported in randomized controlled trials.

Disclosures: Jason Grebely - Grant/Research Support: Schering-Plough; Grant/Research Support: Roche; Speaker’s Bureau: Schering-Plough; Speaker’s Bureau: Roche; Fiona Duncan - Grant/Research Support: Schering-Plough; Grant/Research Support: Roche; Consultant/Adviser: Schering-Plough; Consultant/Adviser: Roche; Mark Vlijmen - Grant/Research Support: Schering-Plough; Grant/Research Support: Roche; Consultant/Adviser: Schering-Plough; Consultant/Adviser: Roche; Brian Conway - Grant/Research Support: Schering-Plough; Grant/Research Support: Roche; Consultant/Adviser: Schering-Plough; Consultant/Adviser: Roche; Speaker’s Bureau: Schering-Plough; Speaker’s Bureau: Roche

The following people have nothing to disclose: Krista Genoway, Doug Elliott, Milan Khara, Jesse D. Raffa, Stanley deVlaming

296 INFREQUENT HEPATITIS C VIRUS (HCV) RE-INFECTION AFTER SUSTAINED VIROLOGICAL RESPONSE (SVR) AMONG CURRENT AND FORMER INJECTION DRUG USERS (IDUS) HAVING RECEIVED TREATMENT FOR HCV INFECTION
Jason Grebely1,3, Jesse D. Raffa2, Krista Genoway1,3, Grey Shover4, Fiona Duncan2, Mark Vlijmen3, Milan Khara2, Stanley deVlaming3, Chris Fraser5, Brian Conway1,3; 1Anesthesiology, Pharmacology and Therapeutics, University of British Columbia, Vancouver, BC, Canada; 2Department of Statistics and Actuarial Science, University of Waterloo, Waterloo, ON, Canada; 3Pender Community Health Centre, Vancouver Coastal Health, Vancouver, BC, Canada; 4Cool-Aid Community Health Centre, Victoria, BC, Canada

Purpose: To evaluate HCV re-infection following SVR among IDUs having received directly observed IFN alpha-2b or PEG-
IFN alpha-2a/b in combination with self-administered ribavirin in a directly observed therapy program. Methods: Viremic HCV-infected IDUs, with ALT > 1.5x ULN, received 24 or 48 week therapy (based on HCV genotype) with ribavirin and interferon alpha-2b, replaced by PEG-interferon alpha-2a/2b. Following treatment, subjects were encouraged to return to the clinic at follow-up intervals of ~1 year and were asked about their use of illicit drugs. HCV RNA testing by PCR was performed and positive results were genotyped. Results: Overall, 28/51 subjects (55%) receiving IFN alpha-2b (n=12), Peg-IFN alpha-2b (n=32) or PEG-IFN alpha-2a (n=7) achieved an SVR. Illicit drugs were used by 21/51 (41%) in the 6 months preceding therapy and by 29/51 (57%) during therapy. In total, 28 subjects were followed for a mean of 1.1 years (range, 0-3.2 years) following SVR. In this period, 13/28 (46%) reported using illicit drugs [3 – injection heroin/cocaine and crack cocaine, 2 – injection heroin and crack cocaine, 1 – injection cocaine and crack cocaine, 3 – injection cocaine, 2 – injection heroin, 2 – crack cocaine]. Eleven subjects (39%) reported injection drug use. Overall, 25/28 (89%) remained HCV RNA negative, 1 died of hepatocellular carcinoma, 1 was lost to follow-up and 1 was positive for HCV RNA. This subject received 17 weeks of Peg-IFN alpha-2b therapy and had viral reoccurrence with the same genotype (G1), consistent with re-infection or viral relapse. Therefore, viremia re-occurred in 1/28 (3.6%), providing an estimated rate of re-occurrence of 4.0 cases per 100 person-years. Conclusions: These data demonstrate a low rate of HCV re-infection among IDUs successfully treated for HCV and provides a rationale for expanding treatment in this group. Research is required to understand if this is associated with protective immunity against HCV re-infection or reduced risk behaviors for acquisition following successful treatment.

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Jason Grebely - Grant/Research Support: Schering-Plough, Grant/Research Support: Roche
Brian Conway - Grant/Research Support: Schering-Plough, Grant/Research Support: Roche
Fiona Duncan - Grant/Research Support: Schering-Plough, Grant/Research Support: Roche
Mark Viljoen - Grant/Research Support: Schering-Plough, Grant/Research Support: Roche

297 PEGYLATED INTERFERON ALFA-2B PLUS RIBAVIRIN REDUCES INSULIN RESISTANCE AND IMPROVES GLUCOSE METABOLISM IN PATIENTS WITH CHRONIC HEPATITIS C

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Background: Hepatitis C Virus (HCV) infection is linked to insulin resistance which may contribute to fibrogenesis and carcinogenesis in chronic hepatitis C. Glucose intolerance setting of chronic HCV infection could be related etiologically to viral factors. However, IFN has been reported to acutely induce insulin resistance and glucose intolerance. Although HCV is a candidate for the development of insulin resistance, the effects of antiviral treatment on impaired glucose metabolism remain unclear. The aim of this study was to clarify whether insulin resistance was improved on Pegylated Interferon alfa-2b Plus Ribavirin (PegIFN/RBV). Methods: Plasma glucose (PG) level and immunoreactive insulin (IRI) level in the course of 75g oral glucose tolerance test (OGTT) were measured to evaluate glucose intolerance in 0, 4 and 12 weeks after initial PegIFN/RBV. The OGTT was performed after an overnight fast, and blood samples were taken at 0, 30, 60, 90 and 120 min following glucose ingestion. Then PG area under the curve (AUC-G), IRI area under the curve (AUC-IR) and HOMA-IR were calculated. Simultaneously, oxidation rate of carbohydrate (%CHO), fat (%FAT) and nonprotein respiratory quotient (npRQ) were calculated by using an indirect calorimeter. Results: We examined 25 biopsy-proven patients who received PegIFN/RBV. Twelve patients (48%) were found to have diabetes while five (20%) showed impaired glucose tolerance (IGT) at the pretreatment according to OGTT as based on the revised diagnostic criteria of the American Diabetes Association (1997). In the patients of diabetes and IGT (n=17), the AUC-G was significantly decreasing (0w: 582±33 4w: 453±22; p<0.05) at week 4. Although HOMA-IR did not change at week 12 (0w: 2.4±0.3, 12w: 2.3±0.5), the AUC-IR was decreased (0w: 212±25, 12w: 158±20; p<0.05). The %CHO increased (0w: 40±4, 12w: 50±6) and %FAT decreased (0w: 47±4 12w: 36±5), as a result, npRQ increased (0w: 0.837±0.015, 12w: 0.871±0.017; p<0.05) at week 12. No one shows severe weight loss (5% down at pretreatment) and patients with normal glucose tolerance did not change by week 12. Finally, patients with glucose intolerance were divided into two groups by IFN response: Eleven were under detectable in HCVRNA at week 12 as an EVR group and six were non EVR group. The AUC-G and AUC-IR were reduced by 35% (p<0.05) in EVR. However, the AUC-I did not change in nonEVR, although the AUC-G was decreased by 17%. Conclusions: Insulin resistance and energy metabolism improved by the administration of PegIFN/RBV, which had strongest effect against HCV. The effects of rapid viral reduction might be important for restoring of glucose tolerance in chronic hepatitis C.

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The following people have nothing to disclose: Masaaki Korenaga, Keiko Korenaga, Kouichi Uchida, Takahiro Yamasaki, Keisuke Hino, Isao Sakaia

298 WHOLE-BODY, NOT ONLY LIVER, INSULIN SENSITIVITY IS STRONGLY ASSOCIATED WITH AN EARLY AND SUSTAINED VIROLOGIC RESPONSE TO PegIFN/ RBV PLUS RIBAVIRIN TREATMENT IN PATIENTS WITH CHRONIC HEPATITIS C GENOTYPE 1B AND HIGH VIRAL LOAD

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Aim: Recent studies have indicated that insulin resistance (IR) might be an important factor associated with the virologic response to interferon treatment for chronic hepatitis C, but little is known about its mechanism. IR consists of hepatic IR (central IR) and muscle IR (peripheral IR). We analyzed the effect of hepatic and whole-body (hepatic + muscle) IR on the efficacy of peginterferon plus ribavirin treatment. Methods: Forty-three chronic hepatitis C patients with genotype 1b and high viral load treated with peginterferon alpha-2b plus ribavirin for 48 weeks were examined. Early virologic response (EVR) and sustained virologic response (SVR) were obtained in 22 patients (51%) and 18 patients (42%), respectively. We used two methods to evaluate IR; HOMA-IR, a marker of hepatic IR, and the ISI composite, which indicates whole-body insulin sensitivity and is calculated as 10000/VFG × FRI × mean BS (0-120) × mean IRI (0-120) from a 75 g oral glucose tolerance test. Serum adiponectin levels were measured and visceral fat areas at the
umbilical level were evaluated by computed tomography. We analyzed whether the HOMA-IR and ISI composite values before treatment were associated with EVR or SVR Results: There were significant differences in HOMA-IR and ISI composite values before treatment between EVR and non-EVR patients (HOMA-IR: 1.7 vs 3.0, p=0.0005; ISI composite: 5.7 vs 3.3, p=0.02). Similarly, there were significant differences in both between SVR and non-SVR (HOMA-IR: 1.6 vs 2.8, p=0.009; ISI composite: 6.0 vs 3.4, p=0.003). Analyzed according to HOMA-IR classes, EVR and SVR rates were 76% and 62% in <2 (n=21), 32% and 26% in 2-4 (n=19), 0% and 0% in >4 (n=3), respectively. According to ISI composite classes, EVR and SVR rates were 20% and 13% in <3 (n=15), 60% and 45% in 3-6 (n=20), 100% and 80% in 6-9 (n=5), 67% and 100% in >9 (n=3), respectively. A positive predictive value for EVR and SVR when ISI composite was >6 was 87.5% for both. Multivariate analysis showed that >6 in ISI composite was the only factor associated with SVR. ISI composite values were significantly positively correlated with serum adiponectin levels and negatively with visceral fat areas. Conclusions: Whole-body, predominantly liver and skeletal muscle, insulin sensitivity is strongly associated with the efficacy of peginterferon plus ribavirin treatment. These data suggest that lifestyle interventions might enhance the effect of antiviral therapy in patients with chronic hepatitis C.

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329 EVALUATION OF A MULTIDISCIPLINARY SUPPORT PROGRAM IN HEPATITIS C TREATMENT

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Adherence to antiviral treatment has been cited as a potentially important factor in determining the outcome of therapy in hepatitis C patients. Aim: To evaluate the multidisciplinary support program (MSP) efficacy and its impact on the health-related quality of life (HQL) during HCV treatment. Method: One hundred eighty-eight HCV naive patients were consecutively treated in the Liver Section of the Hospital Mar: Group 1, 91 patients included in the MSP (2005), and Group 2, 97 patients with conventional control (2003-2004). All patients were treated with Peg-IFN alpha-2a and ribavirin (RBV). The MSP team includes hepatologists, nurses, a pharmacist, a psychologist and a psychiatrist. Uniform patient informing, open and flexible visits scheduling, continued evaluation of psychiatric risk (PHQ and HADS), active medication control, and standardized management of the secondary effects were carried out during MSP. Patients receiving ≥ 80% of each assigned drug for ≥ 80% of the expected duration therapy were considered as adherent to the treatment. HQL was evaluated by SF-36 before and at 1, 3, and 6 months during treatment. Results: No differences were observed between either group in terms of age, gender, HCV-genotype, viral load and fibrosis degree. Anti-depressives or anxiolytics were prescribed in 34 (37.4%) and 21 (21.6%) patients in each group, respectively (P 0.02). Patients in Group 1 showed better scores in all domain scales of health status than the patients in Group 2. However, the differences only reached statistical significance in terms of bodily pain, and in general and mental health scores. Conclusions: The multidisciplinary support program (MSP) increases the adherence to the antiviral therapy and improves the perception of the quality of life during treatment.

<table>
<thead>
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<th></th>
<th>Group 1</th>
<th>Group 2</th>
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<td>Withdrawal / drop-out (%)</td>
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<td>End of treatment response (%)</td>
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Disclosures: The following people have nothing to disclose: Montserrat Garcia-Retortillo, Maria Dolors Giménez, Carme Márquez, Pere Castellvi, Ricard Navines, Eduard Clot, Isabel Cirera, Esther Salas, Rocío Martín-Santos, Ricard Solà

300 THE TGF-β CODON 19 T/C AND 25 G/C POLYMORPHISMS AFFECTS RESPONSE TO INTERFERON-THERAPY IN ACUTELY HCV-INFECTED HIV-POSITIVE PATIENTS

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Background: Co-infection with the Hepatitis C virus (HCV) in HIV-positive patients is an emerging health problem. Recently, outbreaks of acute hepatitis C (HCV) infections have been reported among HIV-seropositive homosexual men in Amsterdam, London, Paris and Berlin. The factors that predispose some individuals to treatment failure are poorly understood but may involve host genetic factors. Recently, HCV NS5A induced inhibition of TGF-β signaling has been suggested as a potential mechanism involved in HCV pathogenesis. TGF-β is a multifunctional cytokine that displays gene polymorphisms (TGF-β codon 10 T/C and codon 25G/C) associated with differential cytokine secretion. Here, we studied whether the TGF-β gene polymorphisms affects the response to antiviral treatment in acutely HCV-infected HIV positive subjects.

Methods: TGF-β genotypes were determined in 52 HIV-positive patients with acute hepatitis C treated with pegylated interferon-alpha. Genotypes were classified into high and low-intermediate producers. Rates of sustained virological responses (SVRs) were compared between the carriers of the respective cytokine genotypes. As a control 100 healthy subjects were studied.

Results: TGF-β genotype distribution did not differ significantly between HCV/HIV co-infected and healthy individuals. SVR were achieved in 67.3% (35/52) patients, respectively. Carriers of the TGF-β "high producer" genotype had significantly higher SVR rates than patients with a TGF-β "low-intermediate producer" genotype (75% [31/41] vs. 36.4% [4/11]; p=0.027). In a forward-conditioned stepwise regression model TGF-β "high-producer" genotype could be confirmed as an independent positive predictor for SVR in interferon-alpha therapy (Odds ratio: 11.2; 95%CI: 1-125; p=0.027).

Conclusions: Response rates to interferon-alpha therapy are enhanced in acute HCV-infected HIV-positive patients carrying the TGF-β "high producer" genotype. This finding may indicate that a TGF-β "high-producer" state can partially compensate HCV NS5A-induced inhibition of TGF-β signaling.
301 THE IMPACT OF STEATOSIS AND STEATOHEPATITIS ON THE RESPONSE TO TREATMENT IN PATIENTS WITH CHRONIC HEPATITIS C INFECTION

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Background: The impact of steatosis on sustained virological response (SVR) in patients treated with pegylated interferon and ribavirin for chronic hepatitis C (CHC) infection is controversial. Aims: We studied whether finding steatosis with or without steatohepatitis on a pre-treatment liver biopsy (LB) influenced SVR in patients with CHC subsequently treated with pegylated interferon and ribavirin. Patients: We assessed 204 consecutive patients treated for CHC between January 2001 and January 2005. LB was performed in all patients before treatment. The method described by Ishak was used to grade necro-inflammation and stage fibrosis. Steatosis and steatohepatitis were graded using the modified Brunt score. Patients were classified according to the presence or absence of steatosis and steatohepatitis. Patients achieving SVR were labeled as responders and those failing to achieve SVR as non-responders Results: 179 patients were included in the final analysis after exclusions; 72% male, median age 46 years (interquartile range 40 – 52). 127 patients had Ishak Fibrosis stage 3 or less (71%), and 31 patients (17.3%) were cirrhotic (Ishak stage 5-6). 106 patients were responders (59%); the SVR rates by genotype were: genotype 1 (35%), genotype 2 (82%), genotype 3 (79%), and genotype 4 (57%). 98 patients had steatosis (51.9%). The X2 test for trend demonstrated different rates of steatosis between the genotypes (P=0.14). Steatosis was most prevalent in genotype 4, 6/7 (86%), although the numbers were small. Genotype 3 had a high level of steatosis 34/54 (63%, p=0.053) and genotype 2 had a low level, 12/37 (32%, p=0.008). Steatohepatitis was present in 34 patients (19%), who were older (49 years vs. 44 years, p=0.03) and more likely to have severe steatosis (grade 2-3 vs. 0-1, p=0.0001), and cirrhosis (p=0.001). Genotype had no effect on the likelihood of steatohepatitis (p=0.69). Responders did not differ from non-responders in respect to gender, age, the presence or severity of steatosis, necroinflammatory grade or pre-treatment ALT. In a univariate analysis, reduced SVR was associated with genotypes 1 and 4 (p<0.0001), cirrhosis (p=0.0001), steatohepatitis and heavier patient weight (both p<0.009). But on multivariate analysis only genotypes 1 and 4 (p<0.001), pretreatment weight (p=0.002) and cirrhosis (p=0.06) were found to be associated with SVR. Conclusion: In CHC, steatosis has no effect on SVR. Steatohepatitis is associated with severe steatosis and liver disease progression but not SVR, which is mainly determined by genotype, patient weight and the presence of cirrhosis.

Disclosures: The following people have nothing to disclose: Timothy J. Cross, Alberto Quaglia, Jonathon Nolan, Ian Fletcher, Kosh Agarwal, Philip M. Harrison.
Rapid virological response at week 4 is the best predictor of treatment outcome in patients with chronic hepatitis C: A multivariate analysis

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It is well established that HCV genotype and baseline HCV-RNA are good predictors of sustained virological response (SVR) in selected patients included in clinical trials of pegylated interferon alpha (PEG-IFN)+ribavirin (RBV). Limited knowledge is available on predictors of SVR in the general population managed in routine clinical practice. Methods: 408 patients consecutively treated with PEG-IFN α2b+RBV, were studied (221 naïves; 125 non-responders (NRs); 62 relapsers (RRs). Patients with genotypes 1 or 4 and non-naïve were treated 48 weeks and patients with genotypes 2 or 3 were treated 24 weeks (PEG-IFN 1.5µg/kg/week; RIBA 800-1200 mg/day). Serum HCV-RNA was measured at baseline, week 4, week 12, end of treatment and 6 months after the end of treatment with the quantitative VERSANTR HCV 3.0 Assay (bDNA)(Siemens). Samples below limit of quantification were tested with VERSANTR HCV RNA Qualitative Assay (TMA)(Siemens). SVR was defined as undetectable serum HCV-RNA by TMA at the end of 6-month post-treatment follow-up. The characteristics included in the logistic regression analysis were: baseline viral load (≤400 10^3 IU/ml; >400 10^3 IU/ml), HCV genotype (1, 2, 3, 4, 5), gender, age (≤55 vs >55), histology grade (A0-A naive vs A2-A3), fibrosis stage (F0-F1 vs F2-F3) assessed with Metavir score, serum ALT, pre-treatment status, rapid virological response at week 4 (RVR4)(TMA undetectable) and rapid virological response at week 12 (RVR12) (>2 log viral drop) of therapy. Early viral kinetic was analyzed at weeks 1 to 4 in a subgroup of 78 patients. Results. Overall SVR rate was 46%; SVR rates were 53%, 25% and 65% in naïves, NRs and RRs, respectively. In the overall population factors significantly associated with SVR odds ratio (95%CI) were: RVR4: 26.4(6.1-114.4)(p<0001), age 2.6(1.3-5.2)(p=0.005) fibrosis 2.1(1.01-4.40)(p=0.04). Non-RVR12 was significantly associated with non-response 51.2(6.7-538.7)(p<0001). In naïve patients RVR4: 16.2(3.3-79)(p=0.001), age 3.2(1.2-8.7)(p=0.001) fibrosis 3.9(1.2-12.3)(p=0.001) were significantly associated with SVR. In non-responders only non-RVR12 16.9(2.1-132)(p=0.007) was significantly associated with non-response. The slopes of early viral kinetics were significantly different in SVR and in non-SVR patients (p<0001), the slopes were not different in naïves and experienced patients who developed SVR. Conclusions. RVR at week 4 of therapy is the strongest independent factor for prediction of SVR. Non-EVR at week 12 is the strongest predictor of non-SVR. Therefore, monitoring of therapy should include both, detection of serum HCV RNA at week 4 with a sensitive assay (TMA) to predict SVR and quantification of HCV RNA at week 12 to predict non-SVR.

304 ASSESSMENT OF BOTH VIROLOGICAL RESPONSE AT WEEK 4 AND AT WEEK 12 OPTIMIZES PREDICTION OF TREATMENT OUTCOME IN PATIENTS WITH CHRONIC HEPATITIS C TREATED WITH PEGINTERFERON ALFA-2B PLUS RIBAVIRIN

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Pretreatment viral load is an important predictor for treatment outcome in patients with chronic hepatitis C. Recently, Zeuzem et al. (AASLD 2006) proposed 400 000 IU/ml (assessed with the COBAS TaqMan HCV assay) as the optimal cut-off to best discriminate low and high VL, based on the probability to achieve SVR in patients treated with peginterferon (PEG-IFN) alpha 2a+ribavirin (RBV). Our study aimed to analyze the positive predictive value (PPV) of this cut-off value at baseline, the PPV of rapid virological response at week 4 (RVR4) and the negative predictive value (NPV) of non early virological response at week 12 (non-EVR12), in patients treated with PEG-IFN alpha 2b+RBV. Patients-Methods: 408 patients (221 naïve; 187 non-naïve) consecutively treated with PEG-IFN alpha 2b+RBV (PEG IFN 1.5µg/kg/week plus RIBA 800-1200 mg/day according to weight), were included in this study. Patients with genotypes 1, 4 or 5 or non-responders were treated 48 weeks; naïve patients infected with genotypes 2 or 3 were treated 24 weeks. Serum HCV RNA was measured at baseline, week 4, week 12, end of treatment and 6 months after the end of treatment with the quantitative VERSANTR HCV 3.0 Assay (bDNA)(Siemens). Samples below the limit of quantification were tested with VERSANTR HCV RNA Qualitative Assay (TMA)(Siemens). SVR was defined as undetectable serum HCV RNA by TMA at end of 6 months post-treatment follow-up. At treatment initiation PPV was defined with a cut-off set up at ≤400 103 IU/ml; at week 4 the PPV was defined as TMA undetectable or >2 log drop baseline viral load. Results. The overall SVR rate was 46% (53% in naïve patients and 38% in non-naïve patients). Conclusions. Undetectable HCV RNA at week 4 (RVR) when assessed with a sensitive assay (TMA) is more accurate (96%-100%) than baseline cut-off (<400 103 IU/ml) to predict SVR both in naïve and in experienced patients. The absence of EVR at week 12 (less than 2 log reduction of HCV RNA) is a strong predictor (96%-100%) of non response to therapy independently of the patients pretreatment status.
305 PREDICTABILITY OF RESPONSE: POSITIVE AND NEGATIVE PREDICTIVE VALUES OF RAPID AND EARLY VIROLOGIC RESPONSES TO PEGINTERFERON ALFA-2B AND RIBAVIRIN IN THE TREATMENT OF CHRONIC HEPATITIS C

Fred Poordad1, Chrispin Kambili2; 1Cedars-Sinai Medical Center, Los Angeles, CA; 2Schering-Plough Corporation, Kenilworth, NJ

Background: Assessing viral kinetics in response to peginterferon (PEG-IFN) alfa and ribavirin (RBV) can help predict treatment response among patients with chronic hepatitis C. Hepatitis C virus (HCV) RNA levels decrease rapidly after initiation of treatment with PEG-IFN alfa-2b/RBV. To determine the value of assessing early viral kinetics during PEG-IFN alfa-2b/RBV, positive and negative predictive values (PPVs; NPVs) of rapid and early virologic response (RVR; EVR) for sustained virologic response (SVR) were culled from PEG-IFN alfa-2b articles or abstracts. Methods: Published PEG-IFN alfa-2b data were evaluated for PPVs and NPVs for RVR and/or EVR. PPVs and NPVs were calculated if not reported but RVR and/or EVR rates and SVR rates were available. RVR was defined as undetectable HCV RNA at wk 4 of treatment (lower limit of detection <50 IU/mL in most studies). EVR was defined as undetectable HCV RNA or a >2 log10 decrease from baseline at wk 12 of treatment. Results: Patients from applicable trials (N=2000) were monoinfected with HCV or coinfected with HCV/HIV and were treated with PEG-IFN alfa-2b (1.0 or 1.5µg/kg/wk or 50-150µg/wk) and various doses of RBV (800-1400mg/d). Treatment duration was 48 or 24 weeks, generally depending on genotype (G). Attaining RVR was highly predictive of attaining SVR in patients with any genotype who were treated for 48 wks (PPV 89%), in G1/4 patients treated for 48 wks (PPV 81%), and in G2/3 patients treated for 24 wks (PPV 85-90%). Not attaining RVR was a less reliable predictor of not attaining SVR for all genotypes (NPV 59%); NPVs were 84% among G1/4 and 44-63% among G2/3 patients. G2/G3 patients were analyzed separately in 1 study; the PPV and NPV for RVR were 89% and 50%, respectively, for G2 patients and 100% and 57%, respectively, for G3 patients. Failure to attain EVR was a consistent indicator of failure to attain SVR (NPV 95-100%), whereas attaining EVR was a less reliable predictor of attaining SVR (PPV 67-72%) for all genotypes. Among African Americans, NPVs and PPVs for EVR were 100% and 48-83%, respectively. Studies in G4 patients revealed NPVs for EVR of 86-100% and PPVs for EVR of 76-100%. Conclusions: Predictability of response to PEG-IFN alfa-2b/RBV applied to mono- and coinfected patients. EVR was a better negative than positive predictor of SVR and can be used to guide treatment cessation in G1/4 patients who do not respond by week 12 of treatment. RVR was an excellent positive but poor negative predictor of SVR. NPVs and PPVs for EVR can be used as a patient motivator and can reliably be used as part of the treatment algorithm for future studies and in clinical practice.

Disclosures: The following people have nothing to disclose: Michelle Martinot-Peignoux, Sarah Maylin, Marie Pierre Ripault, Rami Mocuari, Nathalie Boyer, Nathalie Giuly, Corinne Castelnu, Patrick Marcellin.
BACKGROUND Patients infected with hepatitis C virus genotype 2 (HCV-2) or HCV-3 respond better to interferon alfa (IFN-α) treatment than HCV-1 or HCV-4 patients. The mean initial decline in HCV RNA during IFN-α therapy is faster for HCV-2 and HCV-3 compared to HCV-1 patients. Little is known about viral kinetics in patients with HCV-4. AIMS The aim of our study was to determinate genotype specific differences in viral kinetics in HCV-1 and HCV-4 patients during a modified treatment regimen with a high initial dose of interferon (induction). METHODS We treated naive patients with HCV-1 (n=42) or HCV-4 (n=12) with triple antiviral therapy consisting of amantadine hydrochloride and ribavirin, combined with 6 weeks of IFN-α2b induction (week 1-2: 18 MU/day, week 3-4: 9 MU/day, week 5-6: 6 MU/day), thereafter combined with weekly Peg-IFNα2b, for 24 or 48 weeks. HCV RNA was assessed at baseline, day 1, 2, week 1, 2, 4, 6, 8, and then every 4 weeks until end of treatment by quantitative bDNA (LLD 615 IU/ml), qualitative PCR (LLD 50 IU/ml), and TMA (LLD 5 IU/ml). Viral dynamics were estimated using the bi-phasic model for HCV during treatment with IFN-α. RESULTS Baseline HCV RNA levels, and the 1st and 2nd phase decline in HCV RNA, were similar in HCV-1 and HCV-4 patients (Figure). Mean time to reach a TMA negative status in patients with subsequent SVR was shorter in HCV-4 (4.3 ± 2.3 weeks) compared to HCV-1 (6.4 ± 4.5 weeks), this difference was not significant. SVR was achieved by 43% of HCV-1 and 50% of HCV-4 patients. CONCLUSION Viral kinetics are similar in HCV-1 and HCV-4 patients, these results confirm that HCV-4 patients should be treated as HCV-1.

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Marcel Beld - Consultant/Adviser: Bayer

The following people have nothing to disclose: Huub C. Gelderblom, Hans L. Zaaijer, Christine J. Weegink, Peter L. Jansen

Background & Aim: The early viral response, defined as undetectable or at least a 2-log decrease in HCV RNA level after 12 weeks of treatment, is very important for predicting which hepatitis C patients are not likely to display a sustained viral response (SVR) to peginterferon (Peg-IFN) plus ribavirin (RBV) combination therapy. In this study, we investigated the relationship between the viral kinetics during the first 8 weeks and the antiviral outcome to identify factors with a high positive predictive value (PPV) or negative predictive value (NPV) for SVR. Patients & Methods: This study was conducted at Osaka University Hospital and institutions of participating in the Osaka Liver Forum. A total of 551 patients with chronic hepatitis C (332 males, 218 females; mean age 55.6 ± 10.2 y.o.) were treated with combination therapy of Peg-IFN alfa-2b at a dose of 1.5 µg/kg/week and RBV each at 600 mg/day (body weight [BW] ≤ 60 kg) or 800 mg/day (60 < BW ≤ 80 kg) or 1000 mg/day (BW > 80 kg) for 48 weeks. All had HCV genotype 1b with over 100 KIU/ml HCV RNA. Serum HCV RNA level was assessed at week 2 and every 4 weeks during therapy and 24 weeks after the therapy by COBAS AMPLICOR HCV test, v2.0 (<50 IU/ml), and COBAS AMPLICOR GT HCV MONITOR test, v2.0 (5 - 5000 IU/ml). Results: At week 2, 33 out of 39 patients who had undetectable (<5 KIU/ml) or at least a 2-log decrease in HCV RNA achieved SVR (PPV = 85%). Ninety-one of 93 patients with less than a 1-log decrease in HCV RNA did not achieve SVR (91/93, NPV = 98%). At week 4, 21 patients had undetectable serum HCV RNA (<50 IU/ml), and achieved SVR (21/21, PPV = 100%). Seventy-five patients had less than a 1-log decrease in HCV RNA, and none achieved SVR (75/75, NPV = 100%). Similarly, at week 8, 73 patients had less than a 2-log decrease in HCV RNA, and none achieved SVR (NPV = 100%). Conclusion: Early viral kinetics in the first 8 weeks can predict PPV and NPV for SVR; especially useful are the 1-log decrease in HCV RNA at week 4 and 2-log decrease at week 8, which are excellent markers for NPV in Peg-IFN and RBV combination therapy.
PPV and NPV for SVR

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<td>2-log decrease or &lt;5 KIU/ml</td>
<td>2</td>
<td>85% (33/39)</td>
<td>87% (101/116)</td>
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<td>1-log decrease or &lt;5 KIU/ml</td>
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309 IMPACT OF REDUCING PEGINTERFERON ALFA-2B AND RIBAVIRIN ON EARLY VIRAL RESPONSE IN GENOTYPE 1 INFECTED PATIENTS WITH CHRONIC HEPATITIS C


Background & Aim: Patients with early viral response (EVR), defined as undetectable or less than a 2-log decrease in HCV RNA level after 12 weeks of treatment, show a high probability of SVR in pegylated interferon (Peg-IFN) and Ribavirin (RBV) therapy for chronic hepatitis C patients with genotype 1. We evaluated the effect of Peg-IFN alfa-2b and RBV dose reduction on EVR in patients with genotype 1 and high viral load. Patients & Methods: This study was conducted at Osaka University Hospital and institutions of participating in the Osaka Liver Forum. A total of 839 patients with chronic hepatitis C (476 males, 362 females; mean age 56.3 ± 10.0 y.o.) were treated with combination therapy of Peg-IFN alfa-2b and RBV for 48 weeks. All had HCV genotype 1b with over 100 KIU/ml HCV RNA. We also evaluated based on the amounts of Peg-IFN alfa-2b and RBV given during the first 12 weeks of treatment. The mean doses of both drugs were calculated as the average dose per body weight: Peg-IFN, μg/kg/week; RBV, mg/kg/day. We also calculated the complete EVR (c-EVR) rate, defined as undetectable serum HCV RNA from 13 to 24 weeks, achieved SVR. In patients who received 12 - 14 mg/kg of RBV, the c-EVR rate was 36% (5/14) for those given 0.75 μg/kg (50%) - 1.2 μg/kg (80%) of Peg-IFN, 54% (15/28) for those given 1.2 - 1.5 μg/kg of Peg-IFN, and 61% (42/69) for those given 1.5 - 1.8 μg/kg of Peg-IFN. Similarly, the c-EVR rate was 30% (13/43), 56% (104/186), 53% (34/64) in patients who received 10 - 12 mg/kg of RBV, 42% (11/26), 49% (35/71), 56% (22/39) in those who received 8 - 10 mg/kg of RBV, and 36% (5/14), 50% (9/18), 60% (6/10) in those who received 6 - 8 mg/kg of RBV. No decline in c-EVR rate was observed among patients with more than 1.2 μg/kg of Peg-IFN, but the c-EVR rate declined among those with less than 1.2 μg/kg of Peg-IFN (p < 0.001, Fisher exact test). Conclusion: Peg-IFN reduction up to 80% (≥1.2 μg/kg/week) can maintain approximately 50% of EVR regardless of the RBV dosage.

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310 EFFECTS OF SYSTEMATIC NURSE-PROVIDED THERAPEUTIC EDUCATION ON ADHERENCE AND EFFICIENCY OF PEG-INTERFERON-A2A(PEGASYS®)-RIBAVIRIN TREATMENT IN CHRONIC HEPATITIS C (PEGOBS PROTOCOL)

Dominique Larrey, Annie Salsé, Georges-Philippe Pageaux, Nathalie Funakoshi, Didier Ribard, Olivier Bouet, Valérie Hyvart, Byram Nwalongo, Emmanuel Vanche, Jean-Pierre Arpurt 7, André-Jean Rémy, Sohiane Dahmouni, Natalia Kharlova, Jean-Pierre Daurès, 1 Liver Unit/School of Medecine, St Eloi Hospital/INSERMU632, Montpellier, France; 2 Liver Unit, Nimes Hospital, Nimes, France; 3 Liver Unit, Beziers Hospital, Beziers, France; 4 Liver Unit, Ales Hospital, Ales, France; 5 Liver Unit, Narbonne Hospital, Narbonne, France; 6 Liver Unit, Avignon Hospital, Avignon, France; 7 Liver Unit, Perpignan Hospital, Perpignan, France; 8 Clinical Research, Laboratory Roche, Paris, France; 9 Bio-statistics Department, IURC, Montpellier, France

Introduction: Failures of PegIFN-a2a/ribavirin combination in chronic hepatitis C are mainly due to a poor adherence and side effects. TT optimization is currently recommended. However no prospective study has still been performed for assessing the effect of systematised therapeutic education. Aim: to assess the effect of systematic nurse-provided therapeutic education on adherence and efficiency of PegIFN-a2a (Pegasys®)-ribavirin in pts with chronic hepatitis C. Patients and methods: multicentric, prospective study, randomized in 2 groups: GrA: systematic nurse consultation after medical consultation at D0, 4, 8, 12, 24, 36; performed with a standardised questionnaire; GrB: experiment in an experimented center (center 1) and newly formed nurses. A total of 239 randomized pts: GrA 123; GrB 116; center 1: an experimented center (center 1) and newly formed nurses. All had chronic hepatitis C (476 males, 362 females; mean age 56.3 ± 10.0 y.o.) were treated with combination therapy of Peg-IFN alfa-2b and RBV for 48 weeks. All had HCV genotype 1b with over 100 KIU/ml HCV RNA. We also evaluated based on the amounts of Peg-IFN alfa-2b and RBV given during the first 12 weeks of treatment. The mean doses of both drugs were calculated as the average dose per body weight: Peg-IFN, μg/kg/week; RBV, mg/kg/day. We also calculated the complete EVR (c-EVR) rate, defined as undetectable serum HCV RNA from 13 to 24 weeks, achieved SVR. In patients who received 12 - 14 mg/kg of RBV, the c-EVR rate was 36% (5/14) for those given 0.75 μg/kg (50%) - 1.2 μg/kg (80%) of Peg-IFN, 54% (15/28) for those given 1.2 - 1.5 μg/kg of Peg-IFN, and 61% (42/69) for those given 1.5 - 1.8 μg/kg of Peg-IFN. Similarly, the c-EVR rate was 30% (13/43), 56% (104/186), 53% (34/64) in patients who received 10 - 12 mg/kg of RBV, 42% (11/26), 49% (35/71), 56% (22/39) in those who received 8 - 10 mg/kg of RBV, and 36% (5/14), 50% (9/18), 60% (6/10) in those who received 6 - 8 mg/kg of RBV. No decline in c-EVR rate was observed among patients with more than 1.2 μg/kg of Peg-IFN, but the c-EVR rate declined among those with less than 1.2 μg/kg of Peg-IFN (p < 0.001, Fisher exact test). Conclusion: Peg-IFN reduction up to 80% (≥1.2 μg/kg/week) can maintain approximately 50% of EVR regardless of the RBV dosage.
48W : 69.7 vs 53.2%; center 1 : 24W : 94.1 vs 90%; 48W : 79.1 vs 48.8%. HCV RNA disappearance was more frequent (p<0.01) in GrA vs GrB at W12 : 72.8 vs 57.1%; W24 : 75.2 vs 59.8%; EOT : 70.6 vs 52%. For center 1 : W12 : 76.9% vs 55.7%; W24 : 84.1 vs 62.9%; EOT : 80% vs 55%. Sustained virological response (SVR) was significantly higher: 37.7 vs 25% for all pts; naive : 46.4 vs 31%; re-TT : 25 vs 16%; center 1 : overall 48.4 vs 24%; naive : 66.7 vs 37%; re-TT : 32.3 vs 7%. SVR according to genotypes: HCV1, 4, 5 : 30.7 vs 14%; HCV 2, 3 : 50 vs 43%. Center 1 : HCV1, 4, 5 : 41.9 vs 10%; HCV 2, 3 : 61.9 vs 52%. No difference according to the stage of fibrosis. Conclusion: The systematic nurse-provided therapeutic education is significantly associated with a better adherence to the TT and a better virological response. The beneficial effect was more significant in pts treated 48 W and in center with nurse particularity experimented for therapeutic education (center 1). Comments: These results show : 1) the importance of therapeutic education in the TT of hepatitis C particularly in the most difficult groups with relatively resistant genotypes and previous therapeutic failures; 2) the importance of nurse experience in therapeutic education. We thank Roche Laboratory for its support.

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311 CYTOKINES-CHEMOKINES NETWORK IS MODULATED BY PEGYLATED INTERFERON MONOTHERAPY IN CHRONIC HEPATITIS C PATIENTS

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Pegylated-interferon (PEG-IFN) monotherapy is needed in hepatitis C virus (HCV)infected-patients with renal complications and post-transplant recurrent HCV. The aim of the study was to evaluate the cytokine-chemokine network in HCV patients, to correlate serum IL-8, RANTES, tumour necrosis factor alpha (TNFα), and transforming growth factor-beta (TGFβ)levels with the severity of fibrosis, and the responses to the PEG-IFNα-2b at baseline, end-of-therapy and follow-up. METHODS: 180-non-cirrhotic patients were part of a randomized, active-controlled, double-blind-dose-finding clinical trial PegIntron™ (Schering-Plough, Kenilworth, NJ, USA) to study the efficacy of monotherapy at doses of 0.5, 1.0 and 1.5 mg/kg/week for 48 weeks. The patients were grouped by PEG-IFNα-dose received. In each group the data was stratified by responses to therapy: sustained-response-SR (HCV-RNA undetectable 6 months after the end of therapy), relapse-response-RR (HCV-RNA undetectable at the end of therapy) or no-response-NR (detectable HCV-RNA at the end of therapy). Serum cytokines were measured by ELISA. Student-Ttest with Bonferroni correction determined the significance between the groups. The χ2 test or Fisher's exact test compared the frequency of data between groups. RESULTS: There were no statistical differences regarding the demographics, viral load, genotypes, IL8, RANTES, TNFα, and TGFβ grade of inflammation as well as fibrosis scores between groups at baseline. Of 180 patients; 3 had 0 histological-activity-index (HAI), 47 mild, 121 moderate and 9 high; [MHAI 1-50; M HAI-2-84; MHA13-46] and had Metavir-fibrosis [MF0-5; MF1-152; MF2-13; MF3-10]. A good correlation was seen between the HAI and TNF-α levels (r=0.92, p<0.001) in all the patients (r=0.85; p<0.001). IL-8 and RANTES increased significantly at MHAI-3 versus lower MHAI-1-2. TGFβ levels increased significantly with the severity of fibrosis. The kinetics of TNFα during the therapy followed the responses. In SR the levels of TNFα and apoptosis decreased significantly at the end-of-therapy and the follow-up. This is the first study to illustrate that in monotherapy there is a good correlation between the reduction of the HAI and the decrease of TNFα levels and apoptosis. Regardless the dose of therapy, TGFβ levels decrease significantly in all the SR-patients versus their initial values. CONCLUSION: Low baseline serum TNFα is a predictor for sustained response to therapy. PEG-IFN reduces stellate cell activation contributing to reduced fibrosis.

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312 VIRAL KINETICS CAN QUICKLY PREDICT SUSTAINED VIROLOGICAL RESPONSE IN HCV PATIENTS WITH NORMAL ALT TREATED WITH PEGYLATED IFN α2B AND RIBAVIRIN: A PROSPECTIVE STUDY

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Treatment is recommended in HCV patients (pts) with normal ALT only for viral eradication. HCV viral kinetics during antiviral therapy comports a two phase decline. The second slow δ phase is related to infected cell loss. δ phase is predictive of sustained virological response (SVR) and may be estimated by reduction of viral load (VL). In pts with normal ALT, a good estimate of the δ phase is required to determine as soon as possible the need to continue treatment. Aim: to determine the earliest and optimal time when decline of logVL may be predicted.

Methods: The prediction of viral kinetics was expressed by the AUROC curves. Viral load were assessed at 1, 4, 8 hours, days 1 to 4, 7, 14, 21, 28 and 2 and 3 months during treatment. Results: 24 pts [17 with genotype 1) were included. On overall patients, the median ALT level was 26±6 IU/L. There was no significant fibrosis. Median baseline VL was 284500 IU/ml (95% CI: 22000-320000). SVR was achieved in 62% (53% for genotype 1 pts). On overall patients, reduction of VL at day 21 was the earliest time estimating SVR (AUROC curve of logVL: 0.848±0.086, p<0.0001) but day 28 has the highest AUROC curve of logVL for estimating SVR (0.981±0.02, p<0.0001). Months 2 and 3 didn't have better AUROC curves of logVL than day 28. In a sensitivity analysis restricted to pts with genotype 1, reduction of VL at day 28 had a better predictive value of SVR than reduction of VL at day 21 (AUROC curves of logVL: 0.962±0.04 vs 0.759±0.13, p=0.02). No SVR was observed in genotype 1 pts with a decline of VL <1 log from baseline to day 28. In terms of optimal cut-off in genotype 1 pts, a decline of VL ≥2 log at day 28 was the best predictor of SVR (sensibility 95%, specificity 76%.
The elimination of infected cells is highly important for response to antiviral treatment in patients with chronic hepatitis C. The mechanisms of the elimination of infected cells are not well understood. Cell death by apoptosis could be highly relevant for the elimination of infected cells. Aim of the present study was to investigate 1. Dynamics of apoptosis during antiviral treatment and the relevance of apoptosis for viral kinetics and infected cell loss in patients with chronic hepatitis C during antiviral therapy and 2. To compare apoptosis dynamics with dynamics of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and gamma glutamyl transpeptidase (GGT).

**Methods.** Apoptotic activity was monitored by quantification of apoptotic cytokeratin-18 neoepitopes in serum from patients with chronic hepatitis C (n=16) before, several times during and after treatment with pegylated interferon alfa-2a and ribavirin. The dynamics of apoptotic activity was compared with ALT, AST and GGT, and the viral kinetic parameter infected cell loss delta. **Results.** During the first week of antiviral treatment only minor changes of apoptotic activity were observed. After 4 weeks of treatment, apoptotic activity declined significantly compared with baseline. Later during treatment, however, apoptotic activity increased again to levels similar to baseline. After treatment, apoptotic activity declined significantly. Overall, GGT levels showed a similar kinetic profile during and after treatment. ALT activity also showed a significant decline at week 4 compared with baseline but, in contrast to apoptotic activity, ALT remained on a reduced level during and after the treatment period. AST showed a slower and weaker decline than the ALT activity. Baseline apoptotic activity was inversely correlated with the infected cell loss while an increase of apoptotic activity within the first 4 treatment weeks compared with baseline was positively correlated with the infected cell loss.

**Conclusion.** Apoptosis appears to be an important form of cell death during interferon alfa based treatment which is associated with infected cell loss and is underestimated by ALT and AST activity.
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Hepatitis C Treatment Outcomes in the Rhode Island Department of Corrections

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Introduction: As many as 83% of the USA’s 2 million injection drug users are incarcerated at some time. An estimated 29-43% of hepatitis C virus (HCV)-infected people in the USA are released from prisons or jails yearly. We report our experience with pegylated interferon (PEG-INT) plus ribavirin treatment of 71 HCV RNA-positive male inmates at the Rhode Island Department of Corrections (RIDOC). Methods: We reviewed charts of all male inmates identified as having initiated HCV treatment between October 2000 and April 2004. HCV-infected individuals were identified by HCV antibody screening at intake for known risk factors, elevated aminotransferase levels, or per individual request. Treatment followed standard guidelines with weight-based dosing of both PEG-INT afo-2b and ribavirin. End of treatment response (ETR) was defined as undetectable serum HCV RNA at end of treatment, and sustained virologic response (SVR) as undetectable serum HCV RNA 6 months post-treatment. Endpoints were completion of therapy plus 6 months for SVR, therapy discontinuation, and loss to follow-up. Results: At data collection, 71 patients had reached an endpoint as defined above. The majority was white (80%). The cohort included genotype 1 (65%), genotype 2 (17%), genotype 3 (13%), genotype 4 (5%). Ninety-nine percent had a history of substance use (drug injection, non-injection drug use, or alcohol use). All 13 African-Americans (AA) had genotype 1. Of 59 patients having liver biopsy, 41 (70%) had early stage disease, defined as stage 0-1, 1-2, or 2. Overall ETR was 39% (28 of 71) and SVR, 28% (20 of 71). Response rate was lower for genotype 1 (ETR 33%, SVR 18%) compared to genotype 2 (ETR 64%, SVR 60%) and genotype 3 (ETR 56%, SVR 50%). Of inmates with genotype 1, no statistical difference existed in ETR or SVR by race (33% ETR in AA vs. 34% in whites; 22% SVR in AA vs. 18% in whites). Thirty-three patients completed treatment and 31 stopped for reasons including side effects (26) or initial non-response (5); 7 did not return for follow-up. Conclusion: Our results support the limited data available that acceptable HCV treatment outcomes can be achieved in prisons. Our small study indicates a difference in treatment response by genotype, but no difference by African-American vs. white race for genotype 1. Incarceration may be the only time that this difficult-to-access population intersects with the health care system long enough for evaluation and treatment. The correctional system has unique potential for HCV management and reducing the reservoir of infection, targeting those at high risk of transmission.

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The Cerebral Metabolic and Cognitive Effects of Pegylated Interferon (PIFN) and Hepatitis C Viral (HCV) Clearance

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Background: Altered cerebral metabolites and cognitive dysfunction are common findings in chronic HCV infection. The neurologic benefit of HCV clearance has not been well described. Aim: To assess the effect of PIFN and HCV clearance on cerebral metabolites, cognition and mood. Methods: Fifteen patients with non-cirrhotic HCV were evaluated by MRS/MR spectroscopy, neuropsychometric testing and Beck’s depression inventory before, during and after treatment with PIFN. Three cerebral metabolites, including choline (Cho), myoinositol (MI) and N-acetyl aspartate (NAA), were measured from 3 different brain regions (basal ganglia, left frontal cortex and left dorsal-lateral prefrontal cortex) and expressed as a ratio to the control metabolite creatine (Cr). Seven HCV positive controls (not taking PIFN) were also assessed at 2 time points, 3 months apart to determine (i) the variability of cerebral metabolites over time and (ii) the ‘practice effect’ of repeat cognitive testing. Results: At 12 weeks, 13/15 patients had undetectable HCV RNA (<600IU/ml). Of these, 8 were persistently negative for HCV RNA, 5 relapsed, and 1 was lost to follow up. PIFN therapy had no effect on cerebral metabolites. Cerebral Cho/Cr and MI/Cr were significantly reduced in the basal ganglia at the time of follow up when compared to baseline in sustained virologic responders (SVR) but not in non-responders/relapsers (NR/R), p=0.03 and p=0.03, respectively. There were no significant differences observed in cerebral metabolites over time in controls. Memory and executive functioning significantly improved on PIFN when compared to baseline in treated patients (p=0.006, p= 0.002) and in controls (p=0.03 and 0.02). This improvement was preserved in SVRs (p=0.02 and p=0.04) but not in NR/R. Attention significantly improved on PIFN in treated subjects (p=0.03). Language and motor skills were unaffected by PIFN or viral clearance. There was a significant increase in depression scores on PIFN (p=0.02) but no change when compared to baseline in SVRs and NR/R. Conclusion: PIFN does not alter cerebral metabolites but improves cognition in HCV patients. HCV clearance decreases metabolic markers of cerebral inflammation and activation and improves cognition in SVRs, likely due to the clearance of HCV from brain tissue. This study demonstrates the cerebral benefit to HCV clearance.

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317 ACRYOLEIN, A DERIVATIVE OF ENDOGENOUS LIPID PEROXIDATION AND A COMMON ENVIRONMENTAL POLLUTANT, INHIBITS INTERFERON-ALPHA MEDIATED ANTIVIRAL SIGNALING: IMPLICATIONS FOR HCV THERAPY
Swati Joshi-Barve, Kiranmayi Amancherla, Madhuvanti Patil, Aruni Bhatnagar, Sanjay Srivastava, Matthew C. Cave, Leila Gobejishvili, Craig J. McClain, Shirish Barve

318 THE LEVEL OF PRETREATMENT HCV CORE ANTIGEN IS A NEW AND USEFUL PREDICTOR FOR THE EFFICACY OF PEGINTERFERON ALPHA-2B AND RIBAVIRIN IN PATIENTS WITH CHRONIC HEPATITIS C
Satoshi Shakado, Daisuke Morihara, Shinya Nishizawa, Akira Anan, Takashi Tanaka, Shinjiro Inomata, Syuchi Ueda, Teruo Matsumoto, Yasuaki Takeyama, Makoto Irie, Koaru Iwata, Tetsuro Sakoda, Shotaro Sakisaka

319 HEPATOCELLULAR CARCINOMA IN LONG-TERM SUSTAINED VIROLOGIC RESPONDERS TO ANTIVIRAL TREATMENT FOR CHRONIC HEPATITIS C
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BACKGROUND: Antiviral treatment results in a sustained virologic response (SVR) in 50 to 75% of patients with chronic hepatitis C. Long term follow up studies have observed ongoing SVR in the overwhelming majority. Thus chronic hepatitis C is considered “cured” if a SVR is achieved. Consequently, it is expected that in SVRs long term complications of HCV related chronic liver disease including HCC are eliminated or have a decreased incidence. We report on 5 patients (#1-3 from Austria, #4-5 from USA) who developed HCC during follow up after achieving SVR. During follow up and at diagnosis all were HCV-RNA neg. The key clinical details are summarized in Table 1. #1: After a relapse following a six months interferon (IFN) monotherapy he achieved a SVR after one year treatment with IFNα-2b + IVW and ribavirin (RBV) in 1999. Ultrasoundography in
12/05 showed a normal liver, but a tumour in the right adrenal. The biopsy revealed a metastasis from HCC. At resection the macroscopic appearance of the liver was normal. 8 months later a tumour in the right adrenal was resected and was a metastatic HCC. 

#2: After two 48 week courses (relapse after 1. treatment) of pegIFNα2a/RBV she achieved a SVR in 2001.4 years later an elevated AFP level (47ng/ml) was noted; liver sonography was normal, but revealed a 5 cm HCC in segment 5/6 a year later. At segmental resection the liver was firm but macroscopically did not have cirrhosis. HCV-RNA was undetectable in the tumour tissue. 

#3: Following two courses (relapse after 1. treatment) of pegIFNα2a/RBV she achieved a SVR in 2004. In 2007 she complained of upper abdominal discomfort and sonography showed a multifocal HCC. 

#4: After 2 years treatment with 60 000 IU/week PEG-IFN-2b and 800mg/d RBV he achieved an SVR in 2001. A large HCC was detected in 2006. It was unresectable and he was placed on chemotherapy. 

#5: Pretreatment liver biopsy showed stage 0 fibrosis. He achieved SVR following 1 year PEGIFN α2b/RBV in 2003. HCC was diagnosed in 2006. A staging laparoscopy revealed a non-cirrhotic liver, but tiny peritoneal implants in the peritoneum. He was placed on chemotherapy. CONCLUSION: Successful antiviral treatment in HCV patients does not prevent development of HCC even in non-cirrhotic livers. Long-term follow up of patients with SVR is mandatory and should include screening for HCC.

### Table 1

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<th>Pat.</th>
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<td>2007 (51)</td>
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<td>2006 (55)</td>
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<td>0</td>
<td>1b</td>
<td>i.v. drugs</td>
<td>2003</td>
<td>2006 (52)</td>
</tr>
</tbody>
</table>

*pretreatment

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Peter Ferenci - Consultant/Adviser: Roche; Consultant/Adviser: Roche; Consultant/Research Support: Vertex; Consultant/Adviser: Novartis
The following people have nothing to disclose: Thomas-Matthias Scherzer, Katharina Stauter, Harald Hofer, Petra Steindl-Munda

### 320 SYSTEMIC FACTORS ASSOCIATED WITH VIROLOGIC NONRESPONSE TO PEGINTERFERON/RIBAVIRIN TRETREATMENT OF CHRONIC HEPATITIS C

Karen L. Lindsay1, Chihiro Morishima2, Elizabeth C. Wright2, Jules L. Dienstag2, Mitchell L. Shiffman2, Gregory T. Everson2, Anna S. Lok2, Herbert L. Bankovsky3, Timothy R. Morgan1, Marc Ghany1, HALT-C Trial Group For the

Methods:

- High rates of sustained virological response (SVR) in patients >50 years infected with HCV genotype 1 with positive prognostic factors treated with peginterferon alfa-2a (PEGASYS®) and ribavirin (COPEGUS®)
- The prognosis of pts with chronic HCV is influenced by viral factors such as HCV genotype (G) and HCV RNA level as well as pt factors such as age, gender, race, cirrhosis and drug adherence. The demographics of HCV infection are shifting with an increasing number of pts now in their 50's. To assess the impact of age on SVR rates we analyzed the data from two large phase III studies of PEG-IFN alfa-2a (40KD) plus ribavirin (RBV) (Fried et al. NEJM 2002 and Hadziyannis et al. Ann Intern Med 2004). **Methods:** We included pts who had HCV G1 and were randomised to 48 wks of treatment with peginterferon alpha and ribavirin. Very little information is available to explain why virologic suppression does not occur in some patients.

**RESULTS:** We evaluated potential factors associated with the lack of a week-20 virologic response in a cohort of previous nonresponder patients with advanced fibrosis undergoing retreatment with peginterferon alfa-2a and ribavirin. Among 1,145 patients enrolled in the HALT-C Trial, 588 who received more than 80% of prescribed therapy for 20 weeks were analyzed. Medication compliance was assessed at each visit by patient self-report, interviews, and returned peginterferon vial counts. Factors related to null response in univariate analyses were entered into a multivariate logistic regression with backward stepwise selection to assess their relative importance. Analyses were performed with the SAS version 9.1.

**CONCLUSIONS:** On-treatment variables associated with virologic null response to peginterferon with ribavirin suggest the lack of a systemic response to interferon, perhaps related to host genetic or environmental factors. These data have important implications for the design and analysis of retreatment trials and trials to evaluate new specifically targeted antiviral therapy for hepatitis C. Further studies are needed to discern whether interferon resistance, viral resistance, or both are playing a role in treatment-adherent patients who have no virologic response to interferon treatment.

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The following people have nothing to disclose: Thomas-Matthias Scherzer, Katharina Stauter, Harald Hofer, Petra Steindl-Munda

### 321 HIGH RATES OF SUSTAINED VIROLOGICAL RESPONSE (SVR) IN PATIENTS >50 YEARS INFECTED WITH HCV GENOTYPE 1 WITH POSITIVE PROGNOSTIC FACTORS TREATED WITH PEGINTERFERON ALFA-2A (40KD) (PEGASYS®) AND RIBAVIRIN (COPEGUS®)

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**Background:** The progosis of pts with chronic HCV is influenced by viral factors such as HCV genotype (G) and HCV RNA level as well as pt factors such as age, gender, race, cirrhosis and drug adherence. The demographics of HCV infection are shifting with an increasing number of pts now in their 50's. To assess the impact of age on SVR rates we analyzed the data from two large phase III studies of PEG-IFN alfa-2a (40KD) plus ribavirin (RBV) (Fried et al. NEJM 2002 and Hadziyannis et al. Ann Intern Med 2004). **Methods:** We included pts who had HCV G1 and were randomised to 48 wks of treatment with
PEG-IFN alfa-2a (40KD) 180µg/wk plus RBV 1000/1200mg/d. SVR was defined as undetectable HCV RNA by qualitative PCR after 24 wks of untreated follow-up. Results: Overall, the SVR rate in pts ≤50 years was 52.3% (n=438) and 38.9% in the 131 pts who were >50 (p=0.007). SVR rates in pts aged >50 years were heterogeneous and varied according to previously well-established prognostic factors (Table). In particular, SVR rates were higher in pts without advanced fibrosis, those with low baseline serum HCV RNA levels, those pts who received ≥80% of the planned dose of PEG-IFN and those patients who received ≥60% of the planned dose of RBV. Overall, age did not influence whether pts completed therapy (75.6% of pts ≤50 vs 73.3% of pts >50 years [p=0.5954]). However, there was a trend towards lower cumulative PEG-IFN exposure in older pts (7235µg in pts ≤50 vs 6868µg in pts >50 [p=0.0098]) and a significantly lower cumulative RBV exposure in older pts (304g in pts ≤50 vs 252g in pts >50 [p=0.0001]). Decreasing RBV exposure to <60% of target has been associated with a dramatic reduction in SVR rates (Reddy et al. Clin Gastro Hepatol 2007). In our study more pts >50 years had dose reductions resulting in <60% of target RBV exposure than pts ≤50 years (38% vs 22%). Discontinuation rates due to any AE was similar in those ≤50 (11.4%) compared to those >50 years old (11.5%). Conclusions: SVR rates in certain subgroups (those without advanced fibrosis, low HCV RNA levels) of older pts are comparable to younger pts, especially if high rates of adherence can be maintained. Although dose reductions of RBV are more common in >50 years of age, these pts can be effectively treated.

Characteristics and treatment outcomes in 131 pts aged >50yrs

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Prevalence (%)</th>
<th>SVR (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male : Female White : Black : Other race</td>
<td>BMI &lt;30 : ≥30</td>
<td>60 : 40</td>
</tr>
<tr>
<td>Cirrhosis/bridging : Minimal Fibrosis</td>
<td>54 : 66</td>
<td>28.9 : 44.2**</td>
</tr>
<tr>
<td>HCV RNA ≥400000 &lt;1000000 IU/mL</td>
<td>23 : 77</td>
<td>56.7 : 33.3**</td>
</tr>
<tr>
<td>ALT Quotient ≤5 : &gt;5</td>
<td>63 : 37</td>
<td>38.6 : 39.6*</td>
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<td>25 : 14</td>
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*p=NS; p<0.01; **p<0.05
Disclosures:
Rajender Reddy - Grant/Research Support: Roche; Consultant/Adviser: Roche; Speaker’s Bureau: Roche
Diethelm Messinger - Consultant/Adviser: Roche
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Stephanos J. Hadziyannis - Consultant/Adviser: Roche; Speaker’s Bureau: Roche

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LOW DOSE PEGINTERFERON ALFA-2A AND RIBAVIRIN FOR CHRONIC HEPATITIS C, GENOTYPE 2 & 3: VIRAL KINETICS, EFFICACY AND SAFETY

Yaron Rotman, Brian B. Borg, Alejandro Soza, Rohit Loomba, Apurva A. Modi, Jordan J. Feld, Elenita Rivera, Edward Doo, Theo Heller, Marc Ghany, Avidan U. Neumann, T. Jake Liang, Jay H. Hoofnagle

Introduction: Chronic infection with hepatitis C virus (HCV) genotypes 2 & 3 is highly responsive to the combination therapy with peginterferon and ribavirin. Use of lower doses of peginterferon and ribavirin may reduce side effects without loss of efficacy. Aim: To determine whether a lower dose of peginterferon is better-tolerated and just as effective as the standard dose and to compare the viral kinetics of the two regimens.

Methods: Patients were treated with peginterferon alfa-2a (kindly provided by Roche Laboratories, Nutley, NJ) in a dose of 90 μg/week (low-dose regimen, LD) or 180 μg/week (standard dose, SD) in combination with ribavirin (800 mg/day) for 24 weeks. Patients who failed to become HCV RNA negative by week 12 or who relapsed after treatment were eligible for a 48-week course of standard dose (extended treatment, ET).

Results: 30 patients were treated with the LD and, to date, 18 of a planned 30 patients with the SD regimen. Among those receiving LD, 19 (63%) achieved an SVR compared to 10 of 12 patients (83%) receiving SD who have completed treatment and follow up. The 3 non-responders and 4 of 8 relapsers after LD therapy were retreated with ET, and all but one (86%) achieved an SVR. Viral kinetic analyses showed that the mean reduction in HCV RNA levels at 48 hours (first phase) was -1.1 log with LD vs. -2.5 log with SD (p=0.0011). This difference persisted up to day 14 (-2.6 vs. -3.6, p=0.04) and the mean second phase slope was less with LD (-1.2 log/week) than SD (-2.3 log/week) (p=0.08). Patients receiving SD became HCV RNA negative earlier than those in the low-dose group (log rank, p<0.01), such that by week 12, all patients treated with SD (100%) but only 86% treated with LD were HCV RNA negative. Viral kinetics could not predict relapse, the most common cause for treatment failure. In the 7 LD patients who were retreated with ET, viral kinetic measurements were superior with the standard dose of the ET regimen than the initial low dose. Side effects were measured using a visual analogue scale. Patients on LD peginterferon had significantly less fatigue and felt better overall during therapy than patients receiving SD. Depression scores, however, did not differ. Serious adverse events occurred in 2 patients in both groups (sarcoidosis and acute coronary event with LD; fatal drug overdose, and severe mastoiditis and anemia in SD group). Conclusion: Although better tolerated, a reduced dose of 90 μg/week of peginterferon is significantly less potent and, in combination with 800 mg of ribavirin, provided an unacceptably lower SVR rate in patients with chronic hepatitis C, genotypes 2 and 3.

Disclosures:
The following people have nothing to disclose: Yaron Rotman, Brian B. Borg, Alejandro Soza, Rohit Loomba, Apurva A. Modi, Jordan J. Feld, Elenita Rivera, Edward Doo, Theo Heller, Marc Ghany, Avidan U. Neumann, T. Jake Liang, Jay H. Hoofnagle

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IMPACT OF PEGYLATED INTERFERON α AND RIBAVIRIN THERAPY OF CHRONIC HEPATITIS C ON MAJOR SUBSETS OF PERIPHERAL BLOOD AND INTRAHEPATIC LYMPHOCYTES

Krzysztof Tomaszewicz, Romana Modrzewska; Department of Infectious Diseases, Medical University of Lublin, Lublin, Poland

Objective: The role of cellular immune response in pathogenesis of liver cell injury and course of chronic hepatitis C has been previously confirmed. The aim of this study was to determine the modification of T-lymphocyte major subsets during pegylated interferon α and ribavirin treatment and its correlation with response to the treatment. Methods: The assessment of subsets of intrahepatic lymphocytes (IHL) and peripheral blood lymphocytes (PBL) was done in 62 patients with chronic hepatitis C using flow cytometry and immunohistochemical study. CD8+ specific cells were measured using Dimer-X HLA-A2.1: fusion protein [Becton Dickinson] and PepMix HCV (NS3) system prepared by JPT Peptide Technologie GmbH. PepMix includes 143 peptides deriving from NS3 region [serine protease, NTP-dase and helicase] of HCV. Patients were classified as (1) complete responders (CR) with early viral response (EVR) and sustained viral response (SVR); (2) partial responders (PR)
with EVR, no SVR but ALT normalization, and [3] non-responders (NR) with neither EVR nor SVR and with elevated ALT levels 6 months after the treatment. Results: Significant increase in peripheral blood CD4+ cells, was observed in week 12 of treatment in patients with both complete and partial response to therapy (45,9±5,4% versus 57,3±8,4%, p<0,001 and 42,8±6,6% versus 49,4±7,1%, p<0,01, respectively). There were no significant modifications in nonspecific CD8+ subsets, but after 12 week of treatment in patients with response to therapy, decrease or lack of HCV-specific CD8+ cells were observed (0,26±0,14% versus 0,07±0,04%, p<0,0001). The assessment of subsets of intrahepatic lymphocytes revealed a significantly larger percentage of CD8+ cells in pretreatment liver biopsies from patients with sustained viral response (36,6±5,5% in CR versus 29,8±7,1% in PR, p=0,001 and 26,9±6,3% in NR, p<0,001). Conclusions: The results confirmed the impact of interferon α and ribavirin treatment on cellular response. Some immunological host factors should be considered in the early prognosis of successful treatment. Decrease of HCV-specific CD8+ cells during PEG-Interferon treatment may predict complete response to therapy. Increase in nonspecific CD4+ PBL has been documented both in complete and partial responders and may support continuation therapy and better prognosis.

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PREVALENCE OF BIPOLAR AFFECTIVE DISORDER IN PATIENTS WITH A POSITIVE DEPRESSION SCREEN AT THE INITIATION OF INTERFERON THERAPY FOR CHRONIC HEPATITIS C
Jeffrey S. Olson1, Atif Zaman1, Kenneth D. Ingram1, James R. Phelps2; 1Gastroenterology, OHSU, Portland, OR; 2PsychEducation.org, Corvallis, OR

Purpose: Interferon therapy is associated with worsening of underlying depression, and clinicians frequently screen for depression before starting interferon. Bipolar affective disorder (BAD) patients often present with symptoms of depression rather than mania, and as a result these patients can be misdiagnosed with unipolar depression if a positive depression screen is not followed up with an appropriate screen for subtle or inactive manic traits. Our aim was to assess the prevalence of BAD in hepatitis C patients with a positive depression screen, and to determine if BAD is associated with an increased rate of psychiatric complications during interferon therapy. Methods: Retrospective study performed at the OHSU Hepatology Clinic from 12/04 to 9/06. All adult patients initiating interferon therapy (n=112) were given the Physicians Health Questionnaire (PHQ-9); a validated, self-administered survey of depressive symptoms used as a sensitive screening tool for unipolar depression. Patients with a positive PHQ screen were asked to complete the Mood Disorders Questionnaire; a validated, sensitive and specific screening tool for bipolar disorder. Chart review was performed on all 112 patients for the six month period following initiation of interferon. Psychiatric complications (change in psychiatric medications, referral to psychiatry, visits to the ER, hospital admissions, and discontinuation of interferon therapy) were recorded. Results: Three groups were formed: Group A: 89 patients had a negative PHQ depression screen; and adverse psychiatric events occurred in 22 patients (24.7%). Group B: 16 patients had a + PHQ depression screen, but a negative MDQ screen for manic traits; and adverse events occurred in 3 patients (18.8%). Group C: 7 patients had a + PHQ depression screen, and a + MDQ screen for manic traits; and adverse events occurred in 5 patients (71.4%). Chi Square comparison of groups showed a statistically significant increase in adverse events in group C compared with group A (p=0.045). The increased rate of complications in group C compared with group B approached statistical significance (p=0.169). Conclusions: This is the first study of its kind to evaluate the rate of BAD in interferon patients with a positive depression screen. BAD was defined by a + MDQ screen was common (7/23 patients), and was also associated with a markedly higher rate of psychiatric complications during interferon treatment when compared to both patients without depression, and to patients with depression without manic traits. This preliminary study highlights the importance of follow up screening for BAD in patients with a positive depression screen.

Disclosures:
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PATIENT EDUCATION IMPROVES ADHERENCE TO PEGINTERFERON α-2B AND RIBAVIRIN IN CHRONIC GENOTYPE 2 OR 3 HEPATITIS C VIRUS INFECTION: A PROSPECTIVE, REAL-LIFE STUDY (CHEOBS STUDY)
Patrice Cacoub1, Denis Ouzan2, Thierry Fontanges3, Jean-Philippe Lang4, Pascal Melin5, Michel Rothöf6, Marina Varaster2, Patrick Marcellin7, Michel Chousterman7; 1Hôpital Pitié Salpêtrière, Paris, France; 2Institut Arnaud Tzanck, Saint Laurent du Var, France; 3Centre de l’Appareil Digestif, Bourgoin Jallieu, France; 4Centre Hospitalier Erstein, Erstein, France; 5Hôpital Général, Saint Dizier, France; 6INSERM, Bagneux, France; 7ClinSearch, Bagneux, France; 8Hôpital Beaujon, Clichy, France; 9Hôpital de Créteil, Créteil, France

Objective: The CHEOBS study is a French multicenter, prospective, observational study designed to analyse the factors related to compliance with the combination treatment with peginterferon α-2b (peg-IFN) and ribavirin (RBV) in patients with chronic hepatitis C virus (HCV) infection. Main results in patients with a genotype 2 or 3 infection are presented. Patients and Methods: From January 2003 to December 2004, 705 out of 2001 patients included were infected with a genotype 2 or 3 virus among which 674 patients had sufficient data to be analyzed: 370 patients [group 1] received an educational program to optimize the tolerance and the efficacy of HCV treatment whereas 304 pts [group 2] had no specific formation. Baseline characteristics, impact of the educational program on compliance and sustained virological response (SVR = 6 months after stopping therapy) were assessed. Results: The mean age was 45 ± 11 years, 59% were male, 17% were unemployed and 6% had poor socio-economic conditions. Patients were excesive alcohol consumers (170, 25%), smokers (354, 53%), drug abusers (current [28, 4%], past [332, 50%]), past depressive alcohol consumers (170, 25%), smokers (354, 53%), drug abusers (current [28, 4%], past [332, 50%]), had past depression (180, 27%) and/or current psychiatric disorders (158, 24%). HCV viral load was >800,000 IU for 173 patients (38%) and genotype was 2 [202, 30%] or 3 (472, 70%). A high stage of fibrosis was frequent: Metavir F2-F3 (211, 42%), cirrhosis (69, 14%). Co-morbidities included HIV co-infection (26, 4%), HBV co-infection (9, 1%), or other chronic diseases (153, 23%). There was no significant difference on all these parameters between the groups, except that patients of group 1 had more frequently a past depression (31% vs 22%, p = 0.014), current psychiatric disorders (27% vs 20%, p = 0.044) and a current drug use (6% vs 2%, p = 0.01). At month 6, adherence to bitherapy was 61% in educated and 48% in non-educated patients (p=0.01). Adherence to pegIFN was 78% and 69% (p=0.06); adherence to RBV was 76% and 56% (p=0.006), respectively. The overall SVR was 72% (308/511).
The rate of SVR tended to be higher (75% vs. 68%) and the rate of relapse lower (11% vs. 16%) in educated patients. Education was associated with improved adherence (odds ratio 1.58, p=0.04) but not with SVR (odds ratio 1.43, p=0.12). Conclusions: In clinical practice, patients with a genotype 2 or 3 HCV chronic infection have a difficult-to-treat profile as they frequently experience past or ongoing mental disorders, drug abuse or cirrhosis. In such patients, a high rate of SVR (72%) can be obtained with a combination of peg-IFN plus RBV. The therapeutic education helped maintain adherence to HCV therapy. The beneficial impact on virological response was not significant.

Disclosures: The following people have nothing to disclose: Patrice Cacoub, Denis Ouzan, Thierry Fontanges, Jean-Philippe Lang, Pascal Melin, Michel Rotily, Marina Varastet, Patrick Marcellin, Michel Chaoumtarian

326 PEGINFERON ALFA-2B AND RIBAVIRIN TREATMENT OF PATIENTS WITH CHRONIC HEPATITIS C AND NORMAL VERSUS ELEVATED AMINOTRANSFERASE LEVELS – FINAL RESULTS OF A PROSPECTIVE OPEN TRIAL

Wolfgang Vogel1, Harald Brunner2, Andreas Maierson3, Ivo Graziaidei1, Martha Rosenbeiger2, Rudolf E. Stauber2, Daniela Wolkersdorfer1, 1Medical University Innsbruck, Innsbruck, Austria; 2KH Kitzing, Vienna, Austria; 3KH Elisabethinen, Linz, Austria; 4KH Leoben, Leoben, Austria; 5Graz Medical University, Graz, Austria

Prognosis of chronic hepatitis C (CHC) seems to be similar in patients with persistently normal transaminases (PNALT) and in those with elevated ALT (EALT). In addition, the response to therapy with interferon and ribavirin is similar in both groups of patients. However, further studies are needed. In this prospective, multi-center, open-labeled clinical trial 231 treatment-naive patients with biopsy-proven CHC with normal or elevated ALT levels received PegIntron 1.5 µg/kg/wk & Rebetol 0.8-1.2g/d for 48 weeks (genotype 1/4) or 24 weeks (genotype 2/3). Study design: Patients were stratified according to elevated or normal (median of 3 measurements over >3 months) ALT, high or low viral titer (cut-off 800,000 IU/ml, bDNA HCV RNA 3.0 assay; Bayer Diagnostics) and genotypes (GT) 1/4 or 2/3. From these 231 patients 47.6% had PNALT and 52.4% had EALT. 76.6% with GT 1 or 4 (77.3% of the PNALT and 76% in the EALT group) and 23.4% patients with GT 2 or 3 (22.7% of the PNALT and 23.9% in the EALT group) were enrolled. The median ALT in the PNALT group was 28 U/ml (GT 1 or 4) and 24 U/ml (GT 2 or 3) and 78 U/ml (GT 1 or 4) and 131 U/ml (GT2 or 3) in the EALT group. Results: The pretreatment characteristics were comparable between the two groups. 65.5% of patients with PNALT had a viral load lower or equal 800,000 IU/ml vs. 66.4% of patients with EALT. Results of liver biopsies at baseline were available from 147 patients. 60.5% of these patients had fibrosis grade 0 or I (70.8% PNALT, 50.6% EALT). There was a difference in the fibrosis grade between the two groups (p=0.0188). From 119/231 patients viral load was determined after week 4: 45.4% had undetectable RNA (46.9% PNALT and 43.6% EALT); after week 12 177/231 pts. were evaluated: 74.6% had undetectable RNA (80.7% PNALT and 69% EALT). 70.6% had undetectable RNA at end of treatment (73.6% normal ALT and 67.8% EALT; 65.5% GT 1/4 and 87% GT 2/3). 6 months after end of treatment (SVR) 49.4% had undetectable RNA (50.9% normal ALT and 47.9% EALT; 65.5% GT 1/4 and 87% GT 2/3). SVRs were significantly worse in the PNALT and EALT group in patients with high viral load (PNALT vs. EALT: 34.5% vs. 33% and 65.5% vs. 67%) but not between groups. Conclusions: Sustained virological response rates after treatment with PegIntron 1.5 µg/kg/wk & Rebetol 0.8-1.2g/d in patients with CHC is independent of elevation of transaminases and are the same in groups stratified for genotype and viral load. In this study patients with EALT had higher levels of fibrosis.

Disclosures: Daniela Wolkersdorfer - Employee: Schering-Plough

327 COMPARISON OF EFFICACY OF TREATMENT WITH PEGINFERON ALFA-2A PLUS RIBAVIRIN VS PEGINFERON ALFA-2B PLUS RIBAVIRIN AMONG PATIENTS CHRONICALLY INFECTED WITH NON 2/3 HCV GENOTYPES WITH LOW AND HIGH PRETREATMENT VIRAL LOAD

Hanna Berak1, Anna Kolakowska-Rzadzka1, Marek Wasilewski1, Janusz Stanczak2, Katarzyna Szamatulska3, Krzysztof Bardadin3, Andrzej Horban1, 1Hospital for Infectious Diseases, Warsaw, Poland; 2Institute of Mother and Child, Warsaw, Poland; 3Medical Centre for Postgraduate Education, Warsaw, Poland

PATIENTS: 212 patients chronically infected with non 2/3 HCV genotypes were treated 48 weeks with peginterferon alfa-2a (Pegasys, group A) and peginterferon alfa-2b (Peginteron, group B), both plus standard doses of ribavirin. A and B pts were divided into groups with low (<600,000 IU/ml) and high (>600,000 IU/ml) pretreatment viral load (VL) to compare the efficacy of treatment. 17 pts were lost to observation (9 in group A and 8 in group B). The analysis concerned 195 (92%) pts: 92 (91.1%) treated with Pegasys and 103 (92.8%) treated with Peginteron. METHODS: Liver biopsies were analyzed according to the Knodell’s and Scheuer’s scores. The main study outcome was undetectable HCV RNA on week 72 (SVR), 24 weeks after end of treatment. HCV RNA was performed, determined with HCV RNA ASSAY and viral load (VL) with CA HCV MONITOR TEST (both of ROCHE DIAGN SYS.). Statistical analysis was performed with Chi-squared. RESULTS: No statistically significant difference was found on the positive SVR in group A vs B and low pretreatment VL: 20 (66.7%) pts in group A and 17 (58.6%) in group B (p=0.523). Ten (33.3%) pts in group A and 12 (41.4%) in group B had detectable HCV RNA. Thirty (48.4%) pts with high pretreatment VL in group A and 32 (43.2%) in group B had positive SVR (p=0.549), and 32 (51.6%) in group A and 42 (56.8%) in group B had detectable HCV RNA. There were higher proportions of patients with SVR treated with peginterferon alfa-2a (Pegasys). The low pretreatment VL is a good predictor of SVR.

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<td>HCV RNA baseline virelia</td>
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gender: *p=0.895, **p=0.118
PEG INFERENCE ALFA-2A MONOTHERAPY IN DIALYSIS PATIENTS INFECTED WITH HEPATITIS C VIRUS

Reinhart Zachoval1, Peter Buggisch2, Petra Reinke3, Rainer Waitas4, Ann Michelsen5, Michael Fuchs6, Wulf Böcher7, Ewert Schulte-Frohlinde8, Jens Encke9, Birgit Kallinowski9. 1Department of Internal Medicine, Ludwig-Maximilians-University, Munich, Germany; 2Department of Internal Medicine, University of Hamburg, Hamburg-Eppendorf, Germany; 3Department of Nephrology, University of Mainz, Mainz, Germany; 4Department of Internal Medicine, Technical University of Munich, Munich, Germany; 5Department of Internal Medicine, University of Heidelberg, Heidelberg, Germany

AIM: Hepatitis C is an important cause of liver related morbidity and mortality under hemodialysis. Data about the efficacy of antiviral treatment with pegylated interferons (PEG-IFNs) are limited in this difficult to treat patient group. Theoretically Peg-IFN alfa-2a should be preferable to Peg-IFN alfa-2b due to the fact that <10% is eliminated by the kidneys. We conducted a multicenter controlled trial to assess the efficacy and tolerability of Peg-IFN alfa-2a monotherapy 135 µg s.c. qw for 48 weeks in treatment naive patients with hepatitis C virus(HCV) infection under hemodialysis. PATIENTS & METHODS: Thirty-eight patients were included: 23 males(61%), mean age 48 years(25-68), mean HCV-RNA 872.749 IU/ml(1523-5.560.000), 29 patients(83%) genotype 1, 3 patients(8%) fibrosis stage 3/4. Patients received 135µg Peg-IFN alfa-2a s.c. qw after dialysis for 48 weeks. Treatment was discontinued at week 24 in those patients without HCV-RNA decline ≥2 log. The primary end-point was sustained viral response(SVR) defined as undetectable HCV-RNA(<50 IU/ml) after 24 weeks of follow-up. In a subgroup the pharmacokinetics of 2 different doses of Peg-IFN alfa-2a (135µg N=5, 180µg N=6) during the first 12 weeks of treatment were assessed by weekly serum measurements (ELISA). RESULTS:Thirty-seven percent (14/38) of the patients had a sustained viral response, 17/38(45%) of the first 12 weeks of treatment were assessed by weekly serum measurements. Dose reduction of Peg-IFN alfa-2a was necessary in 4 patients due to hematologic abnormalities; in 10 patients treatment was discontinued (SAEs N=8, patients' decision N=2). Twelve SAEs were noted during the study including fatal myocardial infarction, coronary bypass operation, retinal infarction, seizures, pneumonia, decomplementation of cirrhosis (1 patient each). In the pharmacokinetic substudy tolerability was comparable in both dosage groups. The observed side effects in this patient cohort were comparable to those observed in patients with normal renal function. Stable concentrations of Peg-IFN alfa-2a were reached within 4-7 weeks in both groups (13 ng/ml with 135µg and 24 ng/ml with 180µg respectively). CONCLUSION: Peg-IFN alfa-2a monotherapy (135µg qw) has a good efficacy in hemodialysis patients (SVR 37%). The 180µg weekly dose which might be even more efficient should be evaluated in a larger trial; repeated injections of Peg-IFN alfa-2a lead to safe and constant drug concentrations in the serum of patients with end stage renal disease. The high rate of SAEs even with Peg-IFN-monotherapy in this group of patients with considerable comorbidity warrants meticulous patient selection and calculation of the potential risk versus the benefit.

Disclosures:
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PEGINTERFERON ALPHA-2B VS PEGINTERFERON ALPHA-2A IN THE TREATMENT OF CHRONIC HEPATITIS C INFECTION

Ihab Hammoud, Mary Ann H. Sherbondy, Dilip Moonda, Stuart C. Gordon, Rodolfo Guevara, Kimberly Brown; gastro, Henry Ford Hospital, Detroit, MI

BACKGROUND: Pegylated interferon alfa 2a with ribavirin and pegylated interferon alfa 2b with ribavirin are both approved therapies for the treatment of hepatitis C. Although there is no head to head comparison, the efficacy of the two regimens is reported to be similar. OBJECTIVE: We attempted to compare the efficacy of peginterferon alpha-2b and peginterferon alfa-2a based regimens with weight-based dose ribavirin in the treatment of patients with HCV. METHODS: Naive patients with HCV treated with pegylated interferon and ribavirin from 2001 to 2005 at our center were included. Patients received peg 2a or peg 2b and weight-based dose ribavirin for 48 weeks (genotype 1) or 24-48 weeks (genotype 2/3). PCR was checked 24 weeks following therapy to determine SVR. Patients coinfected with HIV, HBV or on dialysis were excluded from analysis. Comparison of baseline characteristics and sustained viral response (SVR) between the two groups were performed. RESULTS: 259 patients were included in the analysis. 212 received peginterferon alfa-2a and ribavirin (Group A) and 47 received peginterferon alfa-2a with ribavirin (Group B). Baseline characteristics of the two groups were comparable as shown in table 1. Dose reductions for either peginterferon or ribavirin were similar for the two groups, 8% for group A and 2% for group B (p=NS). Overall SVR was 38% and 40% in groups A and B (p=NS). For genotype 1 patients, SVR was 23% vs 26% in groups A and B (p=NS). In genotype 1 patients with high viral load, SVR was 20% and 30% in groups A and B (p=NS). In genotype 1 patient weighing greater than 105 KG, SVR rates were 20.7% and 20% for groups A and B (p=NS). CONCLUSION: In a relatively difficult to treat population we found no difference in the sustained response rates between patients treated with peginterferon alfa 2a vs peginterferon alfa-2b and weight based dosed ribavirin.

Table 1

<table>
<thead>
<tr>
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<th>Group A (n=212)</th>
<th>Group B (n=47)</th>
<th>p-value</th>
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<td>% male</td>
<td>62% (132)</td>
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<td>42% (90)</td>
<td>38% (18)</td>
<td>NS</td>
</tr>
<tr>
<td>% genotype 1</td>
<td>74% (156)</td>
<td>74% (35)</td>
<td>NS</td>
</tr>
<tr>
<td>% genotype 1, HVL</td>
<td>62% (96)</td>
<td>59% (20)</td>
<td>NS</td>
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<tr>
<td>% &gt; 105 kg</td>
<td>21% (45)</td>
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<tr>
<td>Ribavirin dose mg/kg</td>
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Disclosures:
Dilip Moonda - Speakers Bureau: Schering-Plough; Speakers Bureau: Roche; Speakers Bureau: Gilead
Stuart C. Gordon - Grant/Research Support: Coley; Grant/Research Support: Gilead; Grant/Research Support: GlaxoSmithKline; Grant/Research Support: Indexx; Grant/Research Support: Bristol-Myers Squibb; Grant/Research Support: Human Genome Sciences; Grant/Research Support: Merck; Grant/Research Support: Novartis; Grant/Research Support: Roche; Grant/Research Support: Schering-Plough
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GM-CSF MAY MODULATE THE RESPONSE TO THERAPEUTIC IFN-α IN CHRONIC HEPATITIS C VIRUS (HCV) INFECTION

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Haematological growth factors, particularly GM-CSF and G-CSF, are frequently used to treat anaemia in HCV-infected patients receiving therapeutic interferon alpha. However, targeted use of these factors may also have important immunoregulatory roles in patients who fail to respond to conventional therapy. Previous studies have shown that these non-responsive patients can be identified, prior to treatment, by high levels of the chemokine, CXCL10. It has also been shown that GM-CSF can inhibit CXCL10 expression. In preliminary studies, we found that PBMCs from non-responsive HCV patients secreted low levels of the haematological growth factor GM-CSF compared with patients who responded to interferon alpha. The aim of this study was to determine the relationship between GM-CSF, CXCL10 and HCV infection in chronically infected patients with a view to developing a rationale for targeted therapeutic use of GM-CSF. Circulating GM-CSF was measured in twenty-six women who had been exposed to HCV-contaminated anti-D immunoglobulin and thirteen healthy controls by ELISA. HCV infected patients had detectable levels of circulating GM-CSF (controls: 0 pg/ml vs. 20.7, PCR+ 25 pg/ml ± 18.7); the PCR+ group had significantly more circulating GM-CSF than the healthy controls (p= 0.04), although there was significant inter-individual variation in levels. Peripheral blood mononuclear cells (PBMCs) stimulated with toll-like receptor (TLR-3 and TLR-7) agonists secreted significant quantities of GM-CSF indicating that the raised levels of circulating growth factor in HCV-infected patients were virally induced. GM-CSF suppressed interferon alpha-mediated induction of the chemokine CXCL10 by PBMCs. These data suggest that targeted use of therapeutic GM-CSF may overcome CXCL10-mediated inhibition of interferon alpha responsiveness in chronic HCV infection.

Disclosures:
The following people have nothing to disclose: Tariq Tajuddin, Elizabeth Ryan, John Hegarty, Cliona O’Farrell

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HEPATITIS C ASSOCIATED SYSTEMIC CRYOGLOBULINEMIA: SUCCESSFUL TREATMENT WITH PLASMA EXCHANGE

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Introduction: Acute treatment of mixed cryoglobulinemia associated with hepatitis C is indicated for systemic disease characterized by renal dysfunction, cutaneous vasculitis, or peripheral neuropathy. Methods: We reviewed the medical records of 21 patients (pts) who were diagnosed with moderate to severe hepatitis C associated cryoglobulinemia from January, 2005 to May, 2007 and referred for plasmapheresis treatment. The mean age of pts was 54 years (46-64 years old); 11 pts (52%) were male. 18 pts (86%) had HCV genotype 1A or 1B. The median HCV RNA was 733,900 IU/ml (73,620-9,370,000 IU/ml). All pts had positive cryoglobulin studies; median cryocrit was 1.7% (0.3-5.0%). 18 pts (86%) had significant elevation of rheumatoid factor (RF). 79-90% of pts had low complement levels. Clinical Presentation: 17 pts (81%) presented with renal disease. 11 pts (52%) had chronic renal insufficiency and 6 pts (29%) presented with acute renal failure requiring hemodialysis (HD). Of 17 pts with renal disease, thirteen (76%) had a renal biopsy; 12 pts (92%) had MPGN. 17 pts (81%) had elevated 24-hour urine protein; 13 of these pts (76%) had nephrotic range proteinuria. 13 pts (62%) presented with active vasculitic skin lesions. 7 pts (33%) had peripheral neuropathy. Treatment: 19 pts (90%) received a course of inpatient plasma exchange (PE) every other day; mean number of PE treatments (txs) was 8.4 (5-12 txs). 2 pts (9.5%) received outpatient PE. After completion of inpatient PE txs, 8 pts (38%) received low dose cyclophosphamide to prevent rebound of immune complex production. 5 pts (24%) underwent weekly rituximab (4-6 doses). 15 pts (71%) started weekly pegylated alpha interferon after completion of PE txs; 12 pts (57%) started daily ribavirin. Results: 20 pts (95%) experienced clinical improvement. Of 13 pts with nephrotic range proteinuria, 9 pts (69%) had significant decline in urinary protein. 3 of 6 pts (50%) on HD were able to stop dialysis. 17 pts (94%) had a marked decrease in RF with twelve pts (67%) normalizing their RF levels. 12 pts (92%) demonstrated significant improvement in vasculitic skin lesions. 6 pts (86%) experienced slight improvement in symptoms of peripheral neuropathy. 3 pts (14%) with refractory disease required several inpatient courses of PE. 6 pts (29%) received weekly maintenance PE txs. Conclusion: Plasma exchange is an effective therapy for hepatitis C associated cryoglobulinemia, especially in acute treatment of progressive renal disease and cutaneous vasculitis. Some patients may require maintenance plasmapheresis treatment. Successful long-term response to antiviral therapy is essential in controlling this disease.

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COMPARISON BETWEEN THE TWO PEGINTERFERONS ALFA IN THE TREATMENT OF CHRONIC HEPATITIS C

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In order to have the evaluation of efficacy, safety and to detect the predictors for sustained viral response of two Peginterferons, we have implemented a matched pair study on treatment naive chronic hepatitis C patients treated with weight -based Peginterferon alfa-2b or fixed dose Peginterferon alfa-2a plus Ribavirin. There are 238 naive chronic hepatitis C patients aged from 18 to 68, divided into two equal groups in clinical manifestations. Group I (N=131; 77 genotype 1, 15 genotype 2, 39 genotype 6) were treated with Peg-IFN alfa-2b, 1.5mcg/kg qweek plus Ribavirin 15mg/kg/day; Group II (N=107; 64 genotype 1, 11 genotype 2, 32 genotype 6) were treated with peg-IFN alfa-2a 180mcg/week plus Ribavirin 15mg/kg/day. The time of treatment was 48 weeks. Sustained viral response was undetectable HCV RNA after 24 weeks of follow-up. The treatment outcome can be predicted by analyzing various data on age, sex, weight, serum ALT, genotype and virus load. The sustained viral response rate of Group I was not
different from of GroupB on total patients (62.59% vs. 60.74%, p>0.05). The relapse in two groups were also similar (18.81% vs. 19.75%, p>0.05). The sustained viral response with treatment of Peg-IFN alfa-2b was better than with Peg-IFN alfa-2a in group of high-weighted patients (28.57% vs. 61.9%, p<0.01). Baseline ALT, age, sex, genotye, virus load were not statistically significant predictors of sustained viral response between two Peginterferons. In conclusion, the sustained viral response rate is similar in naive chronic hepatitis C patients treated with Peg-IFN alfa-2a or Peg-IFN alfa-2b. Peg-IFN alfa-2b seems to be better in high-weighted patients. The relapse in two groups are also similar. Side effect of thrombocytopenia more frequently occurs in treatment with Peg-IFN alfa-2a, however it should be further studied in future.

Analysis of Features Associated with The Response of Treatment

<table>
<thead>
<tr>
<th>Features</th>
<th>Group I (N=138) Peginterferon alfa-2b + Ribavirin</th>
<th>p</th>
<th>Group II (N=107) Peginterferon alfa-2a + Ribavirin</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex Male</td>
<td>53/86 (61.62%)</td>
<td>&gt;0.05</td>
<td>42/70(60.0%)</td>
<td>23/37 (62.16%)</td>
</tr>
<tr>
<td>Age &gt;60</td>
<td>38/78 (48.71%)</td>
<td>&gt;0.001</td>
<td>31/48 (64.53%)</td>
<td>4/34 (11.76%)</td>
</tr>
<tr>
<td>Body weight &gt;75 kg</td>
<td>26/42 (61.9%)</td>
<td>&gt;0.001</td>
<td>20/35 (57.14%)</td>
<td>15/27 (55.56%)</td>
</tr>
<tr>
<td>ALT&gt;1.5 ULN</td>
<td>35/52 (66.9%)</td>
<td>&gt;0.05</td>
<td>26/42 (61.9%)</td>
<td>30/45 (66.66%)</td>
</tr>
<tr>
<td>HCV RNA &gt;20M copies/ml</td>
<td>62/100 (62.0%)</td>
<td>&gt;0.05</td>
<td>47/81 (58.02%)</td>
<td>18/26 (69.23%)</td>
</tr>
<tr>
<td>Genotype 1 and 6</td>
<td>42/77 (54.43%)</td>
<td>&gt;0.05</td>
<td>35/64 (54.62%)</td>
<td>32/54 (59.26%)</td>
</tr>
</tbody>
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Disclosures:
The following people have nothing to disclose: Pham T. Thuy, Ho Tan Dat

333 IMPAIRED SENSITIVITY IN NON-RESPONDERS TO PEGYLATED INTERFERON PLUS RIBAVIRIN THERAPY ASSESSED BY 2'-5'OLIGOADENYLATE SYNTHETASE ACTIVITY

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[Aim] Approximately half of patients who were treated with pegylated interferon plus ribavirin remain HCV RNA positive. In order to understand host response to exogenous interferon was a determining factor in the outcome of therapy, we assessed 2'-5' oligoadenylate synthetase activity (2-5AS) which is induced by interferon and is an accurate indicator of antiviral effect of interferon. To prevent dose reduction (IFN) dosage by adverse effects leads to unfavorable results in the treatment of chronic hepatitis C. To prevent dose reduction by alleviating adverse effects is important to increase sustained virological response (SVR), Granulocyte colony-stimulating factor (G-CSF) is often used to increase neutrophils, but is costly. We conducted a trial to evaluate whether 8-week oral administration of meloxicam, a COX-II specific non-steroidal anti-inflammatory drug, prevents dose reduction of pegylated interferon alfa-2a in the treatment of chronic hepatitis C.

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Background & Aim: Reduction or discontinuation of interferon (IFN) dosage by adverse effects leads to unfavorable results in the treatment of chronic hepatitis C. To prevent dose reduction by alleviating adverse effects is important to increase sustained virological response (SVR). To conduct a trial to evaluate whether 8-week oral administration of meloxicam, a COX-II specific non-steroidal anti-inflammatory drug, would decrease the rate of patients requiring dose reduction of pegylated (PEG) IFN in the treatment of chronic hepatitis C. Patients & Methods: The enrollment criteria was (1) presence of serum HCV-RNA, (2) neutrophil count ≥ 1,500 /µl, (3) platelet count ≥ 90,000 /µl, and (4) serum hemoglobin ≥ 10 g/dl. Sixty patients given weekly subcutaneous administration of PEG-IFN alfa-2a (180 µg) for 48 weeks were allocated into the meloxicam group (n = 22) and the control group (n = 38) before treatment. Meloxicam was given orally at a dose of 10 mg once a day for 8 weeks since IFN treatment started. Patients were not allowed to receive G-CSF. Dose of PEG-IFN was reduced to 90 µg when neutrophil and platelet counts fell down below 750 /µl and 50,000 /µl, respectively. Results: Age, pretreatment neutrophil and platelet counts, and body weight were not significantly different between groups. Any adverse effects of meloxicam were not observed. Cumulative rate of patients who required dose reduction was significantly lower in the meloxicam group [Kaplan-Meier plot, P < 0.05]. In the meloxicam group, 9.1% and 40% of patients required dose reduction until week 8 and throughout the treatment, respectively. On the other hand, 44.7% and...
71.9% of the control group required dose reduction until week 8 and throughout the treatment, respectively. Major cause of dose modification was neutropenia. Meloxicam relieved a decline of neutrophils within first 8 weeks from 54.2% to 44.2% (P < 0.05). The COX proportional hazards regression model revealed that lower pretreatment neutrophil count (adjusted HR: 3.0 per 1,000 /μl-decrease, 95% C.I.: 1.5-6.1, P = 0.002) and not receiving meloxicam (adjusted HR: 3.6, 95% C.I.: 1.4-8.9, P = 0.007) were significantly associated with dose reduction. SVR was obtained in 42.9% of the control group and 68.2% of the meloxicam group, however, multivariate logistic analysis revealed that viral serotype and viral load were only independent factors associated with SVR. Conclusions: Eight-week administration of meloxicam prevented dose reduction of Peg-IFN by relieving a decline of neutrophil count in the treatment of chronic hepatitis C.

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335 GENE EXPRESSION BIOMARKERS PREDICTING RESPONSE TO Pegylated Interferon Alpha (Peg-IFN) and Ribavirin (RBV) IN THE PERIPHERAL BLOOD OF PATIENTS WITH CHRONIC HEPATITIS C (CH-C), Non-Responder (NR) TO PREVIOUS TREATMENT

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Re-treatment of previously NR with another course of Peg-IFN and RBV is associated with low sustained virologic response (SVR). AIM: To develop a biomarker profile predicting sustained virologic response (SVR) after antiviral therapy. METHODS: Thirty nine CH-C patients who had previously failed combination therapy were enrolled. Patients were being started on Peg-IFN+RBV (standard doses of PEIFN alpha 2a or 2b and RBV). Blood samples were collected into PAXgene™ RNA blood tubes (PreAnalytix) prior to treatment, 1 day, 1 week, 4 weeks, and 8 weeks after the first dosing. Total RNA was extracted and quantified using RiboGreen™ Quantitation Kit ( Molecular Probes) and was used in PCR. One-step RT-PCR was used to profile 317 mRNAs [160 genes consisting of interferon-inducible, interferon pathway, immune response, and housekeeping genes]. The expression levels of mRNAs of interest were normalized with 6 "housekeeping" genes and a Human Universal Reference RNA (Stratagene). Multiple regression analysis and stepwise selection were performed to assess differences in gene expression at different time points and predictive performance was evaluated for each model. RESULTS: Demographic data of patients included age: 49 ± 6 years, 59% Male, 69% Caucasian, 78% HCV genotype 1, 16% G3 and 6% G4. In all patients prior to treatment, IFI2 expression level predicted SVR (Model p-value = 0.04; AUC = 0.718, Sensitivity = 0.769, Specificity = 0.611). Immediately after initiation of treatment, SVR in G1 was predicted by IFI2 and JAK1 expression levels (Model p-value = 0.0005; AUC = 0.917, Sensitivity = 1.000, Specificity = 0.750). After a week of treatment, SVR in G1 patients was predicted by IRF4, BAG1, SOCS6, GMIP, LYN and SDCPB expression levels (Model p-value = 0.0014; AUC = 0.997, Sensitivity = 1.000, Specificity = 0.923), while for the entire CH-C cohort, it was predicted by NMI, PF4, BAG1, SOCS1, PDGFA and B2M expression levels (Model p-value < 0.0014; AUC = 0.989, Sensitivity = 1.000, Specificity = 0.929). At day 56, SVR in the entire CH-C cohort was predicted by the expression levels of NMI, IKBKB, RHOC, CD58 and PDGFA (Model p-value = 0.004, AUC = 0.917, Sensitivity = 0.750, Specificity = 1.000). On the other hand, in G1 patients, SVR was predicted by IL15 and COX17 expression levels (Model p-value = 0.00034, AUC = 0.962, Sensitivity = 0.875, Specificity = 1.000). CONCLUSIONS: A panel of non-invasive biomarkers is developed that can accurately predict SVR in NR CH-C patients. If validated, this biomarker panel can be useful in the management of HCV.

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Chris Santini - Employee: Other
Chris Sigua - Employee: Other
Joanne Chan - Employee: Other
Ayuko Iverson - Employee: Other
Sheng-Yung Chang - Employee: Other

The following people have nothing to disclose: Zobair M. Younossi, Rochelle Collantes, Ancha Baranova, Amy Kim, Ganiraju Manyam, Maria Stepanova

336 TREATMENT AND OUTCOME OF GENOTYPE 4 CHRONIC HEPATITIS C PATIENTS WITH PegINTERFERON Alfa 2B AND Ribavirin IN THE CLINICAL SETTING IN GERMANY

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BACKGROUND: Pegylated interferon (Peg-IFN alfa) and Ribavirin (RBV) are standard of care for hepatitis C (HCV) treatment. Since there are no large controlled studies for genotype (GT) 4 patients (pts), available cohort data are of interest in order to better define response rates and suitable treatment strategies. METHODS: 280 sites (75 with GT 4 pts) have treated a total of 3948 HCV pts, of whom 123 were GT 4. The results of GT 4 pts who had reached an observational period beyond 72 weeks after baseline are described. Pts without available HCV-RNA data 24 weeks post therapy were counted as treatment failures. Data are analyzed using standard summary statistics. RESULTS: GT 4 pts had a mean age of 41 years, 61% (n=75) were male. 48% (n=59) were German, 7% (n=8) Italian, 12% (n=15) Egyptian, 4% (n=5) Ethiopian, and 4% (n=5) Turkish. 3% (n=4) of the pts showed cirrhosis and 6% (n=7) had HBV or HIV co-infection. 88% (108/123) of the pts received peg-IFN and RBV as primary therapy, 12% (15/123) were retreated after unsuccessful IFN mono-therapy. 39% (48/123) received standard PEG-IFN dose (1.5 mg/kg body weight), 33% (41/123) received lower and 25% (31/123) higher doses. 70% (87/123) received standard weight-dosed RBV (≥10.6 mg/kg body weight). Since the study is ongoing, only 74 pts have reached the end of therapy, yet, 28% (21/74) were treated for less than 24 weeks, 7% (5/74) were treated
24 weeks, 45% ([33/74]) were treated > 24 - ≤ 48 weeks and 20.3% ([15/74]) were treated for ≤ 48 weeks. As of yet, 50 pts have reached the 24 weeks follow-up after end of therapy. Normalization of ALT was seen in 50% ([25/50]) of the pts at follow-up. 42% ([21/50]) of the pts analyzed thus far showed sustained virologic response (SVR), 16% ([8/50]) relapsed, 24% ([12/50]) were non-responders, and 18% ([9/50]) were lost to follow-up or missing. The BMI index class ≥ 18 < 25 showed the highest SVR rate with 59% ([13/22]) followed by ≥ 25 < 30 with 50% ([7/14]) and the class ≥ 30 with 0% ([0/2]). In patients with baseline HCV-RNA < 600,000 IU/ml (LVL) SVR rate was 60% ([12/20]) and 47% ([8/17]) in the HVL group (≥ 600,000 IU/ml). More nonresponders were seen in the HVL group (41.2%; 7/17) than in the LVL group (15%; 3/20). No differences in SVR were observed between different treatment durations ≥ 25 weeks ≤ 48 weeks ([62%; 13/21]) and >48 weeks ([63%; 5/8]), whereas in the group <25 weeks only 25% ([3/12]) of the patients showed SVR. CONCLUSION: Efficacy of PEG-IFN alfa-2b and RBV in GT 4 patients is comparable, but not superior to results in genotype 1 patients.

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Demographics and results

<table>
<thead>
<tr>
<th>PEG-IFN alfa-2a plus RBV</th>
<th>CIFN plus RBV (800mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male (n)</td>
<td>29</td>
</tr>
<tr>
<td>Female (n)</td>
<td>23</td>
</tr>
<tr>
<td>GT 2/3 (n)</td>
<td>26</td>
</tr>
<tr>
<td>GT 1/4 (n)</td>
<td>26</td>
</tr>
<tr>
<td>SVR 2/3 (%)</td>
<td>85</td>
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<tr>
<td>SVR 1/4 (%)</td>
<td>58</td>
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<tr>
<td>Cirrhosis (n)</td>
<td>4</td>
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<td>Drop-outs (n)</td>
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The following people have nothing to disclose: Thomas Witthöft

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BONE MINERAL DENSITY AND METABOLISM IN NON-CIRRHOTIC PATIENTS WITH CHRONIC HEPATITIS C BEFORE AND AFTER ANTIVIRAL THERAPY WITH PEGYLATED INTERFERON α AND RIBAVIRIN: A PROSPECTIVE CONTROLLED STUDY

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Introduction: The importance of osteoporosis as a complication of end-stage chronic liver disease is well known. However, significant osteopenia may already be present in non-cirrhotic patients with chronic hepatitis C (CHC). Furthermore, the administration of antiviral therapy may have an influence on bone metabolism. While interferon alfa has been shown to decrease bone resorption in vitro and may be beneficial for bone mineral density in vivo, less consistent data are available for ribavirin. Patients and Methods: Thirty non-cirrhotic patients with CHC genotype 1 infection were treated with peginterferon alfa and ribavirin for 48 weeks. Dual-energy x-ray absorptiometry was performed at baseline, after 48 weeks of therapy, and at the end of a 24-week follow up period. Bone mineral density (BMD), and T-scores / Z-scores were assessed. Additionally, levels of serum C-terminal propeptide of type I collagen, bone-specific alkaline phosphatase, and osteocalcin were measured. Results: Thirteen of the 30 non-cirrhotic patients had osteopenia (43%) and manifest osteoporosis was present in 4 patients (13%). Antiviral therapy led to a significant increase of femur neck and hip BMD (baseline vs end of treatment, p<0.05) as well as T-scores (p<0.05) and Z-scores (p<0.01). While in patients with sustained virologic response (n=19) most parameters remained highly above baseline values by the end of the 24-week follow up period, patients with virologic relapse (n=11) had a decrease of BMD, T-scores and Z-scores thereafter. Patients with sustained virologic response (n=19) most parameters remained highly above baseline values by the end of the 24-week follow up period, patients with virologic relapse (n=11) had a decrease of BMD, T-scores and Z-scores thereafter. While in patients with sustained virologic response (n=19) most parameters remained highly above baseline values by the end of the 24-week follow up period, patients with virologic relapse (n=11) had a decrease of BMD, T-scores and Z-scores thereafter.

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Efficacy of Interferon-Based Antiviral Therapy in Patients with Chronic Hepatitis C Infected with Hepatitis C Virus Genotype 5: A Meta-Analysis of Two Large Prospective Belgian Clinical Trials

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7NV Roche SA, Brussels, Belgium

Hepatitis C Virus genotype 5 (HCV-5) response to interferon-based antiviral therapy is currently poorly documented and the best treatment regimen for this genotype has still to be determined. Comparing the sensitivity of genotype 5 (GNT 5) to treatment, with other better known genotypes should allow a better evaluation of response to treatment and help defining the optimal treatment regimen for GNT 5. Because of the small size of the GNT 5 population, it seems improbable that a study could be conducted in a large population of HCV-5 patients. For this reason, any prospective clinical trial on CHC patients should be used for a sub-group analysis of G5 response to treatment. So, in order to compare the efficacy of antiviral therapy on HCV-5 with other genotypes, a meta-analysis of two large phase III prospective randomized clinical trials conducted in Belgium in naïve patients and relapsers (BeRNaR 1 and BeRNaR 2) has been performed. Both studies were designed to evaluate the safety and efficacy of various interferon-based therapies in a large population of patients with chronic hepatitis C (CHC). In total, 1139 patients were recruited from October 2000 to May 2005 in both studies from which 48 (4.2%) were of GNT 5. The meta-analysis measured and compared patients’ characteristics, early viral responses, end-of-treatment responses, sustained viral responses (SVR) and relapse-rates between GNT 5 and other genotypes. A subset of HCV-1 infected patients was then selected from the studies database to match the HCV-5 sample according to age category (< vs. > 40), gender, baseline virus load (< vs. > 800,000 IU/mL), cirrhosis status (yes vs. no), pretreatment status (naïve vs. relapsers) and treatment received (only patients treated with the same regimen of pegylated interferon alpha-2a plus ribavirin were selected). In the sample of patients matched for these variables, the “intrinsic sensitivity” of HCV-5 was comparable to HCV-1 with a sustained viral response equal to 56% in both matched groups. The observed SVR in HCV-5 is close to what has been recently published by Bonny et al (2006) in this subgroup. This is the first comparative analysis of HCV-5 response to interferon-based therapy derived from large prospective randomized clinical trials. The study allows better characterization of the Belgian HCV-5 population and its response to treatment. It suggests that HCV-5 patients should be treated with the same treatment regimen as HCV-1 patients.

FACTORs IMPACTING SVR IN HCV GENOTYPE 1 PATIENTS WITH EVR AND WEEK 24 NEGATIVITY

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Background: Current guidelines for treatment of chronic HCV utilize the EVR with ≥2 log decrease in virus as an indicator of patients who are likely to respond to therapy. Reports indicate a 72% SVR rate in patients with an EVR. The purpose of this study was to evaluate factors impacting the SVR rate in chronic HCV genotype 1 patients who have achieved an EVR at treatment week 12 with viral negativity at week 24. Methods: This was a retrospective cohort analysis of HCV genotype 1 patients treated with PEG IFN plus ribavirin. All patients who achieved an EVR and were HCV RNA undetected at week 24 of treatment were included. “Slow viral responders” had a ≥2 log decrease with detectable virus at week 12 achieving undetectable virus at week 24. Results: Total number of patients was 143 (92 men, 51 women). Age range was 22-70 (mean 47.1 ± 8.4). Race was White 112 (78%), Black 17 (12%), and Hispanic 14 (10%). Fibrosis was stage 0-1 in 21, stage 2 in 47, stage 3 in 31, and stage 4 in 34 patients. At treatment week 12, 107 (75%) patients achieved an undetectable HCV RNA with the remaining 26 (25%) patients being slow viral responders (Table 1). SVR rate in the group with undetectable virus at week 12 was 78% (p = 0.0001). In patients with slow viral response, the most important factor impacting SVR was the week 12 HCV RNA <100 IU/mL vs. ≥100 IU/mL with 10/20 (50%) and 0/16 (0%), respectively (p = 0.006). Additional factors demonstrating a trend in different SVR rates were race, baseline viral count, and PEG IFN used. Racial differences comparing White with non-White demonstrated a trend with 9/18 (33%) and 1/9 (11%), respectively (p = 0.2). Patients with high baseline viral load (>400,000 IU/mL) had lower SVR than those with low viral load (0/4 and 10/32, respectively, p = 0.25). In slow viral responders, SVR rate with PEG IFN alpha-2a vs. alpha-2b were 9% and 36%, respectively (p = 0.10). Conclusions: Slow viral responders, with a ≥2 log decrease but delayed viral clearance until week 24, have a significantly reduced chance of SVR with standard 48 week treatment than those with undetectable virus, 27% vs. 78% respectively. Significantly higher SVR rates were found in patients who were virus positive with HCV RNA level <100 IU/mL vs. ≥100 IU/mL at week 12. Trends toward lower SVR rates were also seen in difficult to treat patients: non-White race and high baseline viral count. Further investigation is needed to improve SVR rates in patients who are slow viral responders.

Number of Patients With Undetectable HCV RNA

<table>
<thead>
<tr>
<th>Week 12 Viral Response</th>
<th>n</th>
<th>Week 48</th>
<th>SVR</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>≥2 log decrease and viral clearance at week 24</td>
<td>36</td>
<td>26 (71.2%)</td>
<td>10 (27.7%)</td>
<td>0.001</td>
</tr>
<tr>
<td>HCV RNA negative</td>
<td>107</td>
<td>95 (88.8%)</td>
<td>83 (77.6%)</td>
<td>0.001</td>
</tr>
</tbody>
</table>

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SILYMARIN PHARMACOKINETICS (PK) IS ALTERED IN PATIENTS WITH CHRONIC HEPATITIS C VIRUS (HCV) AND NONALCOHOLIC FATTY LIVER DISEASE (NAFLD) AND CORRELATES WITH CASPASE-3/7 ACTIVITY

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Silymarin is commonly used by liver disease patients and is under investigation as a treatment for HCV and NAFLD. Silymarin, a mixture of 6 flavonoids with antioxidant activity, is metabolized by hepatic conjugating enzymes and the conjugates undergo significant biliary transport. Liver disease can alter the expression and function of conjugating enzymes and biliary transporters, which may impact silymarin’s PK. We studied the PK of silymarin flavonoids and their conjugates in patients and healthy subjects. Caspase-3/7 activity, cytokines, and oxidative stress were also measured as biomarkers of disease activity. Twenty subjects were enrolled: 5 healthy; 10 chronic HCV (5 with cirrhosis by biopsy); and 5 NAFLD by biopsy. Fasted subjects received a single oral 480 mg dose of standardized silymarin and 15 blood samples were obtained over 24 h. Plasma concentrations of silymarin flavonoids and their conjugates were determined by LC-MS before and after enzymatic hydrolysis. PK parameters were determined using a noncompartmental approach with WinNonlin®4.1. Plasma cytokine (R&D Systems®) and 8-isoprostane-F2α (8-isoPGF2α) concentrations were measured by enzyme-linked immunosorbent assay. Plasma caspase-3/7 activity was determined by luminescence (Promega®). Statistical analyses were performed using SAS JMP®6.0.0. Data are presented as means, with p<0.05 considered significant. Silymarin conjugate peak plasma concentrations (16-595 ng/ml) were >8-fold higher than for silymarin (4-76 ng/ml). 24 h exposures (AUC₀₋₂₄) for total plasma conjugates were 2.7, 3.0, and 5.1-fold higher for HCV noncirrhotic, NAFLD, and HCV cirrhotic (p=0.03 vs. healthy) patients, respectively, compared to healthy subjects (AUC₀₋₂₄=23.41 ng/ml*h). Elimination half-lives for total conjugates ranged from 8.10 h in disease cohorts vs. 4 h in the healthy cohort. Plasma caspase-3/7 activity correlated with AUC₀₋₂₄ for total silymarin conjugates among cohorts (R²=0.52) and was elevated in HCV cirrhotic patients compared to other cohorts (p<0.005). No differences were observed in plasma concentrations of 8-isoPGF2α or IL-6 among cohorts. Our results indicate that cirrhosis and elevated caspase-3/7 activity are associated with highest silymarin exposures in patients with liver disease. Therefore, silymarin may be useful for quantifying changes in disease stage. In addition, chronic dosing with silymarin may result in the accumulation of conjugates. Therefore, future clinical studies should include patients with advanced disease in order to determine the biological effects of silymarin conjugates. These findings will be confirmed in larger Phase 1/2 ongoing trials.

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NEGATIVE IMPACT OF ABACAVIR ON RESPONSE TO PEGIFN PLUS RBV IN HIV/HCV COINFECTED PATIENTS

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Background: The choice of antiretrovirals may influence outcome of pegylated-interferon (pegIFN) plus ribavirin (RBV) therapy. While didanosine and zidovudine have to be avoided due to greater risk of side effects, little is known on the influence of abacavir, which is a guanosine analog as RBV. Methods: All HIV/HCV patients receiving first-line pegIFN plus RBV therapy between 2002 and 2005 in three Spanish hospitals were retrospectively analyzed. Main demographics, HCV features and antiretrovirals were recorded. Baseline liver fibrosis and RBV plasma levels at week 4 were also available for a large subset of patients. Results: A total of 426 HIV/HCV patients (80% males, mean age 41 years, 80% on HAART, mean CD4 count 567 cells/µl) received at least one dose of pegIFN plus RBV. At baseline, mean HCV-RNA 5.8 log IU/ml, 65% genotypes 1 or 4, and 40% Metavir ≥F3. The SVR was 38% (G1/4, 26%; G2/3, 61%). Factors associated with non-SVR in the multivariate analysis [OR [95% CI] p] were: higher baseline HCV-RNA (10.31 [3.70-28.57] <0.001), HCV genotype 1/4 (7.75 [2.56-23.25] <0.001), Metavir (10.31 [3.70-28.57] <0.001), HCV genotype 1/4 (7.75 [2.56-23.25] <0.001), Metavir ≥F3 (4.03 [1.32-12.50] 0.01), and lower RBV plasma trough levels (1.64 [1.03-2.65] 0.04). The use of abacavir predicted non-SVR (2.04 [1.08-3.85] 0.03) when RBV levels were not included in the model. The negative impact of abacavir on HCV suppression was recognized at weeks 4, 12, 48 and 72. However, it was not appreciable in the subset of patients with RBV plasma trough levels >2.2 µg/ml. Conclusions: The use of abacavir is associated with a poorer response to pegIFN plus RBV in HIV/HCV patients. This negative impact may be overcome by high RBV exposure, suggesting that an inhibitory competition exists between RBV and abacavir.

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SUSTAINED VIROLOGIC RESPONSE (SVR) TO PEGIFN PLUS RBV IN GENOTYPE-4 HCV-HIV COINFECTED PATIENTS

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Background: HCV Genotype 1 and 4 respond worse to pegylated interferon (pegIFN) plus ribavirin (RBV) therapy. For this reason most patients with advanced disease are treated interferon (pegIFN) + ribavirin (RBV). For this reason most trials genotype 4 sample is small, and real factors associated with response can be biased. Methods: All genotype 4 HIV/HCV infected patients who received treatment with pegIFN+RBV in...
two different multicentric studies (PRESCO and ROMANCE) were retrospective analyzed. Baseline viral load (BVL), undetectable HCV-RNA at week 4 (RVR), decrease of 2 log at week 12 (EVR) were assessed as predictive factors of response. Results are given median (IQR) and percentage. Univariate and multivariate logistic regression analysis was performed. Results: Overall, 75 patients (60 men) were evaluated. Median age and CD4 cell count were 40 and 598, respectively. 49% of patients had HIV-RNA < 50 copies/ml, 71% had elevated aminotransferase levels and 31% had F3-4 fibrosis. Median HCV BVL was 5.7 log/ml. RVR was obtained in 10 (20%) patients and EVR in 26(42%). In an intention to treat (ITT) and on-treatment analysis (OTT), sustained virological response (SVR) was achieved in 21/75 (28%) and 21/62 (34%) of patients, respectively. In the multivariate analysis (OTT) BVL OR for every log increment 0.8 95%CI:0.008-0.8) and EVR OR 95%CI:1.3-30.8) were independently associated with SVR. HCV baseline cut-off to predict response could not be established, as sensitivity was low: the best cut-off was 5.7 log HCV-RNA with a sensitivity of 0.3. Conclusion: In this large series of patients with HCV-4/HIV coinfected treated with pegIFN+RBV we confirm than genotype 4 is a difficult to treat genotype. BVL and RVR are the best predictors of response. However, we could not establish a good baseline HCV-RNA cut-off to predict response.

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344 DETERMINING SUITABLE INTERFERON TREATMENT PHASE USING ACUTE HEPATITIS C CELL CULTURE MODEL

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Background & Aims: Interferon (IFN) had been widely used in the treatment of hepatitis C. We first reported that IFN treatment is highly effective against acute hepatitis C (Oomata et al. Lancet 1991). However, suitable IFN treatment phase, namely when to start IFN treatment against acute hepatitis C, has not been determined. Moreover, it has become difficult to perform clinical study of acute hepatitis C treatment because of decrease in number of acute hepatitis C after introduction of anti-HCV screening. Recently, a robust genotype 2a hepatitis C virus (HCV) full-length replication and infection system has been established. Thus, we also established this system and tried to determine suitable IFN treatment phase against acute hepatitis C using this system. Methods: First, we transfected JFH1 HCV RNA into Huh7 cells, and then collected supernatant for infection. Huh7 cells were infected with JFH1 HCV, and 20-160 IU/ml of IFNα2b was added to cells for 4 or 8 days at 1, 3, 7, 11, 13, 17, 21, or 25 days after infection. Cells were observed for 3 months after the end of IFN treatment. HCV core protein amount in the supernatant of infected cells was sequentially measured and HCV RNA in the supernatant of infected cells just after IFN treatment was examined. Results: The peak of HCV replication was 11 days after infection. Thereafter, HCV replication was decreased and fluctuated. IFN dose-dependent and duration-dependent decrease of HCV core protein in the supernatant was observed. End of treatment response was achieved when IFN was added 13, 17, 21, or 25 days after infection. Sustained viral response was achieved when IFN was added 13, 17, or 25 days after infection. Conclusions: The most effective phase of IFN treatment in acute hepatitis C cell culture model was after the peak of HCV replication. Full-length HCV infection system is useful not only to examine the effect of antivirals, but also to determine the suitable treatment phase.

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345 RE-TREATMENT WITH PEGYLATED INTERFERON PLUS WEIGHT-ADJUSTED RIBAVIRIN IN HIV+ PATIENTS WITH CHRONIC HEPATITIS C: THE PILOT-NR STUDY

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Background: The efficacy of IFN-based therapy is lower in patients with chronic hepatitis C and HIV coinfection. The number of HCV/HIV patients that failed to clear HCV with suboptimal therapy in the past has increased rapidly. They might be good candidates for re-treatment with pegIFN plus weight-adjusted RBV. Methods: Prospective study of therapy with pegIFNα2a (180 µg weekly) plus RBV (<75 kg: 1000/d; >75 kg: 1200 mg/d) for 12 months in HIV/HCV patients non-responders or relapsers to a suboptimal IFN-based therapy. Main endpoint was the achievement of SVR. Advanced liver fibrosis (ALF) was defined as >9.5 kPa using FibroScan. RBV plasma trough levels were measured by UVHPLC. Results: A total of 51 patients were included (80% males; mean age 42 years; mean CD4 count 696 cells/µl; 90% on HAART; 89% with undetectable HIV-RNA; mean HCV-RNA 6.1 log IU/ml; 72% G1-4; 59% with ALF). Prior suboptimal regimens were IFN monotherapy (23%), IFN+RBV (27%) and pegIFN+RBV 800 mg/d (50%). Mean length of these treatments was 6.6 months. Overall, 64% were non-responders and 36% relapsers. Re-treatment with pegIFN+RBV provided SVR in 39% of patients (27% for G1-4 vs 70% G2-3; p=0.02 ). SVR was lower in non-responders than relapsers (30 vs 58%; p=ns), as in patients with ALF vs non-ALF (18 vs 60%; p=0.02). RBV plasma trough levels at week 4 were comparable between SVR and non-SVR (2.53 vs 2.13 ug/ml). In the multivariate analysis, factors independently associated with the attainment of SVR (OR [95% CI] p) were: non-ALF (50 [2.12-100] p=0.02), lower baseline HCV-RNA log IU/ml [16 (1.5-166)] p<0.05) and G2-3 (33.3 [1.03-166] p=0.05). Conclusions: Re-treatment with pegIFNα2a plus weight-adjusted RBV for 12 months allows HCV clearance in a significant proportion of HIV/HCV patients. The better chances of HCV eradication were seen in patients with mild liver fibrosis, low HCV-RNA, and G2-3.

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346 FEASIBILITY OF COMBINATION THERAPY FOR CHRONIC HEPATITIS C IN IVDU IN THE FRAMEWORK OF AN HEROIN DETOXIFICATION PROTOCOL

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Background and aims. HCV infection is highly prevalent among intravenous drug users (IVDU), but only few receive standard-of-
care antiviral treatment albeit small-scale studies suggest feasibility of treatment in this cohort, but also high rates of withdrawal from therapy affecting its efficacy. We aimed to assess adherence and sustained viral response (SVR) to therapy in a cohort of HCV-infected drug addicts within the framework of an heroin detoxification protocol. Patients and protocols. Sixty-seven consecutive anti-HCV positive IDU patients currently on methadone or buprenorphine were referred to a Liver Unit by 4 Addiction Centres, to considered for antiviral therapy. PEG-interferon alfa-2b 1.5 µg/wk and Ribavirin 800-1,400 mg/day according to body weight were given for 24 (genotype 2 or 3) or 48 weeks (genotype 1 or 4), and observed for 24 weeks thereafter. Patients were followed for the entire period directly by the Addiction Centre. Results. Seven patients were excluded (3 being HCV RNA negative, 3 because of alcohol abuse, one for morbid obesity) and 60 were treated. This analysis is conducted on 41 subjects who have completed treatment and follow-up at the current time. Thirty-nine were males (mean age 32.4 yrs, mean duration of IVDU 9.1 yrs). Sixteen patients had HCV genotype 1, 2 genotype 2, 17 genotype 3 and 1 genotype 4. The overall withdrawal rate under therapy was 44%. Seventeen out of 22 (77%) patients who prematurely discontinued therapy were classified as early drop-outs (discontinuation within the fourth week): in all cases but one, the cause of discontinuation was primary non-compliance. Sustained viral response was 34% (18% in G1-4 and 41% in G2-3) on intention to treat analysis and 73% (40% in G1-4 and 100% in G2-3) by per protocol analysis. None of the patients enrolled was lost to the heroin detoxification program. Both the mean time of abstinence from IVDU and the mean duration of former IVDU were comparable between the patients with adequate and those with suboptimal adherence. Remarkably, adherence to the antiviral regimen was higher in the group treated with buprenorphine than in methadone maintenance (8 out of 14, 57.1% vs. 4 out 17, 23.5%, p = 0.06). Conclusions. Patients with chronic hepatitis C on an heroin detoxification regimen may be effectively treated with PEG-Interferon and ribavirin, without any unexpected side effect or loss of efficacy of the opioid maintenance regimen. The high rate of voluntary discontinuation (mostly within the first 4 weeks of therapy) cannot be predicted by sociodemographic factors, but buprenorphine may be more effective than methadone in helping the patients to adhere to the schedule.

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347 DESCRIPTION OF PATIENTS WITH VIRAL BREAKTHROUGH DURING PEG IFN AND RIBAVIRIN TREATMENT FOR CHRONIC HCV GENOTYPE 1

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Purpose: The purpose of this study was to evaluate factors impacting viral breakthrough in patients undergoing PEG/RBV treatment for hepatitis C genotype 1. Methods: This was a retrospective cohort analysis of HCV genotype 1 patients treated with pegylated interferon alpha-2b plus weight-based ribavirin from 2003 to present. All genotype 1 patients who were HCV RNA undetectable prior to week 24 of treatment were included in the sample. Results: The total number of genotype 1 patients who underwent treatment was 431 with 209 achieving an undetectable HCV RNA prior to week 24 of treatment. The number of patients with documented viral breakthrough was 22 (17 males and 5 females). Age range was 24-58 (mean 45.8 ± 6.6). Race distribution was White 13 (59%), Black 7 (32%), Hispanic 1 (5%) and Asian 1 (5%). Fibrosis was stage 0-1 in 3, stage 2 in 11, stage 3 in 4, and stage 4 in 4 patients. BMI was normal in 6 (27%), overweight in 6 (27%), and obese in 10 patients (45%). Baseline viral load was <400,000 IU/mL in 6 (27%) and >800,000 IU/mL in the remaining 16 (73%). At treatment week 4, 1 patient was HCV RNA undetectable with a viral breakthrough prior to week 12. At week 12, 9 patients had HCV RNA undetectable with viral breakthrough in 4 prior to week 24, 2 prior to week 40, and 3 at week 48. The other 12 patients had undetectable virus on or before week 24 with viral breakthrough in 1 prior to week 24, 8 prior to week 40 and 3 at week 48. During treatment, 7 patients (32%) were noncompliant with dosing (2 with ribavirin, 1 with IFN and 4 with both), 9 patients (41%) had dose reductions (5 with ribavirin, 1 with IFN and 4 with both), and the remaining 5 (23%) patients were all viral undetectable by week 12 and had no known noncompliance or dose reductions of medications. Conclusions: The majority of patients who suffered a viral breakthrough during treatment with Peg/RBV for chronic hepatitis C had experienced either dose modifications or were noncompliant with dosing of one or both medications. However, viral breakthrough happened in some genotype 1 patients with favorable predictors of SVR, including low viral load and week 4 and 12 negativity, when neither dose modification nor noncompliance to medications occurred. This data emphasizes the importance of maintaining compliance during HCV treatment even when undetectable levels of virus have been attained.

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Jeffrey Weinstein - Speakers Bureau: Schering-Plough; Speakers Bureau: Valeant; Speaker’s Bureau: Valeant

The following people have nothing to disclose: Anisha Steephen, Abdullah Mubarak

348 EXTENDING ANTIVIRAL TREATMENT FOR 12 MONTHS AFTER HCV-RNA CLEARANCE IS ASSOCIATED WITH A HIGH RESPONSE RATE IN DIFFICULT-TO-TREAT HCV PATIENTS

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Background: difficult-to-treat patients with chronic hepatitis C constitute a large number of heterogeneous patients characterized to resistance to standard antiviral treatment mainly due to a slow HCV-RNA clearance. Recently, it has been suggested that extension of treatment to 72 weeks improves the response in this subset of pts. Methods: we enrolled 65 difficult-to-treat genotype 1 HCV pts (38 males, mean age: 54±11 yrs) defined by at least one of the following criteria: HCV-RNA serum levels ≥6x10^5 UI/ml (36 pts), presence of cirrhosis (10 pts), nonresponse to previous antiviral treatment (31 pts). Patients were treated with Peg-IFNα2b 1.5 mg/kg plus Ribavirin 800-1,200 mg/dy. HCV-RNA was assayed monthly during the first 6 months and pts resulting HCV-RNA negative (<50 UI/ml) continued the treatment for further 12 months starting since the
moment of HCV RNA negativization. After 6 months pts who did not achieve a HCV RNA negativization, withdrew from the study and were considered non-responders. Study end-point was undetectable HCV RNA 6 months after treatment cessation (SVR). Results: HCV RNA was negative in 5 pts (8%) after one month of treatment, in 8 (12%) after two, in 25 (39%) after three, in 6 (9%) after four, in 4 (6%) after five and in 9 (14%) after six months. Eight pts (12%) did not achieve HCV RNA negativization within the first six months and stopped treatment. Up to now 61 patients completed the treatment period and 46 the 6 month of follow-up. A SVR was obtained in 30/46 pts (65%). The absence of cirrhosis and the naïve status were the only predictive factors of SVR (p=0.009 and p=0.005, respectively). Conclusions: extending antiviral treatment for 12 months after HCV RNA negativization is a rational approach to treat HCV difficult-to-treat patients. Although the study population was heterogeneous, this type of patients are not selected and very commonly encountered in the medical practice and it is crucial to define a treatment strategy in this setting.

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349 ULTRA RAPID VIROLOGIC RESPONSE PREDICTS SUSTAINED VIROLOGIC RESPONSE IN HCV INFECTED PATIENTS WITH GENOTYPE 3 AND HIGH VIRAL LOAD: THE GET-C STUDY

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Aim: To assess the impact of viral clearance at week 2 of therapy on the sustained virologic response (SVR) rate of HCV genotype 3 infected patients with a high viral load. Methods: The GET-C 3 (GET-C trial) is an ongoing study designed to evaluate extended therapy in genotype 3 patients with a baseline viral load >400,000 IU/ml. Patients were randomized to receive Peg IFN alpha 2b 1.5ug /kg per week plus weight based ribavirin for 24 or 48 weeks. We assessed the predictive value of a week 2 and week 4 virologic response on the sustained virologic response rate. Viral load was determined using the Versant HCV RNA 3.0 BDNA assay (Bayer Diagnostics) (limit of detection 615 IU/ml) at baseline, week 2, 4, 12, 24, 36, 48 and 72 where applicable. All data was collected in an electronic case record form. Statistical analysis was performed using the Fisher’s Exact Chi Square two-tailed test. Results: Baseline demographics were similar in responders and non responders. To date, of the first 43 patients who have completed therapy and follow up, 42 patients had week 2 virologic data available. Of 42 patients, 30 (71%) achieved viral suppression to below 615 IU/ml at week 2. Of those who achieved this, 28 had an SVR with a positive predictive value (PPV) of 93%. 12 patients did not clear virus by week 2, of these, 5 did not have an SVR with a negative predictive value (NPV) of 42%. The impact on SVR of achieving a viral load reduction to <615IU/ml at week 2 was statistically significant (p=0.011). Of 42 patients, 39 (93%) were below 615 IU/ml at week 4. Seven patients who were positive at week 2 were negative at week 4 and 4/7 achieved a SVR (57%). Conclusion: An ultra-rapid virologic response at week 2 was highly predictive of SVR (PPV 93%). These data in 42 genotype 3 patients with high viral load suggest that week 2 may be a useful time for predicting SVR. Patients who fail to attain an undetectable viral load by week 2 have a significantly reduced chance of achieving a SVR and may benefit from extended therapy, which is being evaluated in the GET-C study.

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Margaret Perlman - Employee: Schering-Plough
Kylie McCelland - Employee: Schering-Plough
Carmella Law - Employee: Schering-Plough

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350 CLINICAL RELEVANCE OF THE INTERFERON-ACTIVATED 2′-5′-OLIGOADENYLATE SYNTHETASE (OAS)/RNASE L SYSTEM FOR TREATMENT SUCCESS IN CHRONIC HEPATITIS C

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Background: Interferon-alfa (IFN) induces the 2′-5′-oligoadenylate synthetase (2′-5′-OAS)/RNase L system for treatment success in chronic hepatitis C is unknown.

Methods: Nucleotide sequences coding for non-structural and structural proteins of the hepatitis C virus (HCV) have been investigated for an association between response towards an IFN-based therapy and the number of RNase L cleavage sites in patients with HCV genotypes 1b and 3a (HCV 1b: NS5A, n=45; E2/PePHD, n=41; CD81/HVR2, n=38; HCV 3a: NS5A, n=33; E2/PePHD, n=33; CD81/HVR2, n=17). In 5 patients with HCV 1b infection and non-response to IFN-based therapy the frequency of RNase L cleavage sites within NS5A and NS4B has been evaluated during therapy (baseline, treatment weeks 4 and 48; follow up week 12).

Nucleotide sequences of isolates from patients with HCV 1b and 3a infection were compared with respect to the number of RNase L cleavage sites (HCV 1b: NS5A, n=45; E2/PePHD, n=41; CD81/HVR2, n=38; HCV 3a: NS5A, n=33; E2/PePHD, n=33; CD81/HVR2, n=17). Results: No significant association was observed between response to antiviral therapy and the number of RNase L cleavage sites in HCV NS5A, E2/PePHD or CD81/HVR2 nucleotide sequences in patients with HCV 1b or 3a infection. The frequency of RNase L cleavage sites in HCV NS4B and NS5A did not differ significantly at different time points during antiviral therapy and follow up in 5 patients with HCV 1b infection. Patients with HCV genotype 3a infection showed more UU cleavage sites within NS5A and E2/PePHD compared to patients with HCV 1b infection (p=0.001). The number of UA cleavage sites was not different between isolates from HCV 1b and 3a infection.

Conclusions: No association between the frequency of
The occurrence of depression was reported in 20-30 % of cases in the studies which assessed peginterferon alfa and ribavirin in chronic hepatitis C. However, in these studies the diagnosis of depression was based only on the clinical impression of the investigators. Aims: The aims of our study were to evaluate the incidence of depression during the treatment with peginterferon alfa-2a plus ribavirin in chronic hepatitis C in the real life with the help of the Mini-International Neuropsychiatric Interview (MINI), a short structured diagnostic interview for DSM-IV and ICD-10 psychiatric disorders and to make the correlation between the MINI results and the diagnosis made by the clinician and with the Beck Depression Inventory score (BDI). Patients and methods: 150 HCV mono-infected patients treated with peginterferon alfa-2a plus ribavirin are expected to be enrolled in this multicentric study. The incidence of depression will be assessed at baseline, at weeks 4, 12, 24, at the end of the treatment and at the end of follow up with the MINI, the clinician feeling and the BDI score. Results: The results from baseline week 12 of the 123 first enrolled patients are presented in this intermediate analysis. The baseline patients characteristics were as follows: mean age: 48 years, male: 73 (59 %), history of psychiatric events 30 (24 %), genotype 1: 67 (54 %), F3-F4 fibrosis: 45 (37 %), naive patients: 73 (59 %). At baseline, the MINI revealed a current major depression in 9 patients (7 %), 7 received anti-depressant therapy before (n=5) or after (n=2) the beginning of peginterferon. Between the baseline and week 12, 21 (18 %) major depressions were diagnosed by the MINI. In 16 cases (76 %), the clinicians considered that there was no depression. Finally, an anti-depressant therapy was started in 17 cases (81 %). On the other hand, in 7 cases (6 %) the diagnosis of major depression was made by the clinician but not confirmed by the MINI. The correlation between the MINI and the BDI was poor. Conclusion: The incidence of depression between the baseline and week 12 was of 18 %. Major depression seems to be under-diagnosed by the clinicians. The systematic use of Mini-International Neuropsychiatric Interview (MINI), a short structured diagnostic interview for DSM-IV psychiatric disorders may be of interest during peginterferon therapy. This study was supported by Roche.

Disclosures:
Jean-Pierre Bronowicki - Consultant/Adviser: Roche
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The following people have nothing to disclose: Nathalie Talbodec, Dominique Capron, Jean-Jacques Raabe, Isabelle Beurthun, Valérie Cansva, Anne-Marie Weiss, Damien Lucidarme, Serge Fratte, Catherine Chandelier

The following people have nothing to disclose: Donna Evon, Amit Verma, Kelly Simpson, Karen Dougherty, Michael W. Fried
353 COMBINATION THERAPY WITH PEGYLATED INTERFERON ALFA-2B AND RIBAVIRIN FOR PATIENTS WITH CHRONIC HEPATITIS C AND NORMAL AMINOTRANSFERASE LEVELS

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Background/Aim: Combination therapy with pegylated interferon (PEGIFN) and ribavirin is standard therapy for patients with chronic hepatitis C (CHC). There is evidence that patients with CHC and persistently normal alanine aminotransferase (PNALT) levels respond similarly to those with elevated alanine aminotransferase (ALT) levels to treatment with interferon and ribavirin, however, such data with PEGIFN alfa-2b and ribavirin are currently few. The aim of this study was to evaluate whether patients with PNALT levels and patients with elevated ALT levels respond similarly to the combination therapy with PEGIFN alfa-2b and ribavirin. Methods: 299 chronic hepatitis patients infected with hepatitis C virus (HCV) genotype 1 were treated with PEGIFN alfa-2b 1.5 µg/kg/wk plus ribavirin 600-1000 mg/d for 48 weeks. Among this group 54 patients, had at least 3 normal ALT levels over the 3 months prior to treatment, were defined as patients with PNALT levels. The evaluation of efficacy was sustained virologic response (SVR), defined as undetectable serum HCV RNA at 24 weeks after completion of treatment. Results: Liver histology in patients with PNALT levels were significantly milder compared with that in patients with elevated ALT levels. Overall, 50.0% of patients with PNALT levels and 43.7% of those with elevated ALT levels were SVR (p=0.395). In male, SVR rates of 46.4% and 51.8% were obtained in the PNALT group and elevated ALT group, respectively (p=0.605). In female, SVR rates of 53.9% and 31.7% were obtained in the PNALT group and elevated ALT group, respectively (p=0.036). Conclusions: The efficacy of PEGIFN alfa-2b and ribavirin combination therapy in CHC patients with PNALT levels is similar to that in patients with elevated ALT levels. In particular, female patients with PNALT levels should be considered for curative therapy.

Disclosures: The following people have nothing to disclose: Yoshiaki Katano, Masatoshi Ishigami, Isao Nakano, Kazuhiko Hayashi, Takashi Honda, Hidemi Goto

354 THE ROLE OF STEATOSIS AND DIABETES AS A PREDICTOR OF TREATMENT RESPONSE TO INTERFERON BASED THERAPIES IN LATINO AMERICANS WITH CHRONIC HEPATITIS C

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Background: The role of nonalcoholic steatohepatitis (NASH) must be considered as a predictor of response to interferon based therapies in the Latino population. The aim of this study is to determine if steatosis and diabetes influence sustained virologic response (SVR) rates. Understanding the pathophysiology of NASH could help clarify pharmacological options and defining the role of treatment of NASH in overall treatment of HCV. Methods: Retrospective chart review of 205 Latino Americans referred to the HMRI Liver Center. Steatosis was defined as having fatty infiltration or balloon degeneration on liver biopsy. Diabetes was defined as having a fasting sugar greater than 126. SVR was defined as being RNA negative 6 months after interferon based therapy. Chi-square, Hest and logistic regression were performed. Results: Of the 205 patients, 67% were born in the USA, 23% were born in Mexico, and 10% were from a country in Central or South America. The mode of acquisition were: 42% tattoo, 40% transfusion, 37% IVDA, and 37% cocaine. The mean age this population was 52 yrs (range 25-82), mean weight 178 lbs (range 114-296), and the mean BMI was 28 (range 21-44). In 136 with liver biopsies; 67% had steatosis. Also ultrasound reports in 177 patients showed that 50% had radiographic evidence of steatosis. The most common genotype was 1 65%, 2 24%, and 3 10%. There were 79 patients treated for Hepatitis C. The majority of patients received pegylated interferon with weight based ribavirin 66%. The sustained virologic response rate to interferon based therapies for all genotypes was 36%. The nonresponder rate (NR) was 44% and the rebound rate (RR) was 14%. In Genotype 1 the SVR 23%, NR 60%, RR 17%. For Genotype 2 the SVR 76%, NR 18%, RR 6%. In Genotype 3 the SVR 38%, NR 50%, RR 13%. We found no statistical difference for SVR between age, weight, BMI, steatosis, diabetes, and cirrhosis. However there were trends of clinical relevance. Table 1 Summary: There were no statistical differences in SVR with respect to weight, BMI, diabetes. This may be due to the low samples size of the population. However, there were trends that need to be further analyzed. 67% of our population had steatosis on liver biopsy. Patients with steatosis on biopsy tended to have a lower SVR (33%) compared to those with no steatosis (46%). The diabetic patients had a lower SVR of (26%) compared to our non-diabetic patients with an SVR of (40%).

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<th>SVR (%)</th>
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Disclosures: The following people have nothing to disclose: Edward A. Mena, Orlinda E. Ventura, Lawrence M. Blatt, Myron J. Tong

355 HEPATITIS C VIRUS (HCV) TREATMENT OUTCOMES WITH CONSENSUS INTERFERON WITH OR WITHOUT RIBAVIRIN IN PEGINTERFERON/RIBAVIRIN NON-RESPONDERS/RELAPSERS: RESULTS FROM NATIONAL CLINICAL PRACTICE SETTINGS

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Background: Approximately 50% of chronic hepatitis C patients who receive current standard of therapy (peginterferon alfa plus ribavirin) are nonresponders to treatment. Currently, there is no FDA-approved treatment for HCV nonresponders or relapsers to peginterferon alfa plus ribavirin. Despite limited published data and limited criteria for use, some providers may consider using consensus interferon (CIFN, Infergen®) in combination with ribavirin as a treatment option in these patients. To date, there have not been any large studies evaluating provider practice patterns and treatment outcomes of CIFN in HCV nonresponders or relapsers to peginterferon alfa plus ribavirin. Objective: To evaluate treatment outcomes and utilization patterns of CIFN with or without ribavirin in chronic HCV
CARCINOMA IN PRIMARY SCLEROSING CHOLANGITIS

TECHNIQUES FOR THE DETECTION OF CHOLANGIOCARCINOMA

Cholangiographic findings suspicious for the diagnosis of CC included a polypoid bile duct lesion or a dominant stricture with or without proximal biliary dilatation. Serum CA19-9 combined with either US, CT, or MRI provided a sensitivity of 91%, 100%, and 96%, specificity of 62%, 38%, and 37%, positive predictive value (PPV) of 23%, 22%, and 24%, and negative predictive value (NPV) of 98%, 100%, and 98%, respectively, if at least one method was positive. In the clinical context of patients with biliary strictures plus either CA19-9 > 20.4 U/ml or suspicious findings on cross-sectional images, routine cytology, aneuploidy detection by digital imaging analysis, and aneusomy detection by fluorescent in situ hybridization yielded a sensitivity of 50%, 57%, and 86%, specificity of 97%, 90%, and 75%, PPV of 88%, 80%, and 71%, and NPV of 83%, 75%, and 88%, respectively. Conclusions: No individual test is a perfect tool. Serology combined with radiologic method provides high sensitivity and NPV whereas biliary cytologic examination provides high specificity and PPV for the diagnosis of CC in PSC. These may be useful for the early detection of CC in patients with PSC.

<table>
<thead>
<tr>
<th>Test</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>PPV (%)</th>
<th>NPV (%)</th>
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<tr>
<td>CA 19-9</td>
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<td>67</td>
<td>23</td>
<td>96</td>
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Disclosures:
The following people have nothing to disclose: Phunchai Charatcharoenwitthaya, Keith D. Lindor

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THE VALUE OF SERUM CA 19-9, ULTRASONOGRAPHY, COMPUTED TOMOGRAPHY, MAGNETIC RESONANCE, CHOLANGIOGRAPHY, AND BILIARY CYTLOGIC TECHNIQUES FOR THE DETECTION OF CHOLANGIOCARCINOMA IN PRIMARY SCLEROSING CHOLANGITIS

Phunchai Charatcharoenwitthaya, Keith D. Lindor; Division of Gastroenterology and Hepatology, Medicine Department, Mayo Clinic, Rochester, MN

Background & Aims: There is limited information on test performance for detecting cholangiocarcinoma (CC) in primary sclerosing cholangitis (PSC). This study aimed to characterize diagnostic performance of serum CA19-9, US, CT, MRI, cholangiography, and biliary cytologic techniques for detecting CC in PSC. Methods: Two hundred and thirty patients with PSC were followed until the development of CC from 2000 through 2006. During this time, 23 of these patients developed cytopathologically confirmed CC. Patients were considered free of CC if they had no evidence of CC at least 1 year of follow-up. Results: The annual incidence of CC was 2.0%. According to the TNM classification for CC, 15 (65%) patients had stage I-II, one had stage III, and seven had stage IV. Operative characteristics of each test for predicting CC in PSC were summarized in the Table. The optimal cutoff value for serum CA19-9 is 20.4 U/ml. Bile duct cancers should be suspected if there is a liver mass, marked bile duct wall thickening, marked biliary dilatation, or focal intrathoracic biliary dilatation associated with ipsilateral lobe atrophy on cross-sectional images of the liver. Cholangiographic findings suspicious for the diagnosis of CC included a polypoid bile duct lesion or a dominant stricture with or without proximal biliary dilatation. Serum CA19-9 combined with either US, CT, or MRI provided a sensitivity of 91%, 100%, and 96%, specificity of 62%, 38%, and 37%, positive predictive value (PPV) of 23%, 22%, and 24%, and negative predictive value (NPV) of 98%, 100%, and 98%, respectively, if at least one method was positive. In the clinical context of patients with biliary strictures plus either CA19-9 > 20.4 U/ml or suspicious findings on cross-sectional images, routine cytology, aneuploidy detection by digital imaging analysis, and aneusomy detection by fluorescent in situ hybridization yielded a sensitivity of 50%, 57%, and 86%, specificity of 97%, 90%, and 75%, PPV of 88%, 80%, and 71%, and NPV of 83%, 75%, and 88%, respectively. Conclusions: No individual test is a perfect tool. Serology combined with radiologic method provides high sensitivity and NPV whereas biliary cytologic examination provides high specificity and PPV for the diagnosis of CC in PSC. These may be useful for the early detection of CC in patients with PSC.

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VALIDATION STUDY OF THE PROGNOSTIC IMMUNOHISTOCHEMICAL MARKER GLYPICAN-3 IN HEPATOCELULAR CARCINOMA

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Distinguishing an early hepatocellular carcinoma (HCC) from benign and pre-malignant small focal lesions (dysplastic foci, low-and high grade dysplastic nodules) that occur in liver cirrhosis can be difficult but important to provide patients with the most appropriate treatment. Several groups reported the potential use of glypican-3 (GPC-3) in the diagnosis of small early HCC. For clinical use it is important to validate the reproducibility of the immunohistochemical staining of GPC-3 in the same lab as in different pathology labs around the world. We performed immunohistochemistry for GPC-3 on 8 low-grade dysplastic nodules, 19 high-grade dysplastic nodules, and 38 HCCs with a diameter less or equal to 3 cm present in the cirrhotic liver of patients. We tested the reproducibility of the GPC-3 marker on the same set of biopsies inside the same institution (Leuven, Belgium) with a 2 year interval and in a second institution (Milan, Italy). The sensitivity and specificity of a positive GPC-3 staining for the diagnosis of small HCC is summarized in Table 1. The intra- and inter-laboratory reproducibility of the staining was > 95%. Because GPC-3 staining is easily interpretable and internationally reproducible, immunohistochemistry for GPC-3 is a valuable ancillary tool in the histopathological diagnosis of small focal lesions in cirrhosis.
Table 1.

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THE ANGIOGENIC MAKE UP OF HUMAN HEPATOCELLULAR CARCINOMA DOES NOT FAVOUR VEGF / ANGIOPOIETIN Driven SPROUTING NEOVASCULARIZATION

Wenjiao Zeng1,2, Annette S. Gouw1, Marius van den Heuvel1, Sibrand Poppema1, Nong Zhang2, Inge Plateel1, Koert P. De Jong2, Grietje Molema2; 1Dept. of Pathology & Laboratory Medicine, University Medical Center Groningen, Groningen, Netherlands; 2Dept. of Pathology, Shanghai Medical College, Fudan University, Shanghai, China; 3Dept. Pathology & Laboratory Medicine, University College of Medicine, Kangdong Sacred Heart Hospital, Seoul, South Korea; 4Dept. of HPB Surgery & Liver transplantation, University Medical Center Groningen, Groningen, Netherlands

Background: The first hopeful results of anti-angiogenic treatment of HCC has been announced, yet knowledge regarding the angiogenic status of HCC is still limited. This study investigated the balances in gene and protein expression levels of members of the Vascular Endothelial Growth Factor (VEGF) and Angiopoietin/Tie-2 system that are responsible for angiogenic sprouting of blood vessels. Methods: We studied gene and protein expression levels and cellular localization of VEGF-A, VEGFR-1 and VEGFR-2, Angiopoietin (Angpt)-1 and -2, and their receptor Tie-2 in human HCC from non-cirrhotic and cirrhotic livers, using real-time RT-PCR, Western blot and immunohistochemistry. The data were compared with those in the highly angiogenic human renal cell carcinoma (RCC), and normal liver and kidney. Results: HCC expressed VEGF-A, VEGFR-1, and VEGFR-2 to a similar extent as in normal liver. VEGF-A was mainly expressed by tumor endothelial cells (EC), while their receptors were weakly expressed in the tumor. The Angpt-1 expression was slightly increased in HCC compared to normal liver, while its receptor Tie-2 was strongly downregulated in tumor vasculature. The vessel-stabilization factor Angpt-2, was undetectable in HCC. VEGF-A levels were one order of magnitude higher in RCC than in HCC: there was a 100-fold increase in RCC compared with normal kidney, whereas no differences were seen between HCC and normal livers. Expression of Angpt-2 was > 30 fold increased compared to normal liver, while Angpt-1 expression was decreased in the tumor. As a consequence, Angpt1/2 ratios fell from 3.6 in normal kidney, to 0.06 in RCC. In comparison, in HCC these ratios ranged between 0.4 (non-cirrhotic) and 1.5 (cirrhotic). HCC from non-cirrhotic and cirrhotic background showed similar expression profiles of the angiogenic factors studied. VEGF-A in normal livers was seen in vascular- and sinusoidal EC, in cholangiocytes, but not in hepatocytes. In tumor EC VEGF-A was less pronounced, and tumor cells were negative. In HCC, VEGFR-1 and VEGFR-2 were only weakly expressed in some tumor EC. Angpt-1 cytoplasmic staining was seen in HCC cells and normal hepatocytes, and weak staining was seen in vascular endothelium. Angpt-2 staining was hardly detectable in HCC. In normal liver Angpt-2 positive sinusoidal ECs were present around terminal hepatic venules, while vascular endothelium was weakly expressing Angpt-2. Tie-2 expression was sinusoidal in normal liver and more scattered in HCC. Conclusions: In contrast to findings in RCC, HCC tumor vascularization is likely not VEGF / Angpt/Tie-2 driven.

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LOW-DOSE PEGYLATED INTERFERON THERAPY IN COMBINATION WITH TRANS-ARTERIAL CHEMOINFUSION IN HEPATOCELLULAR CARCINOMA PATIENTS WITH MAJOR PORTAL VEIN THROMBOSIS OR EXTRAHEPATIC METASTASIS

Hyun Deok Shin1, Young-Hwa Chung1, Dong Dae Soo1, Sung Eun Kim1, Danbi Lee1, Sae Hwan Lee1, Yoon-Seon Lee1, Myoung Kuk Jang2, Soo Hyung Ryu3, Kang Mo Kim1, Yong Suk Lim1, Han Chu Lee1, Yung Sang Lee1, Dong Jin Suh1; 1Department of Internal Medicine, University of Ulsan College of Medicine, Asan Medical Center, Seoul, South Korea; 2Department of Internal Medicine, Hallym University College of Medicine, Kangdong Sacred Heart Hospital, Seoul, South Korea; 3Department of Internal Medicine, University of Inje College of Medicine, Seoul Paik Hospital, Seoul, South Korea

Backgrounds/Aims: Patients with hepatocellular carcinoma (HCC) associated with major portal vein thrombosis (PVT) or extrhepatic metastases (EHM) have few effective therapeutic options so far. In this study, we intended to examine the safety and efficacy of pegylated interferon (Peg-IFN) therapy in combination with trans-arterial chemoinfusion (TACI) in these patients comparing with TACI alone. Methods: One hundred and twenty consecutive HCC patients with major PVT or EHM [Etiology of underlying liver disease (HBV:HCV:NBNC) = 103:5:12; PVT = 88/120 (73%); EHM = 63/120 (53%); Child-Pugh class (A:B:C) = 94:24:2] were randomly allocated to combined therapy group with Peg-IFN and TACI (n = 60) and TACI group (n = 60). Peg-IFN (PEG-INTRON®, Schering-Plough Corporation, Kenilworth, NJ, USA) was administered at a dose of 50 mcg per week subcutaneously. Cisplatin was infused through the proper hepatic artery at a dose of 2mg/Kg of body weight every 4 or 8 weeks as long as it was tolerable. The subjects were followed with serum biochemistry, alpha-fetoprotein (AFP) levels and spiral CT scan at 4-weeks intervals. Results: Between the groups, there was no significant difference in age, gender, etiology of underlying liver disease, serum AFP level, size or type of HCC, the frequency of major PVT or EHM and Child-Pugh class. In addition to anorexia/nausea/vomiting and mild azotemia, flu-like symptoms, leucopenia or throbocytopenia were also observed in a substantial number of patients in combined therapy group. But, most of them were transient and tolerable. Compared with baseline levels, at 4 weeks after treatment, the median value of serum AFP level was decreased in combined therapy group, but rather increased in TACI group. The proportion of progressive disease evaluated at 4 weeks after the treatments was significantly lower in combined therapy group than TACI group (44% vs. 58%; P<0.05). Consequently, the 1-year survival rate of patients treated with combined therapy was much higher than that of TACI group (46% vs. 18%; P<0.05). Also, the median survival period of combined therapy group was much higher than that of TACI group (38 weeks vs. 25 weeks; P<0.05). Conclusion: Thus, low-dose Peg-IFN therapy in combination with TACI may be a safe and useful palliative treatment in HCC patients with major PVT or EHM.

Disclosures:
Background/ Aims: Cholangiocarcinoma (CC) is a primary malignant liver tumor with poor prognosis, partly because of its poor response to chemotherapy. Recent studies have shown that hepatic progenitor cells (HPCs) play a role in the development of hepatocellular carcinoma (HCC). Moreover, prognosis of HCC with HPCs features is poor. Since HPCs can differentiate into both hepatocytes and cholangiocytes, we evaluated the presence of HPCs and the hepatocyte differentiation in CC. The second aim of this study was to investigate the presence of the ATP-binding cassette (ABC) superfamily of membrane transporters given their possible involvement in chemoresistance of CC. Material and methods: We studied 93 cases of CC by immunohistochemistry for hepatocellular differentiation markers (Hepa, canicular polyclonal carcinomaembryonic antigen (pCEA), CD10), biliary/progenitor cell markers (cytokeratin (CK) 7, CK19 and neural cell adhesion molecule (NCAM)) and the ABC transporters (MDR1, MRP1, MRP3 and BCRP). Results: Thirty-seven (39.8%) cases of CC showed hepatocyte differentiation (CCH). Serum AFP values were significantly higher in CCH than in CC (p=0.0449). The morphological features of hepatocyte differentiation was classified into two patterns: (i) a trabecular pattern which show thick trabecular with little stroma consisting of tumour cells with abundant eosinophilic cytoplasm and mild atypia; and (ii) a cord pattern which show atrophic thin trabecular and/or cord structure of small uniform cells. A trabecular pattern was observed in 17/37 cases (45.9%), a cord pattern in 16/37 (43.3%) cases, and both patterns in 4/37 cases (10.8%). In these areas, CK7 positive intermediate-sized cells which showed a submembranous staining pattern was observed in all cases, a canicular staining pattern of pCEA in 30/32 cases (93.8%), CD10 in 21/29 cases (72.4%) and Hepa in 5/24 cases (20.8%). In addition, cytoplasmic CK7 and/or CK19 positive small round oval cells resembling HPCs were present in all cases and also positive to NCAM in 19/24 (79.2%) cases. These immunohistochemical staining pattern were same in both pattern. BCRP, MRP3 and MRP1 staining showed a basolateral staining pattern and MDR1 showed canicular staining pattern in both pattern, but the expression was down-regulated in comparison with surrounding normal hepatocyte. Conclusion: In our study, about 40% cases of CC showed hepatocyte differentiated features. According to both morphological and immunohistochemical features, these findings strongly suggest a possible HPCs origin of a subset of CC. The fact that MRP1, MRP3 and BCRP are up-regulated, may explain chemoresistance of CC.

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Health plan population incidence rates were age-adjusted to the year 2000 standard US population. We identified 716 HCC cases from the selected years. The median age at diagnosis was 63 years; 71% of cases were men; 47% were non-Hispanic white and 29% were Asian. The age-adjusted yearly incidence of HCC rose steadily from 31 per million in 1997-8 to 46 per million in 2004-5. The proportion of cases in which viral hepatitis B and C had not been evaluated dropped from 27% in 1997-8 to 11% in 2000 and all later years studied, and may in part explain the corresponding 1.5-fold increased incidence of viral-hepatitis-related HCC. Although the absolute numbers were small, the incidence of apparent FLD-associated HCC increased more than ten fold from 0.3 per million in 1997-8, to 4.6 per million in 2004-5. This increase was not attributed to changes in surveillance and testing for the factors defining a FLD diagnosis. The proportion of HCC for which an etiology could not be determined varied between 16-25% in the study years. Fluctuations, but no chronologic trends, were found in the relative contributions of hepatitis B (15-22%), of hepatitis C (33-46%), and of alcohol with no viral component (10-20%) to HCC. However, the proportion of HCC cases with probable FLD and no other discernable CLD rose from 1% to 10% during the study years. In this diverse population, HCC incidence rates increased by over 50% in 8 years. The trend was due to a modest increase in the incidence of viral-hepatitis-related HCC, and in part to steadily increasing rates of HCC apparently related to FLD.

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HIV-INFECTED PATIENTS WITH HEPATOCELLULAR CARCINOMA HAVE WORSE SURVIVAL PROGNOSIS THAN UNINFECTED PATIENTS ADJUSTED BY BCLC STAGE

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HAART has changed the natural history of liver disease in HIV-infected patients. Although hepatocellular carcinoma (HCC) in non-HIV patients is well described, few data are available of HCC in HIV-infected persons. AIMS AND METHODS: The aim was to assess the survival of HIV-seropositive patients with HCC and to compare them with HIV-seronegatives. A retrospective analysis from October 1998 until January 2007 in a single tertiary care center found 25 HIV(+)/HCC patients. Controls were 99 consecutive assisted HIV(-)/HCC patients. The main clinical and epidemiological characteristics of patients with HCC were described and their survival was compared between HIV-positive and uninfected subjects. Survival was evaluated by Kaplan-Meier curves and compared by log-rank test. RESULTS: HIV(+)/HCC patients had a mean CD4+ cell count of 309 ± 229/mm3 (28% < 200) and a median HIV RNA load of 162,5 copies/mL (undetected in 48%). Eight patients (32%) had AIDS disease. The main H$\text{I}$V infection way was through intravenous drug use. In HIV(+) tumor was solitary in 8 (32%) patients, multinodular in 7 (28%) and diffuse/massive in 10 (40%). In 64% of patients tumor size was > 5 cm, both liver lobules were affected in 24% and portal vein invasion was present in 36%. Four patients (16%) had extra-hepatic metastases. HIV(+) HCC patients were younger than HIV(-) controls (45.5 ± 4.6 vs 69.6 ± 9.4 years; p=0.001), had more viral etiology (100% vs 70%; p=0.001) mainly due to hepatitis C infection (84% vs 64%; p=0.062), and had more advanced Child-Pugh grade (A grade 36% vs 67%; B-C grade 64% vs 33%; p=0.001). BCLC stage was more advanced in HIV(+)/HCC patients than controls (A stage 16% vs 60%; B stage 12% vs 19%; C stage 36% vs 20%; D stage 36% vs 1%; p=0.001). The median overall survival was 11,1 ± 10,9 months in HIV(+)/HCC patients compared with HIV(-) controls (36,5 ± 3,7 months; p= 0.001). Adjusted by BCLC stage a significant decreased survival in CD stage HIV(+)/HCC patients compared with HIV-seronegatives was observed (1 ± 0.01 vs 10.2 ± 3.3 months; p = 0.008). The HIV RNA load, CD4+ cell level or AIDS disease had no influence in the survival analysis. CONCLUSIONS: HIV-seropositive patients who develop HCC have a younger age, a more severe liver disease and worse survival prognosis than uninfected patients adjusted by BCLC stage.

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ORAL SUPPLEMENTATION WITH BRANCHED-CHAIN AMINO ACIDS IMPROVES SURVIVAL AND RECURRENCE-FREE SURVIVAL AFTER SUCCESSFUL TREATMENT OF HEPATOCELLULAR CARCINOMA IN PATIENTS WITH CIRRHOSIS: A PROSPECTIVE STUDY

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BACKGROUND: Oral supplementation with branched-chain amino acids (BCAA) is reported to improve event-free survival, serum albumin concentration, and QOL in patients with cirrhosis without hepatocellular carcinoma (HCC). BCAA have shown to improve insulin resistance and protein synthesis in vitro by stimulating the pathway involving mammalian TOR. Although, improvement of insulin resistance using BCAA may result in suppressed hepatocarcinogenesis, the clinical efficacy in preventing development of HCC recurrence is unclear. AIM: To determine whether long-term oral supplementation with BCAA can prevent HCC recurrence after curative treatment for initial HCC and improve survival. METHODS: A cohort of 235 patients with HCC who were successfully treated with radiofrequency ablation (RFA) as an initial curative therapy were prospectively analyzed (mean age 67 yrs; M/F = 147/88; HCV/HBV/Others = 188/20/27; Child A/B = 191/44). Patients were divided into two groups: A BCAA group (12 g/day) and a diet therapy alone group (matched daily energy and protein intake). The cumulative survival and recurrence-free survival rates were analyzed by the Kaplan-Meier method, and the factors associated with survival were determined by the Cox proportional hazard model. RESULTS: The rate of first or second recurrence of new foci of HCC was similar between the two groups; however, the rate of third recurrence was significantly lower in the BCAA group (logrank test: p = 0.02). Among patients with lower albumin level (below 3.5 mg/dL), the cumulative survival rate after initial RFA was significantly higher in the BCAA group compared with the diet alone group (at 3 and 5 years: 100% vs. 67% and 73% vs. 41%, logrank test: p = 0.02). In contrast, no significant difference between the two groups was found in patients with higher albumin level (3.5 mg/dL or more). Univariate analysis used to characterize BCAA supplementation (hazard ratio, 3.6; 95%CI, 1.1-12), alpha fetoprotein higher than 100 ng/mL (hazard ratio, 7.5;
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365 SEPARATE ANALYSIS OF INTRANODULAR BLOOD SUPPLY IN NODULAR LESIONS ASSOCIATED WITH LIVER CIRRHOSIS: A NOVEL ULTRASOUND TECHNIQUE “PURE ARTERIAL PHASE IMAGING”
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Background and Aim: It is well known that premalignant nodular lesions associated with liver cirrhosis such as low grade dysplastic nodules (LGDN) and high grade dysplastic nodules (HGDN) are supplied by portal venous flow, whereas overt hepatocellular carcinoma (HCC) is supplied by pure arterial flow. Until now, separate detection of intranodular blood supply is possible only by CT during hepatic arteriography (CTHA) and CT during arterial portography (CTAP). In this study, we investigated the usefulness of newly innovated ultrasound technology “Pure Arterial Phase Imaging”, which permits separate analysis of intranodular blood supply. Materials and Methods: A total of 50 overt HCCs, 10 LGDNs, and 5 nodule-in-nodule type HCCs were included in this study. Pure Arterial Phase Imaging was performed as follow; 1) Injection of ultrasound contrast agent, Levovist or Sonazoid; 2) Performance of the contrast-enhanced ultrasonography (CEUS), 3) Storage of the “raw data” of CEUS in the GE LOGIQ 7 ultrasound machine, 4) By reviewing the raw data of CEUS study, range of interests (ROI) were set on the extranodular arterial vessel and portal venous vessel, 5) Pure arterial phase was automatically calculated by the time-intensity curves of the 2 ROIs, 6) Accumulation or capture images of maximum intensity projection (MIP) were obtained in order to evaluate whether intranodular blood supply is arterial origin or portal venous origin. Results: Arterial supply was proved in all of 50 HCC nodules, which was consistent with dynamic CT findings. Portal supply was proved in all of 10 dysplastic nodules, and this finding was consistent with that obtained by CTAP. Arterial supply within portal supply pattern was clearly demonstrated (nodule-in-nodule pattern) by Pure arterial phase imaging, and this finding was completely compatible with the findings on CTHA and CTAP. Conclusion: Newly innovated technology “Pure Arterial Phase Imaging” can clearly reveal the origin of intranodular blood supply, which permits definitive diagnosis of premalignant lesions (LGDN, HGDN, and nodule-in-nodule type HCC) and overt HCC. Since CTHA and CTAP are invasive technique, this non-invasive technique “Pure Arterial Phase” imaging may be able to replace both CTHA and CTAP.

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366 GENOME-WIDE MOLECULAR PROFILES OF HCV-INDUCED HEPATOCELLULAR CARCINOMA
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Identification of genomic changes linked to the occurrence of HCC in patients with HCV cirrhosis is important to understand the pathways of hepatocarcinogenesis in these patients. Therefore we compared the gene expression profiles in the liver of patients with HCV cirrhosis according to the occurrence or not of HCC during the short term follow-up. Patients who developed HCC in the 5 years following liver biopsies were compared with patients who did not. Both groups were matched for age and sex. All patients had active viral infection and belonged to Child-Pugh class A. As a whole, 17 liver specimens (G1) of patients who did not develop HCC were compare to 13 liver specimens (G2) of patients who develop HCC during the following 5 years. Using the BRB Array Tools software, of the 22 442 genes tested we identified 194 genes as differentially expressed (according to a p-value < 0.03 for T-test with random variance model). Most of them appear to be involved in angiogenesis, matrix metalloproteinase activation, immune response (up-regulated) or sialidation (down-regulated). Leukocyte extravasations signaling was the most discriminant metabolic signaling pathway (up regulated) in patients who develop HCC. Conclusion. Genes expression involved in regulation of angiogenesis, metalloproteinase activation, immune function, N-Glycan degradation are implicated in hepatocarcinogenesis in HCV cirrhosis. This finding highlights the role of stromal interaction in hepatocarcinogenesis.

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367 THE MELD BASED OBJECTIVE SCORING SYSTEM (MOSS): NEW INSIGHT INTO RISK STRATIFICATION OF CIRRHOTIC PATIENTS WITH HEPATOCELLULAR CARCINOMA
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Introduction: The MELD scoring system is a validated tool to ascertain short term survival in patients with cirrhosis. There is lack of a consensus on an ideal model to predict prognosis among patients with cirrhosis and hepatocellular carcinoma (HCC) treated with modalities other than liver transplantation. We therefore conducted a survival analysis and developed a novel MELD-based objective scoring system (MOSS) to stratify survival of HCC patients treated with non-liver transplant modalities. Methods: A retrospective analysis of 204 patients with HCC diagnosed between 1994 and 2004 was performed. Ninety patients were treated with transarterial chemoembolization (TACE) and 114 patients were treated with hepatic resection. Objective clinical variables that determined survival were obtained using a Cox proportionate hazard regression model. Results: The mean MELD score and tumor size for the
TACE and hepatic resection group were 10.7 vs. 9 and 5.2 cm vs. 5 cm, respectively. The median survival for the TACE group was 1.2 years and the hepatic resection group was 1.9 years. In a multivariate analysis, both MELD score and tumor size were significant predictors of survival in both the TACE and the resection groups. MELD score, tumor size, portal vein invasion and tumor multiplicity were each allocated points for incorporation into the scoring system. Three subgroups were then derived from the MOSS, based on their total points. Table 1 demonstrated the median survival for MOSS Group I, II and III in patients treated with TACE and hepatic resection. Conclusion: MOSS, a scoring system that combines MELD and tumor characteristics, accurately stratifies outcomes in cirrhotic patients with HCC treated with TACE or hepatic resection. The parameters used in MOSS are objective, non invasive and readily available clinically. If validated, this scoring system would be important in predicting clinical outcomes, optimizing resource utilization and designing trials of novel treatments.

Table 1: The median survival for patients with cirrhosis and HCC treated with TACE and hepatic resection according to the MOSS group

<table>
<thead>
<tr>
<th>MOSS Group</th>
<th>TACE (n=90), months</th>
<th>Hepatic Resection (n=14), months</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>23</td>
<td>54</td>
</tr>
<tr>
<td>Group II</td>
<td>15</td>
<td>24</td>
</tr>
<tr>
<td>Group III</td>
<td>5</td>
<td>9</td>
</tr>
</tbody>
</table>

MELD score (6, 7, 8 = 3 points), (9, 10, 11 = 6 points), (12, 13, 14 = 9 points), (>15 = 12 points)

Tumor Size (< 5cm = 0 point), (≥ 5 cm = 2 points)

Portal Invasion (No= 0 point), (Yes= 1 point)

Multiplicity (Single lesion = 0 point), (> 2 lesions = 1 point)

Group I = Total points ≤ 5 points

Group II = Total points = 6 - 8 points

Group III= Total points ≥ 9 points

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368 SEMIANNUAL SURVEILLANCE FOR HEPATOCELLULAR CARCINOMA IMPROVED PATIENT SURVIVAL COMARED TO ANNUAL SURVEILLANCE (KOREAN EXPERIENCE)

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Background/Aims: The use of a surveillance interval of 6 months is recommended for the early detection of hepatocellular carcinoma (HCC) in high risk patients. Previously, Italian investigators reported there was no difference of patient survival between semiannual and annual surveillance in cirrhotic patients. The aim of this study was to elucidate whether semiannual surveillance for HCC affected the clinical outcomes of patients diagnosed with HCC, compared with annual surveillance in Korea. Methods: Between May 1990 and December 2004, a total 400 patients (M:F = 2.6:1) were diagnosed with HCC by surveillance program [ultrasound examination and alpha-fetoprotein (AFP) measurement every 6 or 12 months]. These patients were divided into two groups according to surveillance interval; Group 1 (semiannual, n=219) and Group 2 (annual, n=181). The characteristics of HCC identified and overall patient survival were analyzed and compared between these two groups. Results: The mean age of all patients was 57 years. The etiology of HCC was hepatitis B virus in 289 (72.3%) patients, hepatitis C virus in 76 (19.0%), and non B- non C in 32 (8.0%). AFP levels were ≥400 ng/mL in 109 patients (27.3%), and <20 ng/mL in 147 (36.8%). In comparison between two groups, single nodular HCC was more prevalent in Group 1 than in Group 2 (90.4% vs. 72.9%, P<0.001). On the contrary, diffuse type HCC was more common in Group 2 (4.1%, vs. 11.6%, P<0.001).The frequency of solitary HCC ≤3cm was significantly higher in Group 1 compared with Group 2 (62.1% vs. 51.5%, P=0.003). The application of curative treatments such as resection or local ablative therapy was more frequent in Group 1 compared to Group 2 (18.7% vs. 12.2%, P=0.03). Five-year survival in Group 1 was significantly better than that of Group 2 (25% vs 16%, P=0.006, log-rank test). Conclusion: Our data show that semiannual surveillance resulted in the detection of HCC at an earlier stage and improved survival compared to annual surveillance in Korea.

Disclosures:
The following people have nothing to disclose: Do Young Kim, Kwang Hyub Han, Sang Hoon Ahn, Yong Han Paik, Kwan Sik Lee, Chae Yoon Chan, Young Myoung Moon

369 PROSPECTIVE EVALUATION OF STAGING SYSTEMS FOR HEPATOCELLULAR CARCINOMA DETECTED DURING SURVEILLANCE OF CIRRHOTIC PATIENTS

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Background: The role of staging systems for hepatocellular carcinoma (HCC) was never prospectively evaluated in cirrhotic patients with HCC detected during surveillance. Aim: We prospectively evaluated the impact of BCLC, CLIP and GETCH staging systems in predicting the outcome of cirrhotic patients with HCC detected during a surveillance program. Patients and methods: Between 1997 and 2005, among 1383 cirrhotic patients (Child-Pugh A/B), HCC was detected in 248, 173 (70%) males, mean age 65 yrs (range 43-76), 211 (85%) patients (27.3%), and <20 ng/mL in 147 (36.8%). In comparison between these two groups. Results: The mean age of all patients was 57 years. The etiology of HCC was hepatitis B virus in 289 (72.3%) patients, hepatitis C virus in 76 (19.0%), and non B- non C in 32 (8.0%). AFP levels were ≥400 ng/mL in 109 patients (27.3%), and <20 ng/mL in 147 (36.8%). In comparison between two groups, single nodular HCC was more prevalent in Group 1 than in Group 2 (90.4% vs. 72.9%, P<0.001). On the contrary, diffuse type HCC was more common in Group 2 (4.1%, vs. 11.6%, P<0.001).The frequency of solitary HCC ≤3cm was significantly higher in Group 1 compared with Group 2 (62.1% vs. 51.5%, P=0.003). The application of curative treatments such as resection or local ablative therapy was more frequent in Group 1 compared to Group 2 (18.7% vs. 12.2%, P=0.03). Five-year survival in Group 1 was significantly better than that of Group 2 (25% vs 16%, P=0.006, log-rank test). Conclusion: Our data show that semiannual surveillance resulted in the detection of HCC at an earlier stage and improved survival compared to annual surveillance in Korea.

Disclosures:
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months (range 1-107), the yearly mortality rate was 12.1%. By univariate analysis, age (p=0.012), etiology (p=0.01), Child-Pugh (<0.0001), performance status (p=0.0001), BCLC (p=0.0001), CUP (p=0.012), and GETCH (p=0.002), were significantly related to survival. BCLC system had the best homogeneity among patients in the same stages (LHR Chi-square = 18.9), the highest discriminatory power (linear trend = 22.2), and the lowest AIC (737.0), indicating the best stratification power. By multivariate analysis, age (p=0.001), Child-Pugh (p=0.0001), BCLC (p=0.0006), and GETCH (p=0.035) were the only variables independently related to survival. When multivariate analysis was performed stratifying for the treatment received, Child-Pugh (p<0.0006) and GETCH (p=0.022), were the only variables related to survival. Conclusions: GETCH and BCLC are both independent predictor of survival in cirrhotic patients with HCC diagnosed during surveillance program. GETCH has the best predictive value after treatment choice.

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370 HEPATOCELLULAR CARCINOMAS (HCC) IN PATIENTS WITH METABOLIC SYNDROME: A ROLE FOR MALIGNANT TRANSFORMATION OF TELANGIECTATIC ADENOMAS?

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Metabolic syndrome (MS) is a well-known risk factor for chronic liver disease and HCC. Higher prevalence of MS is associated with a significant increase in HCC. The aim of the study was to report the pathological features of HCC and background liver tissue in patients having MS as the only risk factor for liver disease. Twenty-eight patients with MS underwent liver resection for HCC in a single center and were retrospectively studied. Other potential etiological factors of HCC were ruled out. Tumor features including size, differentiation grade, presence of capsule, satellite nodules and vascular invasion were reported, as well as characteristics of the non-tumoral liver: fibrosis was staged according to Kleiner: 0: no fibrosis, 1: perisinusoidal or periportal fibrosis, 2: perisinusoidal and periportal fibrosis, 3: bridging fibrosis and 4: cirrhosis, presence of steatosis and steato-hepatitis. Results: All patients were caucasian males with a mean age of 68 ± 8 years. Mean BMI was 30±5. Dyslipidemia, diabetes, obesity, and hypertension were present in 61%, 75%, 79%, and 82%, respectively. Mean size of HCC was 86±12 mm (20-350 mm). Most of the patients had a unique HCC (n=22, 79%). Histological analysis of the tumor showed presence of a capsule in 71% of cases. In 12 patients, vascular invasion was noted. Presence of satellite nodules was observed in 9 cases (32%). HCC was well and moderately differentiated in 22 and 6 cases, respectively. Adjacent liver showed significant fibrosis (stage >3) in 10 patients (35%), with 7 patients having cirrhosis. Significant steatosis (≥30%) and steato-hepatitis were observed in 19 (65%) and 4 (14%) patients, respectively. When pathological characteristics of the HCC were compared according to the presence of fibrosis in the adjacent liver, size of tumor was significantly smaller in the group of significant fibrosis (54±8 mm versus 104±17 mm, p=0.01). In the same group, HCC were less differentiated than in the group without significant fibrosis (40% versus 16 % of moderately-differentiated). No difference was observed regarding preexistence of satellite nodules or vascular invasion. In the group of HCC occurring on normal liver, 5 out of 18 (28%) arised on a pre-existing liver cell adenoma, 3 of them being telangiectatic adenomas (TA). Conclusion: HCC in patients with MS predominantly develop in the absence of significant fibrosis. Since TA are often associated with MS (Paradis V et al, Hepatology in press), and remnants of TA are observed inside HCC in some of these cases, one may suggest that malignant transformation of TA might be the most relevant pathway of HCC development in the context of MS.

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371 PROGNOSTIC FACTORS IN PATIENTS WITH HEPATOCELLULAR CARCINOMA AND MACROVASCULAR INVASION

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Purpose: To analyze the outcome and prognostic factors of a cohort of patients with hepatocellular carcinoma (HCC) and macrovascular invasion (MVI) and establish subgroup categories with different outcomes. Methods: This is a single center retrospective study from March 2001 to December 2005 of patients with newly diagnosed HCC with MVI. The study included patients with HCC and MVI diagnosed by imaging studies (dual phase CT scan or MRI) and reliable clinical variables available. HCC was diagnosed according to the consensus statements established by the EASL and AASLD. Predictors of survival were identified by Kaplan Meyer analysis and Mantel-Cox comparisons. Multivariate Cox proportional hazard analysis was conducted with variables showing significant association with survival at univariate analysis. Results: During the study period, 231 patients out of the 936 HCC patients diagnosed in our center presented with MVI (24.6%). Among them, 199 pts met the inclusion criteria: Seventy were Child Pugh-A (35%), 86 Class B (43%) and 42 Class C (21%). 114 (58%) were ECOG 0 (asymptomatic) and 84 (42%) were symptomatic. HCC was solitary in 110 patients (53.3%). Main portal trunk was involved in 110 (51%), first portal branch in 79 (40%) and a second branch in 18 (9%). Hepatic vein invasion occurred in 39 (20%). Extra hepatic spread was present in 47 patients(23.6%). 143 patients were Barcelona Clinic Liver Cancer Program (BCLC) stage C and 56 BCLC stage D. 115 (57.8%) received treatment after MVI diagnosis, including resection in 34 cases. Median survival for the entire cohort was 4.6 months, the 1 and 2 years survival rate was of 17% and 5%, respectively. Median survival of BCLC C was 6.2 months and BCLC D was of 2.2 months. In BCLC C patients, ECOG 0 (p<0.01), absence of extra hepatic spread (p<0.01) and resection (p=0.01) were independent prognostic factors. Two subgroup of patients with good [asymptomatic patients without extra hepatic spread; n=79] and poor prognosis [n=64] were established. Median survival of these groups was of 8.1 months vs 4.5 months, respectively (p<0.001). Median survival of the 34 patients resected in the good prognosis group was of 10.2 months. Conclusion: HCC patients with macrovascular invasion have a heterogenous outcome. Independent prognostic factors (ECOG and extrahepatic spread) enabled us to recognize two outcome subgroups, and provide the rationale for stratification of patients included in clinical trials. Whether resection might benefit a subgroup of patients with MVI and
TELOMERE AND MITOTIC INSTABILITY AS MARKERS FOR THE PROGRESSION OF HEPATOCELLULAR CARCINOMA AND POSSIBLE IMPLICATIONS IN STEM CELL CARCINOGENESIS

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Purpose: Telomere dysfunction is associated with chromosomal instability, and telomerase reactivation with stabilization of telomere length is crucial in carcinogenesis. However, the role of telomere parameters with respect to chromosomal instability in hepatocarcinogenesis is not clearly understood. In this study, the relationships between telomere parameters, chromosomal instability and progenitor cell differentiation were evaluated in hepatocellular carcinomas (HCCs) along with their impacts on patient prognosis. Methods: Telomere length, telomerase activity, and hTERT mRNA levels were measured in 49 hepatitis B virus-related HCCs and non-HCCs by hybridization with 3'-end DIG-labeled d(TTAGGG)4 probe, TRAP assay and reverse-transcriptase polymerase chain reaction, respectively. The results were compared with clinicopathological data, including differentiation, multipolar mitosis, anaphase bridge, DNA ploidy, immunohistochemical staining results for cytokeratin 19 (CK19), and patient outcome. Results: Telomere lengths of HCCs ranged from 4.7 to 13.1 kb, and 44.4% of HCCs showed telomere lengthening. hTERT mRNA levels and telomerase activities were closely related (p=0.008), and were significantly higher in HCCs than non-HCCs. Telomere length was significantly higher in HCCs with strong telomere activity (p=0.048), high hTERT mRNA levels (p=0.001) and poor differentiation (p=0.041). Poorly differentiated HCCs had more frequent multipolar mitosis (p=0.007). Telomerase activity was positively correlated with multipolar mitosis and anaphase bridges (p=0.019, p=0.017). 13 (28.3%) HCCs were CK19-positive; these demonstrated longer telomeres than CK19-negative HCCs (p=0.046). Overall survival was poor in HCCs with multipolar mitosis>0.4 per field (p=0.016), high telomerase activity (p=0.009), and high telomere length ratio (HCC/non-HCC) >0.8 (p=0.044). Conclusion: Although telomere shortening and chromosomal instability characterize earlier stages of carcinogenesis, cancer progression is associated with telomere maintenance or even elongation. Our results show close associations between high telomerase activity, telomere lengthening, chromosomal instability, and poor prognosis, suggesting that regulation of telomere stabilization may be important for progression to more aggressive HCCs. In addition, the finding that CK19-positive HCCs showed longer telomeres may support previous observations that progenitor cell differentiation in HCCs is associated with a poor prognosis.

PREDICTIVE ABILITY OF HEPATOCELLULAR CARCINOMA STAGING SYSTEMS ACCORDING TO THE TREATMENT MODALITY IN A COHORT OF PATIENTS PROSPECTIVELY FOLLOWED AT A SINGLE REFERRAL CENTRE

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Staging systems are essential to assess prognosis of patients with hepatocellular carcinoma (HCC), to guide the therapeutic approach and to design clinical trials. The impact of treatment on natural history of HCC can modify the predictive ability of HCC staging systems. Aim: to evaluate, according to the type of treatment, the predictive ability of the three main HCC staging systems (BCLC, CLIP and TNM), in a large cohort of HCC patients treated and followed prospectively at a single referral Centre. Methods: Demographic, clinical and histological data were prospectively collected, together with liver imaging, at baseline and at 3 month intervals after enrolment, in 332 consecutive HCC patients referred to our Unit from January 2000 to June 2006. HCC was diagnosed according with EASL criteria (histologically confirmed in 68%). Median follow-up was 2 years. 59 pts were resected, 68 pts underwent liver transplantation inside Milan Criteria (age < 65 yrs); 93 pts received ablative treatments (percutaneous ethanol injection or radiofrequency thermal ablation) and 46 pts were treated with chemoembolization (TACE). 66 pts remained untreated, because of advanced disease, refusal of therapy or co-morbidities. The Kaplan-Meyer method and Cox regression models were used to analyse survival and prognosis factors for each staging system. Results: Median age of the cohort was 64 yrs, range 32-87, M:F ratio was 3.9:1; 86.6% of the HCC arose on cirrhotic liver, 60.7% were monofocal at diagnosis. Overall probability of survival at 1, 3 and 5 years was 76.6%, 53.7% and 34.5% respectively. To compare the performance of the three prognostic systems, the χ2 test for trend (discriminatory ability), and the likelihood ratio (LR) χ2 test (homogeneity) and the Akaike's (AIC) information criteria (overall goodness of fit) for the univariate Cox Model were used. Data are shown in the table. (Significant values are in bold). Conclusions: BCLC performs better than other staging systems in predicting overall survival among patients treated with ablative therapies or TACE; survival of untreated patients or of patients treated with resection is predicted more accurately by CLIP rather than TNM. No system accurately predicts prognosis in patients undergoing liver transplantation. These data indicate that in predicting the prognosis of patients with HCC, we should choose the staging system according to the planned treatment modality.
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THE EFFECT OF SUSTAINED HBV VIREMIA ON THE RECURRENTENCE OF HEPATOCELLULAR CARCINOMA AFTER CURATIVE RESECTION

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Background: An elevated serum HBV DNA level is a risk factor for developing hepatocellular carcinoma (HCC). Rather than the instantaneous serum HBV DNA, the duration of viremia is more important in carcinogenesis. Nevertheless, most investigations have evaluated the DNA level at study entry as a risk factor. We assessed the effects of sustained HBV viremia on the recurrence of HCC after curative resection. Methods: A total of 230 patients undergoing curative resections between 2000 and 2006 were initially included. Patients who received antiviral therapy at diagnosis or during follow-up, or experienced recurrence within 6 months after resection were excluded. In addition, those who had fluctuating DNA levels (cutoff value: 100,000 copies/mL) were also excluded. Ultimately, 157 patients were enrolled. The serum HBV DNA level, biochemical tests, alpha-fetoprotein, and liver dynamic computed tomography were obtained at 3-month intervals after surgery. The primary endpoint was the recurrence of HCC. Results: With a median follow-up duration of 33.4 months, 75 (47.7%) of 157 patients experienced recurrences. Eighty-nine (non-viremia group) were considered positive when at least one marker had a value above the 75th percentile, the sensibility was 90%, specificity 80%, positive predictive value 92%, negative predictive value 72%, positive likelihood ratio 4.45, and negative likelihood ratio 0.13, and the area under ROC curve 0.9173. Conclusions: Higher expression of serum DCP and GP73 antibody, and low expression of GP73 antigen are new promising markers for the diagnosis of HCC. Combination of these markers provides useful non invasive method for the diagnosis of HCC.

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EARLY DIAGNOSIS PANEL OF SERUM BIOMARKERS OF HBV-INDUCED HEPATOCELLULAR CARCINOMA DURING 3 YEARS FOLLOW-UP

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Objective: To investigate the combined value of α-fetoprotein (AFP), des-γ-carboxy prothrombin (DCP), α-feto-protein-L3 (AFP-L3), γ-glutamyl transpeptidase-II (GGT-II), CA125, CA19-9 in patients with liver cirrhosis who had been followed for the development of hepatocellular carcinoma (HCC). And using SELDI-TOF-MS to investigate proteomic markers of these patients. Patients and Methods Eighty-one patients with HBV-induced liver cirrhosis were monitored by serum AFP, DCP, AFP-L3, CA125, CA19-9, GGT-II and ultrasonography every 6 months for 3 years. Proteomic spectra by SELDI-TOF-MS
were generated in 10 patients who were diagnosed as HCC during follow-up. At the same time, all the serum markers were measured in 99 patients with HBV-induced HCC, 30 with secondary liver metastasis, and 20 healthy controls. Results During 3 years following up, 12(15.9%) of the 81 patients with HBV-induced liver cirrhosis were diagnosed as HCC. Each marker’s positive rate in patients who developed HCC during follow-up before HCC was diagnosed showed in table. The diagnostic value of DCP was the highest, with 75.8% sensitivity, 88.6% specificity, and 81.4% accuracy. The accuracy of other markers was reduced in order of AFP, AFP-L3, CA125, GGT-II, and CA19-9. Logistic regression analysis identified DCP, GGT2, AFP, and CA125 as independent markers for HCC. When compared the accuracy with the combination panel of all the serum markers, AFP, DCP plus CA125 showed the highest of 88.2% while the accuracy of AFP alone was only 76.2%. Spearman correlation analysis showed that DCP and CA125 were related with tumor size, DCP, CA125 and CA19-9 were related with Portal vein thrombosis, but no markers were related with extrahepatic metastasis. In addition, the proteomics analysis showed the 4350.8 m/z protein increased from cirrhosis to HCC, while the level of the 6860.5, 7571, 15125, 15893 m/z proteins decreased from cirrhosis to HCC. Conclusions DCP and GGTII emerged earlier in HCC patients than other markers. The best combination of the markers for early diagnosis of HBV-induced HCC was AFP, DCP plus CA125.

Each marker’s positive rate in patients who developed HCC during follow-up before HCC was diagnosed

| markers   | 18months pre-HCC | 12months pre-HCC | 6months pre-HCC | 3months pre-HCC | 0months pre-HCC | Total developed HCC patients
<table>
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<tbody>
<tr>
<td>DCP</td>
<td>2 (16.7%)</td>
<td>4 (33.3%)</td>
<td>6 (50%)</td>
<td>9 (75%)</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>AFP(&gt;15ng/ml)</td>
<td>0</td>
<td>4 (33.3%)</td>
<td>8 (66.7%)</td>
<td>8 (66.7%)</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>AFP(&gt;200ng/ml)</td>
<td>0</td>
<td>3 (25%)</td>
<td>5 (41.7%)</td>
<td>5 (41.7%)</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>AFP-L3</td>
<td>0</td>
<td>2 (16.7%)</td>
<td>4 (33.3%)</td>
<td>6 (50%)</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>GGT-II</td>
<td>2 (16.7%)</td>
<td>4 (33.3%)</td>
<td>6 (50%)</td>
<td>8 (66.7%)</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>CA125</td>
<td>0</td>
<td>0</td>
<td>1 (12.5%)</td>
<td>2 (16.7%)</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>CA19-9</td>
<td>1 (12.5%)</td>
<td>3 (25%)</td>
<td>6 (50%)</td>
<td>7 (58.3%)</td>
<td>12</td>
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</tbody>
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377 GRADING OF MICROSCOPIC VASCULAR INVASION FOR RESECTED HEPATOCELLLULAR CARCINOMA
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Purpose: Vascular invasion, usually classified as gross or microscopic (MVI), is a risk factor for recurrence of hepatocellular carcinoma (HCC) after resection; however, MVI represents a wide spectrum between no invasion and gross vascular invasion. The purpose is to determine if any histologic findings can stratify patients with HCC and MVI in terms of recurrence and survival after resection. Methods: We reviewed the records of all resections done for HCC at a single institution between 1/90 and 3/06 to identify those with MVI proven on histology; cases with gross vascular invasion were excluded. Number and size of vessels invaded, invasion of a vessel with a muscular wall, distance from the tumor, and presence of satellite nodules was recorded. Log rank test was used to determine correlation with recurrence and survival. Cox regression analysis was used to identify independent predictors of survival. Results: MVI was present in 131 of 384 resected patients (34.1%). Median follow-up was 28.9 mo. There were 88 recurrences and 54 deaths. On univariate analysis, invasion of a vessel with a muscular wall (p=0.0181), invasion > 1 cm from the tumor (p=0.0378), and invasion of > 5 vessels (p=0.0491) were associated with recurrence; invasion of a vessel with a muscular wall (p=0.002) and invasion > 1 cm from the tumor (p=0.001) were also associated with decreased survival. Both were also significant predictors of survival on multivariate analysis: Vessel w/ muscular wall p=0.018 Exp [B]=2.2 95% CI 1.1-4.2 Vessel >1cm from tumor p=0.015 Exp [B]=2.1 95% CI 1.2-3.7 A risk score assigning one point for the presence of each variable correlated significantly with both recurrence (p=0.0379) and survival (Figure1). Conclusion: Patients undergoing resection for HCC with MVI are a heterogeneous group; the proposed grading system can accurately stratify patients according to their risk of recurrence and survival.

378 SURVEILLANCE CT SCANS FOR RECURRENT OF HEPATOCELULAR CARCINOMA POST-LIVER TRANSPLANT
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BACKGROUND: Recurrent hepatocellular carcinoma (HCC) diminishes overall survival in liver transplant (LT) recipients. Established risk factors for recurrence include larger tumor burden, tumor dedifferentiation and presence of vascular invasion. Excellent tumor free survival has been reported for patients whose tumor burden falls within the Milan criteria. Generally, some form of surveillance is undertaken to detect recurrent HCC for patients within and beyond the Milan criteria, although the optimal strategy remains undefined both in terms of frequency and duration of surveillance. At our center, the current practice is to define whether a LT recipient is at high or low risk for recurrence based on the explant characteristics and to tailor accordingly the intensity of post-LT surveillance. OBJECTIVE: This study was designed to test the hypothesis that surveillance dual phase CT scans for low risk LT recipients is low yield. MATERIAL AND METHODS: 267 adult patients underwent LT for HCC at our institution between January 1998 and December 2005. Patients were stratified into a low risk (within the Milan criteria, well or moderately differentiated tumor, and no vascular invasion) and a high risk (outside the Milan criteria, poorly differentiated, or presence of vascular invasion) group. For the low
risk group, surveillance CT scans were obtained at 3 months post-transplant and then yearly for 5 years. For the high risk group, CT scans were performed every 3 months for the first year, every 6 months for the second and third years, and then yearly for years 4 and 5. Time to recurrence was defined as the point at which a mass was first discovered on dual phase CT scan of the abdomen or CT scan of the chest consistent with recurrent HCC. RESULTS: Of the 267 patients, 110 were designated as low-risk, and 157 as high-risk. HCC recurrences developed in 49 cases (18.4%). 4/110 (3.6%) considered low risk cases, and 45/157 (28.7%) high risk cases recurred. Median time to recurrence was 10.7 months for the entire cohort, and 8.1 months in the high-risk group. 4/49 (8%) of recurrences were beyond 3 years, with only 2% occurring after 4 years. CONCLUSIONS: For patients at low risk for HCC recurrence based on clinical and explant characteristics, regular post-LT surveillance is low yield. For high risk patients, the yield of surveillance with dual-phase CT scans beyond 3 years post-LT is also low. Surveillance for recurrent HCC need not be continued beyond 3 years post-LT.

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COMPREHENSIVE GENOME ANALYSIS FOR ETIOLOGY-DEPENDENT AND ETIOLOGY-INDEPENDENT MOLECULAR MECHANISMS IN HUMAN HEPATOCARCINOSIS

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Hepatocellular carcinoma (HCC) represents one of the most common cancers worldwide and is characterized by aggressive tumor formation with poor prognosis. Various etiologies have been linked to HCC development, most prominently chronic hepatitis B (HBV) and C (HCV) virus infections as well as chronic alcohol consumption. About 10% of HCCs are so-called cryptogenic, but likely derive from nonalcoholic steatohepatitis (NASH). In order to identify host changes related to tumor etiology as well as novel driver genes of human hepatocarcinogenesis, we combined high-resolution fine-mapping of genomic imbalances with expression and functional analyses. Array-based comparative genomic hybridization (aCGH) of a panel of etiologically well-defined HCCs (n=68) revealed DNA copy number gains of chromosome arm 8q to be significantly less frequent in cryptogenic HCCs compared to other etiologies. Expression analyses validated MYC as the potential target gene of 8q gains. Moreover, our gene expression data show a upregulation of MYC target genes in these HCCs. In contrast, FGF19, one of the most overexpressed genes in our cryptogenic HCCs, may replace the growth promoting effects of MYC in this etiological subgroup. In addition, we were able to fine-map frequent etiology-independent chromosomal imbalances on 1q32.1 and 20q13.33. From several candidates of these regions, we validated MDM4 and eEF1A2 as the likely candidate genes using expression and functional analyses. In conclusion, molecular hepatocarcinogenesis of cryptogenic HCC differs from the other etiological subgroups at least with respect to 8q gain and MYC activation. Furthermore, activation of the oncogenes MDM4 and eEF1A2 represent an etiology-independent mechanism of human hepatocarcinogenesis in a subset of HCC by presumably overcoming TP53-dependent growth control.

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TUMOUR M2-PYRUVATE KINASE HAS A DIAGNOSTIC AND PROGNOSTIC ROLE IN BILIARY TRACT CANCER

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Background: A reliable tumour marker which could differentiate between benign biliary disease and biliary tract cancer (BTC) would be a useful addition to current diagnostic tests. One such potential marker is the M2 type of the glycolytic isozyme pyruvate kinase (M2-PK), but there are few data on plasma M2-PK in biliary disease. Methods: We used a commercially available ELISA (Schebo-Biotech, Germany) to measure plasma M2-PK levels in 23 patients with benign non-primary sclerosing cholangitis (PSC) biliary diseases (12M, 11F; mean age 50 yr), 11 with PSC (4M, 7F; mean age 59 yr), and 41 with BTC (14M, 27F; mean age 68 yr). Results: The mean ± SD M2-PK level (U/ml) in non-PSC benign disease was 34.3 ± 20.6, compared with values of 71.7 ± 81.6 in PSC (p = 0.031) and 114.8 ± 160.9 in BTC (p = 0.001) (Figure 1). There was also a significant difference in mean M2-PK levels between the 11 patients with PSC alone and three with PSC+BTC (586.7 ± 208.1 U/ml, p = 0.005). Receiver operating characteristics analysis identified a M2-PK cut off of 31 U/ml, corresponding to a sensitivity and specificity of 76% and 61%, respectively, for BTC. However, of the 11 patients with M2-PK levels > 100 U/ml, all but one had BTC. There was a significant correlation between CA19-9 and bilirubin in the PSC and BTC groups, whereas M2-PK was not affected by cholestasis. Patients with BTC who had high levels of both M2-PK and CA19-9 had significantly (p = 0.049) worse survival compared with the other BTC patients; this was the only independent prognostic indicator on multivariate analysis. Conclusions: M2-PK may be a useful diagnostic and prognostic marker in BTC, and warrants further study in the screening of PSC patients for BTC.
Risk factors for HCC occurrence were male gender and absence of diabetes. After a mean follow-up of 50 months, 20 patients developed HCC: 6 in the group treated by beta-blockers alone (n=12) or in association with band ligation (n=35). There was no statistically significant difference between patients treated with beta-blockers or not, in terms of age, sex, alcohol abuse, BMI, platelet counts, presence of diabetes. Recent studies have shown that stress and catecholamine secretion may influence cancer occurrence and progression by modulating the expression of matrix metalloproteinases and vascular endothelial growth factor both inhibited by beta blockers. We assessed the impact of beta-blockers on HCC occurrence in patients with viral C cirrhosis and oesophageal varices periodically screened for HCC Among 420 patients with Child-Pugh A or B viral C cirrhosis, 99 had oesophageal varices and were included in this study, 3 were secondarily excluded due to previous viral eradication. Therefore, the analysis was performed in 96 patients. Clinical and biological data were collected at the time of diagnosis of oesophageal varices and decision to treat Results The mean age of patients was 60.5 ± 12 yrs, 52 men, mean BMI 25.5 ± 4 kg/m2, 60 were diabetics, 38 had alcohol abuse, 52 received a treatment by beta-blockers alone (n=12) or in association with band ligation (n=40). The others (n=44) had no treatment (n= 9) or were treated by band ligation (n=35). There was no statistically significant difference between patients treated with beta-blockers or not, in terms of age, sex, alcohol abuse, BMI, platelet counts, presence of diabetes After a mean follow-up of 50 ± 34 months, 20 patients developed HCC: 6 in the group treated by beta-blocker and 16 in the other group. In univariate analysis, risk factors for HCC occurrence were male gender and absence of beta-blockers use. In multivariate analysis, beta-blockers use was the sole independent risk factor of HCC; HR=0.12 (0.04-0.38) p=0.0003. Conclusion In this retrospective study, Beta-blockers use was associated with a lower incidence of HCC in patients with HCV cirrhosis and oesophageal varices. Beta-blockers may have a preventive effect on the development of HCC that deserve a long term randomised trial. 

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the etiology of HCC recurrence, i.e., metastases or de novo secondary.

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383 SORAFENIB IN MULTIFOCAL HEPATOCELLULAR CARCINOMA AND ADVANCED LIVER DYSFUNCTION
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Background: So far, no established treatment for advanced hepatocellular carcinoma (HCC) was available. Encouraging results of a multicenter, placebo-controlled phase III trial using the multikinase inhibitor sorafenib in unresectable HCC suggest a survival benefit and good tolerability in Child-Pugh A patients. No data are available regarding the safety and efficacy of sorafenib in patients with multifocal HCC and advanced liver cirrhosis. Patients and Methods: Between May 2006 and May 2007 patients with unresectable HCC were treated with oral sorafenib (daily target dose: 400 mg bid). Data were collected retrospectively. Survival curves were calculated via the Kaplan-Meier method. Results: Of 32 patients analyzed (m/f = 25/7, age 27 to 82), the predominant etiology of cirrhosis was alcohol (n = 15). Child-Pugh A/B/C was 15/11/6; Barcelona Clinic Liver Cancer (BCLC) stage B/C/D was 2/19/11. The most common side effects were low-grade and manageable. A total of 14 patients died during the observation period (median survival: 1.6 months; range: 0.4 – 7.4); the median overall survival (OS) of the entire intent-to-treat group was 6.5 months (range: 0.4 – 11.4 months). Median follow-up for Child-Pugh A (alive: 14/15) was 3.4 months; median survival was 3.8 months for Child-Pugh B (alive: 4/11) and 1.5 months for Child-Pugh C (alive: 0/6). The differences between Child-Pugh class A, B and C were statistically significant on univariate analysis (log rank, p = 0.0001) (Figure 1). Median follow-up for BCLC stage B/C (alive: 16/21) was 3.8 months; median survival for BCLC stage D (alive: 2/11) was 1.5 months, which is not different from the expected survival in this patient group. The differences between both groups were statistically significant on univariate analysis (log rank, p = 0.0001). Conclusion: Sorafenib is safe in patients with multifocal HCC and advanced liver dysfunction. Diarrhea was the most troublesome side effect in our series similar to the phase III randomized controlled trial (SHARP-trial) of sorafenib in Child A patients. Efficacy could be limited in Child-Pugh C and BCLC stage D patients. Prospective controlled trials are warranted to evaluate the safety and efficacy of sorafenib in large HCC and advanced liver dysfunction.

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384 GLOBAL DNA METHYLATION INDEX (GDMI) PROFILE IN PATIENTS WITH CIRRHOSIS: CORRELATION TO UNDERLYING CAUSE OF DISEASE AND ASSOCIATED HEPATOCELLULAR CARCINOMA.
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Background: Changes in hepatic DNA methylation have been demonstrated in specific genes and their promoters in association with cirrhosis due to alcoholic liver disease (ALD) and hepatitis C virus (HCV), and in hepatocellular carcinoma (HCC). We hypothesize that the quantitative global DNA methylation status of peripheral white blood cells (WBC) may reflect epigenetic alterations at the tissue level. Methods: A Global Methylation-Restriction Enzyme Sensitive Assay (GM-RESA) was performed in triplicate on WBC of patients with cirrhosis obtained from a tissue repository from one liver transplantation center, and on commercially available control WBC [n=10]. GM-RESA measures the density of methylated CpG dyads throughout the genome using methyl-sensitive (Hpa II) and insensitive (Msp I) restriction enzymes to calculate the Global DNA Methylation Index (GDMI). The GDMI was correlated to cause of liver disease and presence of HCC. Results: 93 patients were included: 59% male; 48% Hispanic, 38% Caucasian, 8% Asian; 55±11 years old. Etiologies (alone or in combination) included: HCV (53%), ALD (35%), HBV (8%), Cryptogenic (11%) and other (9%). 33% of patients were transplanted, and 56% had a tissue diagnosis. 18 (19%) patients had HCC, 13 with tissue diagnoses. Clinical indices of liver disease were: MELD score 14±6, Child-Pugh score 8±2; albumin (g/dL) 3.1±0.7, INR 1.3±0.3, creatinine (mg/dL) 1.1±1.0, total bilirubin (mg/dL) 3.9±6.6, and platelet count (x1000) 110±75. Mean GDMI of patients (0.24±0.06) was similar to controls (0.22±0.02), but with a greater variance (F test: variance ratio 6.2, p=0.003). GDMI did not correlate with any clinical index. To limit bias from type II error and heterogeneity of etiologies, the GDMI was studied within a subgroup of 77 subjects: 10 controls vs. 24 HCV, 21 ALD, 10 ALD+HCV and 12 HCV+HCC. The GDMI variance of each patient group was significantly greater than that of controls. The GDMI of HCV+HCC (0.27±0.06) was greater than that of controls (p=0.046) and ALD (0.23±0.05; p=0.06); and that of ALD+HCV (0.26±0.05) was greater than controls (p=0.039). Overall, there was an increase in GDMI: ALD < controls < HCV < ALD+HCV < HCV+HCC. Conclusion: This pilot study suggests that compared to the controls, quantitative global DNA methylation (GDMI) of peripheral WBC is lower in patients with cirrhosis due to alcohol alone but higher in HCV associated with HCC. Thus GDMI may provide insight into epigenetic alterations involved in the pathogenesis of liver disease and subsequent carcinogenesis. Larger studies and correlation with tissue GDMI are warranted.

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COST-EFFECTIVENESS OF NEW SCREENING STRATEGIES FOR HEPATOCELLULAR CARCINOMA (HCC) COMPARED TO ULTRASOUND (US)

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US is recommended for screening for HCC. However, a positive US is frequently followed by a second imaging study which increases the cost of a screening program. The utility of newer blood tests with better sensitivity and specificity in detecting HCC at relatively low cost for identifying patients who should proceed directly to CT or MRI and reduce the costs of a screening program are unknown. Methods: We conducted a cost-effectiveness analysis of 9 screening strategies to detect HCC in a hypothetical cohort of hepatitis C (HCV) cirrhotics. The outcome was the incremental cost effectiveness ratio (ICER) to detect each additional cancer. Annual screening with US was compared to annual screening with CT, AFP+AFPL3%, DCP+AFP+AFPL3%, and DCP+AFPL3%. Biannual screening strategies that were modeled included US every 6 months (base case), CT every 6 months, US alternating with AFPL3%+DCP, CT alternating with AFPL3%+DCP. We assumed patients with a positive blood test or lesion seen on US would undergo CT. The sensitivity and specificity used were: US 0.6 & 0.8, CT 0.8 & 0.9, AFP+AFPL3% 0.69 & 0.66, AFPL3%+DCP 0.62 & 0.83, AFP+AFPL3%+DCP 0.77 & 0.59. The cost of US, CT, AFP+AFPL3%, DCP+AFP+AFPL3%, DCP+AFPL3% were $200, $590, $90, $180, $180, respectively. Sensitivity analyses were conducted to test the robustness of the model. The annual rate of HCC was assumed at 1.4%. Results: Annual US followed by CT if abnormal was more either more expensive or did not detect more HCC’s than the combination blood screening tests. The most cost-effective strategy was annual screening with AFP+AFPL3% which detected more cancers and cost less than US mostly due to fewer patients proceeding to CT with AFP+AFPL3%. CT detected the most cancers of all the strategies but was the most expensive strategy overall and AFP+AFPL3%+DCP was the blood test that detected the most cancers and was the most expensive blood test. Compared to AFP+AFPL3% the ICER for each additional cancer detected was $85,449 for CT and $147,620 for AFP+AFPL3%+DCP. In sensitivity analysis annual CT would be cost-effective if the cost dropped below $400. The most cost-effective 6 month strategy was CT alternating with AFPL3%+DCP with an ICER of $10,357 for each additional cancer detected compared to biannual US. Conclusion: Annual screening with AFP+AFPL3% is the most cost-effective annual strategy for detecting HCC in HCV cirrhotics; however it detects fewer cancers than AFP+AFPL3%+DCP or CT alone. The most cost-effective 6 month strategy is CT alternating with AFPL3%+DCP; thus, this promising strategy should be prospectively studied in screening of HCC.

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10-YEAR ACTUAL LONG-TERM OUTCOME OF HCC AFTER SURGICAL RESECTION: SINGLE CENTER EXPERIENCES

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Background The aim of this study was to evaluate actual 10-year survival and to identify the prognostic factors affecting long term survival and disease-free survival (DFS) in patients with hepatocellular carcinoma (HCC) after surgical resection. Patients and Methods To obtain the actual data, we confined study period from January 1992 to April 1996. Two hundred eighty-one liver resections for HCC were retrospectively reviewed. There were 237 men and 44 women. Median age was 54 years. Univariate and multivariate analyses were performed on 20 clinicopathological variables to analyze factors affecting survival and DFS. Results The median survival time was 4.8 years. The actual 5- and 10-year survival rates were 48.0% and 28.8% respectively. Eighty-one patients survived over 10 years. Preoperative aspartate aminotransferase (AST) level, cirrhosis, number of tumor, Edmondson-Steiner grade and vascular invasion were identified as independent prognostic factors for survival by multivariate analysis. Tumor recurrence appeared in 217 (77.2%) patients. Of these 25 (11.5%) patients had experienced tumor recurrence after 5 postoperative years. The 5- and 10-year DFS rates were 27.1% and 18.4% respectively. Thirty-four patients (12.1%) did not experience tumor recurrence over 10 years. Preoperative AST and alpha-fetoprotein level, cirrhosis, vascular invasion and satellite nodules were identified as the independent prognostic factors of DFS by multivariate analysis. Conclusions Survival decreased continuously until 10 years after surgical resection because of recurrent disease and decompensated cirrhosis. Hence, to improve long-term outcome, other treatment measures to consider background liver such as transplantation or postoperative adjuvant therapies were needed.

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LOW SERUM APOLIPOTREIN A1 IS PREDICTIVE OF HCC DEVELOPMENT IN PATIENTS WITH VIRAL C CIRRHOSIS

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Low levels of Apolipoprotein A1 (Apo A1) have been reported in patients with extensive liver fibrosis and recently, a genomic study suggested it was predictive of the occurrence of HCC in patients with HCV cirrhosis. This study was aimed to assess the predictive value of serum Apo A1 for the occurrence of HCC in a cohort of 185 patients with HCV cirrhosis, prospectively followed and screened for HCC by US examination performed every 6 months. Sera were sampled and clinical and biological parameters were recorded at the time of inclusion. Results All patients had well compensated cirrhosis. The mean age was 58.5 ± 13.2 yrs, 111 were male, 55 diabetics and 60 have cirrhosis related to HCV + alcohol. The mean BMI was 25.7 ± 3.9 kg/m2., 33 (18%) have a low serum level of Apo A1. After a mean follow-up of 3.3 ± 2.0 yrs, 30 patients developed a
HCC. In univariate analysis, risk factors for HCC occurrence were male gender, high BMI, low level of Apo A1 and low platelet counts. In multivariate analysis, low level of Apo A1 (p<0.02), high BMI (p<0.004), male gender (p=0.01) and platelet counts (p=0.04) were independent risk factors. Conclusion Decrease of serum level of Apo A1 is associated with HCC occurrence in patients with HCV cirrhosis.

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388 A COMPREHENSIVE PROSPECTIVE STUDY FOR THE VALUE OF 11C-ACETATE AND 18F-FDG PET-CT IN THE DETECTION OF HEPATOCELLULAR CARCINOMA

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Background: The value of 18F-FDG positron emission tomography (PET) was found to be low in the detection of hepatocellular carcinoma (HCC) because of a high false negative rate. Recently, 11C-Acetate PET studies showed a relatively high sensitivity for the detection of HCC but there was no sufficient data. Therefore, we evaluated the value of 11C-Acetate and 18F-FDG PET-CT in the detection of HCC. Methods: Between June 2006 and February 2007, 99 patients who had either a newly diagnosed HCC or a new distant metastasis after surgical resection of HCC 6 months prior to this study, were prospectively enrolled. A biopsy was performed on all patients and they all underwent 11C-Acetate and 18F-FDG PET-CT study free of charge before treatment. Results: 1) The number of patients was 5, 37, 16, 13, 28 in modified UICC stages I, II, III, IVa, IVb respectively. 2) Analysis of intrahepatic tumors: A total of 110 countable and above 1 cm-sized intrahepatic tumors from 90 patients were analyzed. The sensitivity of 18F-FDG, 11C-Acetate, and 18F-FDG + 11C-Acetate was 60.9%, 75.4%, and 90% in 11C-Acetate (p<0.001), respectively. The sensitivity according to the size of tumor (<2/2-5/5-10/10-20 cm) was 27.2%/47.8%/85.7% in 11C-Acetate (p<0.001) and 31.8%/78.2%/95.2% in 11C-Acetate (p<0.001), respectively. The standardized uptake value (SUVD) according to the size of tumor (<5/5-10/10-20 cm) was 4.7±1.7/8.0±5.2 in 18F-FDG (p=0.001) and 5.5±2.1/5.5±2.4 in 11C-Acetate (p=0.499). The sensitivity according to the grade of tumor differentiation (Grade 1,2/Grade 3,4) was 50%/90% in 18F-FDG (p=0.001) and 80%/90% in 11C-Acetate (p=0.05). 3) Analysis of distant metastases: We analysed 35 distant metastatic tumors by indexing the largest tumor in each separate organ. The sensitivity of 18F-FDG, 11C-Acetate, and 18F-FDG + 11C-Acetate was 85.7%/77%, and 85.7%, respectively. The sensitivity according to the size of tumor (<2/≥2 cm) was 80%/93% in 18F-FDG (p=0.365) and 65%/93% in 11C-Acetate (p=0.101), respectively. The SUV according to the size of tumor (<2/≥2 cm) was 3.6±2.5/4.6±2.4 in 18F-FDG (p=0.276) and 2.2±2.1/4.1±3.2 in 11C-Acetate (p=0.089). The sensitivity according to the grade (Grade 1,2/Grade 3,4) was 90.9%/76.9% in 18F-FDG (p=0.337) and 90%/53.8% in 11C-Acetate (p=0.032). Conclusions: Both 11C-Acetate and 18F-FDG PET-CT showed low sensitivity in the detection of small intrahepatic HCC but relatively high sensitivity in the detection of extrahepatic metastases. Addition of 11C-Acetate to 18F-FDG PET-CT increases the sensitivity in the detection of intrahepatic HCC tumors, not of extrahepatic HCC metastases.

Disclosures: The following people have nothing to disclose: Shunsuke Ura, Masao Honda, Teruyuki Ueda, Ryuehi Nishino, Hajime Takatori, Mikiko Nakamura, Shuichi Kaneko; Gastroenterology, Graduate School of Medical Science, Kanazawa University, Kanazawa, Japan

Background & Aims: MicroRNAs (miRNAs) are small non-coding RNAs that represent an important mechanism for posttranscriptional gene silencing and have recently been recognized to play important roles in tissue development, cell differentiation and proliferation and apoptosis processes in human diseases and cancers. We have previously reported gene expression profiles of chronic hepatitis (CH)-B and CH-C differ [Gastroenterology. 2001, 120, 955, Hepatology. 2006, 44, 1122]. This study examined miRNA expression profiling in chronic viral hepatitis (CH) and hepatocellular carcinoma (HCC) liver tissues. Methods: We applied real-time detection (RTD)-PCR to detect miRNAs. We used Loopeal-primed RTD-PCR with Taq-Man® MicroRNA Assays Human Panel Early Access Kit (Applied Biosystems), which contains a 180-assay panel covering most known human miRNAs. We analyzed tumor and non-tumor liver tissues from 12 patients with HBV-related HCC (HCC-B), 14 patients with HCV-related HCC (HCC-C) and 9 patients with normal liver (N). Candidate target genes by miRNAs were predicted by Miranda Pro 3.0. To evaluate the regulatory effect of miRNAs on target genes in these samples, whole gene expression profiling was obtained from these samples using an in-house cDNA microarray comprising 9614 clones. Data analysis was performed by BRB Array Tool (NCBI). Results: Hierarchical clustering analysis (unsupervised learning methods) and supervised learning methods using compound covariate predictor revealed significant classification of N, CH and HCC respectively (N vs. CH: 97% accuracy, p<0.001; CH vs. HCC: 79% accuracy, p<0.001). Differences were larger between N and CH than between CH and HCC. Interestingly, miRNA profiles differentiated CH-B and CH-C, and HCC-B and HCC-C (CH-B vs. CH-C: 92% accuracy, p<0.001; HCC-B vs. HCC-C: 77% accuracy, p=0.02), although statistical values for HCC-B and HCC-C were less. Out of differentially expressed miRNAs among these classifications (p<0.01), 18 miRNAs from N vs. CH and 8 miRNAs from CH and HCC regulated target gene expression in host tissue samples, as determined by group comparison using whole gene expression profiling data from cDNA microarrays. Similarly, 4 miRNAs from CH-B vs. CH-C and 2 miRNAs from HCC-B vs. HCC-C were determined and expressions could differentiate HBV- or HCV-infected liver. Conclusions: Expression of miRNAs clearly differentiated N, CH and HCC as well as HBV- and HCV-infected liver. Functionally active miRNAs determined by combined gene expression data can reveal pathogenesis and identify therapeutic targets for liver disease.

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FOCAL NODULAR HYPERPLASIA BUT NOT IN FNH-LIKE

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Focal nodular hyperplasia (FNH) is the second most common benign liver tumor after hemangioma. A central stellate fibrous region containing malformed vascular structures characterizes FNH nodules, which are considered as the result of a hyperplastic response to increased blood flow rather than a neoplastic process. In contrast to typical FNHs that usually develop in normal liver, FNH-like nodules occur in cirrhotic liver but share similar histopathological features. To better understand the pathophysiology of FNH, we searched for deregulated genes in FNH, using a transcriptome analysis exploring more than 15,000 different genes in 8 FNHs compared to 4 non-tumoral liver samples. Selected genes were validated using quantitative RT-PCR in a larger series of 72 benign liver tumors including FNH, adenoma, cirrhotic nodules and FNH-like lesions. Among the 524 genes significantly deregulated in FNH, we identified 20 genes of which the expression is zonated in the normal liver lobule. All 6 genes that are normally highly expressed in the perivenous area were up-regulated in FNH nodules, while 14 genes physiologically expressed in the perportal area were down-regulated. Using immunohistochemistry, we found that overexpression of the β-catenin-activated glutamine synthetase (GS) in FNH extended up to 5 to 10 hepatocytes away from the veins leading to a marked enlargement of the peri-veneinous areas, while in normal liver its expression is restricted to the immediate venous surrounding. In all hepatocytes, β-catenin was membranous without significant cytoplasmic or nuclear over-expression. However, quantitative RT-PCR analyses identified a slight but significant β-catenin mRNA overexpression closely related to the overexpression of β-catenin targeted genes in FNH suggesting an increased gradient of β-catenin expression without activating mutations. We further analysed the expression of 15 genes in a series of 72 benign liver nodules. Interestingly, FNH-like lesions demonstrated an expression profile similar to other related cirrhotic samples but different from the profile observed in typical FNH. However, in FNH-like the two perivenous genes encoding GS and GPR49 were significantly down regulated when compared to normal liver, adenomas and FNH nodules. Consistent with this result, little or no GS staining was observed in FNH-like nodules using immunohistochemistry. In conclusion, our data reveals the presence of an increased zonated expression of β-catenin targeted genes in FNHs, but not in FNH-like nodules. Although they have the same morphological features, our findings indicate that these two types of lesions have a different pathogenesis.

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LIVER RADIODEMOBILIZATION EFFECTIVELY CONTROLS TUMOR GROWTH OF MACROSCOPIC BUT NOT MICROSCOPIC DISEASE IN PATIENTS WITH HEPATOCELLULAR CARCINOMA

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Radioembolization (RE) is a new form of brachytherapy in which radiation is delivered by microspheres labeled with radioisotopes injected into the hepatic artery or its branches. Although anti-tumor effect results from preferential embedding of microspheres in tumor vasculature, non-tumoral parenchyma in targeted areas of the liver also receive a certain dose of radiation that might affect the growth of microscopic disease. Aim: to establish the efficacy of RE in preventing the growth of microscopic lesions in targeted areas, and evaluate possible factors influencing this effect. Patients and Methods: Patients with advanced HCC and preserved liver function were evaluated for RE using resin microspheres labeled with yttrium-90. Previous assessment included MRI or CT to detect extrahepatic disease and to measure liver and tumors volumes, and a hepatic angiogram with radiolabeled macroaggregates of albumin to identify shunt to distant organs and for activity calculation. Patients were followed every 3 months and received no further treatment until progression. RECIST criteria were used for evaluation of tumor response in targeted areas of the liver, where target tumor lesions and new lesions were evaluated separately. Results: Fifty-nine patients with HCC were evaluated for liver RE. Treatment was contraindicated in 19 (32%) mainly due to intense shunting to the lung or to a low efficiency in particle entrapment within the tumor nodules. Forty patients received 43 treatments. Mean age was 60 years (range: 39-75). CLIP scores were distributed as follows: 1 in 25%, 2 in 40%, 3 in 12% and 4 in 5%. Mean tumor volume was 787 mL. Lobar treatments were performed in 65% of cases. The median activity delivered was 2.00 GBq (range 0.75 to 3.25 GBq). A rough estimation of the median dose of radiation received by tumors and liver was 106 Gy (range: 22-1371) and 35 Gy (range: 6-1200), respectively. Mean follow-up was 643 days (range: 65-1314 days). At 3 and 6 months, disease control rates of target lesions were 97% and 94%, respectively. However, new lesions had appeared in the targeted area of the liver in 22% and 27% of the cases, respectively. Patients that developed new lesions did not receive a lower activity in GBq either absolute or relative to targeted liver volume or tumor involvement. One- and 2-y survival rates were 42% and 42% for CLIP-1, 37% and 12% for CLIP-2 and 15% and 0% for CLIP-3 patients, respectively. Conclusion: RE allows an effective control in the treated lesions in the vast majority of cases but did not prevent the emergence of new lesions in the targeted area. This suggests that microscopic lesions are not effectively treated by RE.

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THE SIGNIFICANCE OF OSTEOPONTIN PROMOTER SNPS AT NT-155 AND FOXD3 AS HOST FACTORS TO REGULATE THE DEVELOPMENT OF HEPATOCELLULAR CARCINOMA IN FEMALE PATIENTS WITH HCV INFECTION

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Osteopontin is a cytokine essential for the initiation of Th1 immunity. We identified 4 SNPs (nt-155, -433, -616, -1748) in promoter region of osteopontin gene, and demonstrated that SNP at nt -433 reflected hepatitis grading in chronic hepatitis C patients, while the other 3 SNPs showed linkage disequilibrium to each other. Also, osteopontin is shown to be an extracellular matrix promoting hepatocellular carcinoma (HCC) invasion. Thus, osteopontin promoter SNPs may affect the development of HCC. In the present paper, we tested this hypothesis. [Methods & Results] 1) Clinical examination: 291 patients with chronic liver disease due to HCV infection received ultrasound at 3 months intervals, and HCC was detected in 115 patients (Male 80, Female 35). Osteopontin promoter SNPs at nt -155 and -443 were determined by Invader assay using DNA from blood samples. Mean peripheral platelet levels (×104/mm3) at detection of HCC was less in female than in male (8 vs 11, p<0.05), but the levels in female were higher in those with deletion homozygotes at nt -155 and C/C or C/T at nt -443 than in those showing the other alleles (11 vs 7, p<0.05). 2) Promoter Assay: DNA samples were PCR-amplified between nt -1 and -658. There existed 3 haplotypes, and the alleles at nt -155 and -443 were deletion and C in haplotype-1, deletion and T in haplotype-2 and G and T in haplotype-3, respectively. Each cDNA was subjected to dual-luciferase reporter assay following transfection into HepG2 cells. Promoter activity was greater in haplotype-1 than in haplotype-2, and haplotype-3 cDNA showed minimal activities. 3) Gel-Shift Assay: The assay was performed using nuclear extracts from male HepG2 and female Hela cells. SRY, transfer factor encoded on Y-chromosome, were shown to bind to oligonucleotide around nt -155 irrespective the alleles, but FoxD3 can bind to such region only when the SNP showed deletion mutation. [Conclusion] HCC may develop in female showing deletion homozygotes at nt -155 and C/C or C/T at nt -443 even when progression of liver fibrosis was mild. Promoter activity was greater in case of cDNA showing such haplotype than in those with other haplotypes, and FoxD3 can promote translation by cDNA showing deletion mutation at nt -155 even in female cells. Thus, osteopontin promoter SNP at nt -155 may play a role in the development of HCC through interaction with FoxD3 especially in female patients with HCV infection.

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Introduction: Cholangiocarcinoma (CCA) is the second most common primary liver tumor and its incidence is on the rise. The predictors of survival have not been well defined in patients with CCA. The aims of this study were to evaluate the predictors of survival in patients diagnosed with CCA. Methods: This is a case series of patients with CCA seen in our center from January 2003 to December 2006. CCA was diagnosed by cytology or histopathology. CCA was divided into extrahepatic (ECC) and intrahepatic (ICC). Laboratory and tumor data were obtained at diagnosis and tumor staging was performed according to TNM. Lifetime alcohol and tobacco exposure was quantified as > 1500 gram-years and > 20 pack years, respectively, based on review of the medical record. Kaplan-Meier and Cox regression were utilized for univariate and multivariate analysis, respectively. Results: A total of 123 patients were seen. The median age was 64 years and 70 (57%) were male. Only 15 patients had chronic liver disease (12 PSC and 3 HCV). The median CA 19-9 was 182 U/mL. A total of 55 (45%) had ECC and 68 (55%) had ICC. There was a median of 1 lesion with a median maximum tumor diameter of 6.2 cm, 54 (45%) had vascular involvement and 42 (34%) had extrahepatic metastases. At diagnosis there were 18 stage I, 18 stage II, 42 stage III and 45 stage IV. A total of 29 patients underwent surgical resection, 67 underwent chemotherapy, radiation or combination of these, and 27 did not undergo therapy. The median survival was 8.1 months with a 1-, 3- and 5-year probability of survival of 44%, 17% and 13%, respectively. The 1-year survival for those with stage 1 tumor was 70%. On univariate analysis, CA 19-9 > 100 U/mL, extrahepatic metastases, smoking, alcohol, TNM staging, undergoing therapy and total bilirubin > 1.5 ng/mL were significant predictors of mortality. No difference in survival was observed between patients with ICC and those with ECC. On multivariate analysis, the independent predictors of survival were undergoing therapy (0.44, 95%CI:0.26-.76), CA 19-9 > 100 U/mL (2.8, 95%CI:1.5-4.1) and smoking > 20 pack-years (2.3, 95%CI:1.4-3.7). Presence of underlying liver disease was not a significant factor. Conclusions: In this cohort of CCA patients, 70% had stage III or IV tumors at presentation and median survival was 8.1 months. Smoking, high CA19-9 and undergoing therapy were predictors of survival. Further studies should evaluate whether addition of these factors to tumor burden may improve the prognostic staging of patients with CCA.

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PERSPECTIVE EVALUATION OF ABERRANT P16 METHYLATION IN SERUM OF PATIENTS BEFORE AND AFTER THERAPY FOR LOCALIZED HCC

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Hepatocellular carcinoma (HCC) is a frequent complication of liver cirrhosis and a leading cause of mortality worldwide. Localized HCC in cirrhotic patients may be safely treated with locoregional therapies, but early recurrence is frequent, probably due to dissemination. Aberrant methylation of the tumor suppressor gene p16 plays an important role in hepatocarcinogenesis, and may be detected in serum by methylation-specific PCR (MSP). In this study we evaluated the significance of serum p16 methylation in predicting early HCC recurrence after locoregional therapy. Methods: Twenty-five consecutive adult patients, with HCV-related liver cirrhosis and single HCC nodule < 5 cm in diameter with no macrovascular invasion, underwent percutaneous radiofrequency ablation (RFA) or trans-arterial chemoembolization (TACE). They were evaluated for the presence of p16 methylation in serum by MSP the day before HCC treatment and one month after RFA or TACE. Patients were prospectively followed for at least 24 months with abdominal CT scan, performed one month after therapy and every 3 months thereafter, to assess HCC recurrence. Results: p16 methylation was detected in sera of 8 out of 25 patients (32%) before RFA or TACE. One month after treatment, CT scan showed apparent complete ablation or no residual HCC activity in all of these patients. MSP showed disappearance of p16 methylation in 5/8 HCC patients. No evidence of HCC recurrence was found during follow up in these 5 patients. On the contrary, early recurrence of HCC occurred in the 3 patients who remained positive for p16 one month after therapy (2 RFA and 1 TACE). No correlation was found between p16 methylation and tumor response levels. Conclusions: Results of this pilot prospective study indicates that using MSP, aberrant p16 methylation may be found in one third of patients with localized liver HCC. Patients who achieve an apparent complete destruction of HCC and do not experience early HCC recurrence become negative for p16 methylation in serum one month after successful locoregional therapy. On the contrary, persistence of p16 methylation in serum predicts early recurrence of HCC, despite CT scan evidence of apparent successful treatment. Larger studies are warranted to confirm the clinical implications of our findings.

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POLYMORPHISMS OF DNA REPAIR GENES XRCC3, ERCC5/XPG AND RISK OF HEPATOCELLULAR CARCINOMA IN KOREAN WITH CHRONIC HEPATITIS B INFECTION

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Backgrounds: DNA repair mechanisms are important for maintaining DNA integrity and preventing carcinogenesis. Genetic variations in DNA repair genes are thought to modulate DNA repair capacity and are suggested to be related to hepatocellular carcinoma (HCC) risk. Methods: We investigated the effects of the XRCC3 Thr241Met and ERCC5/XPG His1104Asp polymorphisms on the risk of developing HCC in a case-control study. The XRCC3 Thr241Met and ERCC5/XPG His1104Asp polymorphisms were assayed by PCR-RFLP method for 541 cases with HCC and 405 controls without HCC among hepatitis B virus (HBV) carriers. Allele frequencies of polymorphism were compared between patients with HCC and patients without HCC among HBsAg positives by logistic regression. Results: The observed genotype frequencies of polymorphisms in both cases and controls conformed to the Hardy-Weinberg equilibrium. The frequencies of the ERCC5/XPG His/His, His/Asp and Asp/Asp genotypes of among cases (21.6, 63.9 and 14.5%, respectively) were significantly different from those among controls (9.6, 74.6 and 15.8%, respectively; P<0.001). The ERCC5/XPG His/His genotype was more frequent in cases than in controls, whereas the His/Asp genotype was less frequent in cases than in controls (P<0.001 and P<0.001, respectively). With the adjustment for age and gender, the ERCC5/XPG His/His genotype was associated with a significantly increased risk of HCC (adjusted OR, 2.62; 95% CI, 1.75-3.91; P<0.001) with the combined His/Asp and Asp/Asp genotypes as the reference. However, the XRCC3/XPB Thr241Met genotype was not associated with HCC risk (adjusted OR, 1.24; 95% CI, 0.65-2.37; P=0.52). Conclusions: These results suggest that the ERCC5/XPG His1104Asp polymorphism contributes to genetic susceptibility to HCC, while XRCC3 Thr241Met polymorphism is not associated with HCC risk, in Korean patients with chronic HBV infection.

Disclosures:
The following people have nothing to disclose: Neung Hwa Park, Bo Ryung Park, Jung Woo Shin, Seok Won Jung, Chang Woo Nam, Yang Won Na, Jae Hee Seo, Hyun Duk Shin, Se Hwan Lee, Danbi Lee, Sung Eun Kim, Jung A Kim, Young-Hwa Chung
Background/Aims: The pathogenesis of frequent-intrahepatic recurrence of hepatocellular carcinoma (HCC) after locoregional therapies remains uncertain. Risks and patterns for intrahepatic distant recurrence (IDR) of single, primary HCC after radiofrequency (RF) ablation were examined. Methods: Ninety patients with a single primary HCC less than 3 cm who had complete RF ablation were enrolled the study. Risk factors for IDR and the patterns of IDR after RF ablation were analyzed. Results: The median follow-up was 37.4 months. IDR was observed in 44 (48.9%) patients. Univariate analysis revealed pretreatment serum α-fetoprotein (AFP) level ≥50 ng/ml (P = 0.0324), des-γ-carboxy prothrombin (DCP) level ≥40 mAu/ml (P = 0.006) and prothrombin time (PT) <70% (P = 0.0188) related to IDR. A multivariate stepwise Cox proportional hazards regression model revealed pretreatment serum AFP and DCP levels to be independent risk factors for IDR. The local tumor progression (P = 0.023) and serum DCP level ≥40 mAu/ml (P = 0.025) were related to multiple IDRs. Conclusions: HCC patients with high serum AFP or DCP before RF ablation should be carefully followed up to monitor any IDRs. Multiple IDRs frequently occur in patients with high DCP levels or local tumor progression making complete local control of HCC mandatory.

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patients had perinatally-acquired HBV infection and none had received treatment for HBV. Nested PCR of the full HBx genome was performed on the sera of all cases and PCR products were sequenced to look for mutations. Nested PCR was similarly performed on tumor and non-tumor tissues of the HCC group and PCR products sequenced. Results: In the HCC group, 9/10 had mutations in HBx gene in the sera (6 were PCR negative) compared to 19/21 in the control group (11 were PCR negative), p=NS. The most common mutations in both groups were similar, ie HB6R and I127T. All tumor tissues (16/16) yielded mutations in the HBx gene compared to 13/16 in non-tumor tissues. Concordance of the signature HBx gene mutations between tumor and non-tumor tissues was 43.8% (7/16) and 12.5% (2/16) between sera and tumor tissues (p<0.05). Conclusions: HBx gene mutations are common in the serum of patients with chronic HBV infections with or without HCC. However, in patients with HCC, HBx gene mutations are more common in the liver tissues and further mutations may occur when they integrate into liver tissues, leading to development of HCC.

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401 TRENDS IN THE INCIDENCE OF HEPATOCELLULAR CARCINOMA IN SINGAPORE 1968-2002
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Background and Aims: To describe the trends in the incidence of hepatocellular carcinoma (HCC) in the three main ethnic groups in Singapore over the last 35 years and the prevalence of associated risk factors. Methods: The incidence of HCC was obtained from the Singapore Cancer Registry. Data on the prevalence and incidence of hepatitis B and hepatitis C was obtained from the Ministry of Health Singapore and the prevalence of other risk factors were obtained from published national population surveys. Results: Between 1968 and 2002, 2913 cases of HCC were registered by the Singapore Cancer Registry. The age standardized incidence (ASR) of HCC in the population fell by 58% in men and 47% in women from 1968-1972 to 1998-2002 from 17.1 to 7.1 per 100,000 men (p = 0.004) and from 2.8 to 1.5 per 100,000 women (p = 0.01). Amongst the three main ethnic groups, the incidence for HCC was highest in Chinese compared with Malays (p = 0.048) and Indians (p = 0.023). The overall prevalence of hepatitis B in Singapore fell from 9-10% in 1980-81 to 4% in 1999. The incidence of acute hepatitis C was 0.6 per 100,000 in 2005. The prevalence of regular alcohol consumption rose by 0.8% from 1976-77 to 1998. From 1976 to 2004 the prevalence of smoking dropped by 10%. The prevalence of obesity increased from 4.3% in 1982-85 to 6.9% in 2004 and the prevalence of diabetes mellitus rose from 2% in 1975 to 8.2% in 2004. Conclusions: There has been a significant decrease in the incidence of HCC in Singapore over the last 35 years associated with a decrease in the prevalence of hepatitis B in the population. This is likely to be due to the introduction of hepatitis B vaccination in Singapore in the early 1980’s and the compulsory vaccination of babies born to carrier mothers. The difference in the incidence between different racial groups is likely to be due to the differing prevalence of hepatitis B. The trends in other risk factors suggest that they are unlikely to have contributed significantly.

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402 THE CHANGING EPIDEMIOLOGY AND ETIOLOGY OF HEPATOCELLULAR CARCINOMA FROM 1969 THROUGH 2006 IN ALASKA NATIVE PEOPLE
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Alaska Native people have an increased rate of primary hepatocellular carcinoma (HCC) compared to the overall US population. Hepatitis B virus (HBV) has been identified as a major etiologic agent in Arctic Indigenous people. With the introduction of HBV immunization in 1982, as well as the emergence of hepatitis C virus (HCV) in this population, the epidemiology and etiology of HCC in Alaska may be changing. Methods: We reviewed all cases of viral and non-viral associated HCC in Alaska Native persons from 1969 through 2006. Using ICD-9 codes from the Alaska Native Medical Center, the Alaska Native Tumor Registry, and records from the Liver Disease and Hepatitis Program, all cases of HCC that occurred from 1969 through 2006 were identified and reviewed for demographic and clinical features and etiology. Etiology was categorized as viral (HBV, HCV) and non-viral. Incidence rates per 100,000 population were calculated for HCC overall and by etiologic category. Body mass index (BMI) was compared between non-viral and viral HCC cases. Results. Over the study period, 115 cases of HCC were identified in 110 persons. The age distribution for HCC was bimodal with peaks in the second and sixth decades of life (early peak due to HBV). The overall HCC rate was 3.63 per 100,000 and did not change significantly over the study period (p = 0.54). However, HCV-associated HCC increased from zero cases prior to 1985 to 23 since 1995, representing 30% of viral associated HCC (p = 0.01). The rates of HBV-associated HCC varied widely over time (1.26-3.79 per 100,000) but did not show a significant trend over the study period (p = 0.52). The age distribution of HBV-associated HCC demonstrated a shift towards presentation later in life. From 1969 to 1999, 29% of HBV associated HCC cases were under 20 years of age; after 2000, no cases were under age 20 (p = 0.028). Non-viral HCC rates ranged from 0.3 to 2.04 cases per 100,000 but demonstrated no significant trend over the study period (p = 0.90). Mean BMI was greater in non-viral HCC compared to viral HCC (38.8 vs 26.6, p = 0.002). Conclusions. HCC rates in Alaska Natives remained stable over the study period, but the epidemiology and etiology are changing. HCV has emerged as an important cause of HCC since 1995. Two decades after mass hepatitis B immunization, the HCC age distribution has shifted to cases presenting later in life. This is consistent with the presence of an aging HBV-infected population with few new chronically infected young persons entering the population. The significantly increased BMI in non-viral HCC cases compared to viral HCC cases suggests nonalcoholic fatty liver disease as a likely etiology.

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The following people have nothing to disclose: Marc Connelly, Michael Bruce, Nicholas Kassebaum, Lisa Bulkow, Mary Snowball, Brian J. McMahon
PROGNOSTIC FACTORS AFFECTING 3 YEARS SURVIVAL IN PATIENTS WITH HEPATOCELLULAR CARCINOMA (HCC)

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HCC is heterogeneous cancer with different risk factors, and response to therapy. Overall survival at 3 years is poor, but a subset of patients shows a survival, comparable to that achieved after liver transplantation. The ability to identify these patients would facilitate decision making and organ allocation. 

AIMS: To evaluate the influence of pre-treatment parameters on 3-year survival in a cohort of non-transplanted HCC patients.

METHODS: Out of 400 patients with HCC diagnosed according to the EASL criteria treated and prospectively followed-up at a single referral centre, we selected 228 consecutive patients that did not undergo liver transplantation and were either alive (group A, 91 pts) or dead (group B, 137 pts) three years after diagnosis. Patients were treated with: resection (26 pts in group A, 27 pts in group B), ablative treatments (49 in A and 28 patients in B), TACE (13 pts in A and 28 in B) while 3 patients in A and 55 in B remained untreated because of refusal of therapy, co-morbidities or advanced disease. Qualitative and quantitative variables were first analyzed with the χ² test qualitative or Wald test, respectively, and then with a logistic regression model (multivariate analysis, with stepwise method).

RESULTS: Median survival was 53.4 months (range: 36.2-105.2) in A and 10.7 months (range: 0-35.5) in B. At univariate analysis, ascites, encephalopathy, portal vein thrombosis, HCV, HBsAb/HBeAb, overall tumour burden, number of nodules, ALT, ALP, γGT, Bilirubin, Albumin, PT-INR, Hematocrit, AFP, MELD, Child Pugh, BCLC, TNM and CLIP were significantly different between A and B (p < 0.05). After multivariate analysis, tumor burden, OR:1.332, bilirubin(OR:3.053), portal vein thrombosis (OR:13.208) and HBsAb/HbcAb (OR:3.201) were significant independent predictors of survival. These were used to generate a logistic regression model able to predict the probability of death and survival 3 years after HCC diagnosis. The predictive ability expressed as the Area Under the Curve of the ROC Curve was 83.4%, showing a good accuracy of the model. Depending on the cut-off probability, sensitivity (survival shorter than 3 years correctly classified) of 90.91% and 1-specificity (survival longer than 3 years incorrectly classified) of 16.95% were reached.

CONCLUSION: Prognosis of patients with HCC can be predicted using a mathematical model factoring tumor burden, macrovascular invasion, liver function and previous contact with HBV as independent factors. The ability of the model to stratify patients according to their estimated prognosis three years after the diagnosis may be of help in decision making and organ allocation.

THE CLINICOPATHOLOGICAL AND PROGNOSTIC SIGNIFICANCE OF ALPHA-FETOPROTEIN EXPRESSION IN INTRAHEPATIC CHOLANGIOCARCINOMA. A POSSIBLE PROGENITOR CELL ORIGIN

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Recent studies suggest that some types of human cancers may arise from cancer stem cells. A hepatic progenitor cell which can differentiate into hepatocytes and biliary lineage, has also been suggested to give rise to hepatocellular carcinoma and combined hepatocellular-cholangiocarcinoma. Alpha-fetoprotein (AFP) has been shown to be expressed in liver cells and human progenitor cells and there are some cases of intra hepatic cholangiocarcinomas (ICCs) with seropositive for AFP. Aims: In this study, we investigated the expression of AFP in ICCs. Method: Expression of AFP was examined in 53 patients with ICC by double immunohistochemical and immunofluorescence staining using the antibody against AFP and cytokeratin (CK) 7 or 19 (biliary differentiation marker). The relationship between their positive or negative expression and clinicopathological variables including survival rate after initial hepatectomy was analyzed. Results: Fifty three cases were diagnosed pure ICC because of both morphological aspects, mucus production, and diffuse positivity for CK 7 or 19. Of 53 cases of ICCs, 9 (17.0%) were positive for AFP. In each case, some tumor cells were double positive for CK19 and AFP and showed oval cell like morphology (small size, high nuclear to cytoplasmatic ratio and an oval-shaped nucleus). There were no significant differences between expression of AFP and clinicopathological variables except for elevated serum AFP levels. Although there is no significant difference in survival rate between the patients with AFP positive cases and AFP negative cases, ICCs expressing AFP had a higher rate of recurrence (P = 0.012) after initial hepatectomy compared with AFP negative tumors. Conclusion: In our series, 17% of ICCs contained cells expressing AFP and these ICCs showed higher recurrence rate. Existence of AFP positive oval cell like cells suggests that AFP positive ICCs might originate from hepatic progenitor cells.

THE INTERACTIVE EFFECT OF PROGNOSTIC FACTORS AND PI-88 USE ON THE DELAY OF HEPATOCELLULAR CARCINOMA (HCC) RECURRENCE FOLLOWING CURATIVE PARTIAL HEPATIC RESECTION

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Purpose: Proposed indication for PI-88 is the prevention of cancer recurrence following partial hepatic resection in patients with HCC. PI-88 is a first-in-class mimetic of heparan sulfate (HS) which targets two biological processes critical to the growth & progression of solid tumors: angiogenesis & metastasis. It antagonizes HS interaction with angiogenic growth factors & inhibits cleavage of basement membrane by heparanase. Methods: Efficacy & safety of PI 88 as post-resection adjuvant therapy in pts with HCC was evaluated in a recently completed randomized Phase II study. The primary end point was tumor non-recurrence rate at 48 wks with disease-free survival forming a secondary endpoint. Tumor recurrence rate at 48 weeks was 50% in the control group, 63% in the PI-88 160 mg group & 41% in the PI-88 250 mg group. Time to
recurrence was 78% longer for the treated group than for controls (48 weeks vs. 27 weeks) at the 70th percentile. Literature supports the key prognostic factors affecting HCC recurrence following curative partial hepatic resection including tumor size, number of liver tumors, histopathological differentiation of the tumor, presence of microvascular invasion, & hepatitis serology. In an effort to further delineate the effect of PI-88 on pts with varying degree of HCC recurrence risk as determined by these prognostic factors, a stepwise cluster analysis was conducted for all trial control arm pts. Results: On the basis of this analysis, assessment of HCC recurrence risk for pts in the 160mg & 250mg arms was made, & subsequent Kaplan-Meier disease-free survival analysis was completed for those pts determined to have higher risk of HCC recurrence. Amongst the control arm pts for whom complete 48-week data was available, tumor size is the most powerful predictor of HCC recurrence. Those pts with a (primary) tumor of less than 2.5 cm are unlikely to recur within one year of curative partial hepatic resection, while those with tumors larger than or equal to 2.5 cm are much more likely to recur (approximately 10% versus 60%, respectively). On the basis of tumor size alone, it is possible to correctly predict recurrence in 69% of cases, up from the 43% 12-mth overall recurrence rate. By adding prognostic variables of microvascular invasion & hepatitis serology, it is possible to increase this overall predictive power. Conclusion: This study is consistent with literature on prognostic factors of HCC recurrence following curative partial hepatic resection. Furthermore, the data supports the conclusion that PI-88 has a significant effect on those pts that are likely to recur within 12-mths.

Disclosures:
James Garner - Employee: Other
Justus Homburg - Employee: Other

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COPY NUMBER ANALYSIS OF HEPATOCELLULAR CARCINOMA USING TEN GENES DERIVED FROM GENECHIP DATA SET OF HEPATOMA CELL LINES

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Background/Aim: Hepatocellular carcinoma (HCC) is one of the leading causes of cancer deaths worldwide, and chromosomal copy number alterations could result in aberrant oncogenes activation and tumor suppressor genes inactivation in HCC. In this study, we analyzed 17 hepatoma cell lines for areas of DNA copy number changes (gain, amplification and deletion) and LOH through genome-wide analysis with Affymetrix GeneChip Human Mapping 50 K Set. Moreover, we investigated the copy number of genes that we have chosen from GeneChip data of hepatoma cell lines in HCC clinical samples, and analyzed the association with clinicopathological parameters. Materials/Methods; We analyzed 17 hepatoma cell lines (PLC/PRF/5, Huh-1, Huh-6, Huh-7, HepG2, HepSB, HLE, HLF, SK-Hep-1, JHH-1, JHH-4, JHH-7, SNU398, SNU449, HT17, SSP-25 and RBE) with GeneChip and Copy Number Analyzer for GeneChip (CNAG) (Nannya et al. Cancer Res. 2003) for data analysis. We selected one gene each from highly amplified regions in at least two cell lines, and analyzed copy number of 70 formalin-fixed paraffin-embedded HCC samples using quantitative real-time PCR. Results; Ten high grade amplification regions (n > 4) within 3 Mbp were detected in at least two cell lines, and the size of minimal common amplified region was 129 Kbp. Nineteen homozygous deletion regions were identified in at least one cell line, and the size of minimal deletion was 13 Kbp. Furthermore, LOH at chromosome 4q, 8p, 9p and 13q was observed with high frequency. In regards to clinical samples, copy number was almost normal in liver cirrhosis, and frequently amplified in Edmondson grade II (Ed II) HCC compared with Ed I HCC. Logistic Regression revealed that Gene 1 in 8q22.3 [Ed/I/II 8.3%/39.1%] and Gene 2 in 14q12 [Ed/I/II 4.2%/21.7%] were significantly amplified in Ed II HCC (p<0.05). Multivariate analysis using stepwise variable selection showed that gain of Gene 1 appeared to be associated with HCC recurrence. Conclusion; GeneChip and CNAG enabled us to narrow down the region of copy number changes in hepatoma cell lines. Gain of Gene 1 and 2 were associated with malignant phenotypes of HCC, and gain of Gene 1 might be predictive for recurrence free survival in HCC patients.

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PRETRANSPLANT CHEMOEMBOLIZATION FOR HEPATOCELLULAR CARCINOMA ≤ 3 CM DOES NOT INFLUENCE THE OUTCOME AFTER LIVER TRANSPLANTATION

Mohamed Amer, Mohammed Yousri, Fatma Barakat, Yehia Naga, Fatma Barakat, Yoko Kono, Lisa M. Richards, Elliot Alpert, Claude B. Sirlin, Rose Steven, Marquis Hart, Ajai Khanna, Tarek Hassenin; University of California, San Diego, San Diego, CA

Liver transplantation (OLT) is the favored option of therapy for patients with hepatocellular carcinoma (HCC) who fulfill Milan criteria. Transarterial chemoembolization (TACE) is one of the most widely used treatments for HCC patients awaiting liver transplantation to prevent tumor growth. Aim: To evaluate the impact of pre-transplant TACE in patients with tumor size ≤3 cm on the outcome after liver transplantation regarding HCC recurrence and patient survival. Methods: The explants of 66 consecutive patients with HCC who were transplanted between 2001 and 2007 were reviewed. 15 patients had HCC > 3 cm while 51 patients had HCC ≤3 cm. Patients with HCC of ≤3 cm are the subjects of this analysis. Patients were divided into Group A (n=30) who received TACE pre-OLT and Group B (n=21) who did not receive TACE. Patient charts were reviewed and data was collected. Results: The mean age was 55.2 ± 6.9 and 86% were male. 49% were Caucasian, 43% Hispanic and 8% other. 84% had Hepatitis C Virus, with no significant difference between both groups in terms of etiology of liver disease (p=0.64). Patients in group B had higher MELD score than patients in group A (16.8 ± 5.3 and 12.4 ± 5.2, respectively; p<0.01); 39% of Group B patients had a Child Pugh class C versus 11% of Group A patients (p=0.03). Examining the liver explant showed that there were no differences between both groups in tumor characteristics (Table). 30% of patients in group A who received TACE had complete tumor necrosis. 27% of patients in group A had poorly or moderately differentiated tumor versus 57% in group B (p=0.75). The median duration between diagnosis of HCC and liver transplantation was 146 (8-1313) days with no significant difference between both groups (p=0.44). The median post liver transplant follow-up was 16 (1-54) months for both groups (p=0.87). There was no statistical significant difference between both groups in terms of HCC recurrence or survival. 1 patient in each group had recurrence (p=0.79). 24 (80%) of patients in group A versus 17
408 BILARY INSULIN LIKE GROWTH FACTOR-1 (IGF1) IS A SENSITIVE MARKER FOR THE DIAGNOSIS OF EXTRA-HEPATIC CHOLANGIOCARCINOMA

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Cholangiocarcinoma is a malignant tumour with an incidence and mortality progressively increasing worldwide and, characterized by poor prognosis and scarce response to current therapies. Unfortunately, diagnostic markers for an early diagnosis are virtually lacking. Human and experimental studies showed that cholangiocarcinoma cells express and secrete IGF1 and VEGF (vascular endothelial growth factor). Our aim was to measure IGF1 and VEGF in bile and serum of patients with extrahepatic cholangiocarcinoma and to evaluate their performance as diagnostic markers. Bile samples were collected from 73 patients consecutively submitted to endoscopic retrograde cholangiopancreatography (ERCP) including extrahepatic cholangiocarcinoma (n=29), pancreatic cancer (n=19) and benign biliary pathologies (n=25; bile duct stones (n=16), primary sclerosing cholangitis (n=8), cholangitis (n=1)).

Definitive diagnosis was based on conventional radiology, ERCP and follow-up. IGF1 and VEGF were measured by ELISA, biliary bile salt by 3α-hydroxysteroid dehydrogenase. Biliary IGF1 concentration was 15-20-fold higher (p<0.00001) in extrahepatic cholangiocarcinoma than in pancreatic cancer or benign biliary pathologies, the latter two groups showing similar results. By using ROC analysis and an optimized cut-off point, biliary IGF1 concentration higher than 114 ng/ml showed, as a single marker, a sensitivity and specificity equal to 1 (100%) in the diagnosis of extrahepatic cholangiocarcinoma with respect to either pancreatic cancer or benign biliary pathologies. Biliary VEGF concentration, in contrast, was similar between cholangiocarcinoma, pancreatic cancer and benign biliary pathologies. The serum levels of IGF1 were similar while, serum levels of VEGF were significantly higher (p<0.05) in cholangiocarcinoma and pancreatic cancer with respect to benign biliary pathologies. In conclusion, we demonstrated that the determination of biliary IGF1 concentration in patients submitted to ERCP for biliary obstruction definitively differentiates extrahepatic cholangiocarcinoma from either pancreatic cancer or benign biliary pathologies, suggesting a role in the clinical work-up.

Disclosures:
The following people have nothing to disclose: Mohamed Amer, Mohammed Yousri, Fatma Barakat, Yehia Naga, Fatma Barakat, Yoko Kono, Lisa M. Richards, Elliot Alpert, Claude B. Sirinli, Rose Steven, Marquis Hart, Ajai Khanna, Tarek Hassenin.

<table>
<thead>
<tr>
<th>Group A (TACE)</th>
<th>Group B (No TACE)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>30</td>
<td>21</td>
</tr>
<tr>
<td>Mucinocytality</td>
<td>21 (70%)</td>
<td>11 (52.4%)</td>
</tr>
<tr>
<td>Vascular Invasion</td>
<td>2 (6.7%)</td>
<td>12 (57.6%)</td>
</tr>
</tbody>
</table>

(81%) in group B had disease free survival (p=0.93) during the follow-up period post OLT. Conclusion: Pre-OLT TACE of <3 cm tumor size does not influence rate of recurrence of HCC or survival post-OLT in patient fulfilled the Milan Criteria.

409 RADIOFREQUENCY ABLATION FOR HEPATOCELLULAR CARCINOMA AND INTRATUMORAL PRESSURE

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[Background and Aims] Radiofrequency ablation (RFA) is considered to be a less invasive therapeutic technique for hepatocellular carcinoma (HCC). However, sporadic cases of unexpected recurrence, such as intrahepatic dissemination, peritoneal dissemination and extrahepatic metastasis, have been described. Some of these types of recurrences might be caused by increased intratumoral pressure. We therefore examined basic and clinical studies on the association between RFA and intratumoral pressure. [Subjects and Methods] A Basic study: Under general anesthesia, laparotomy was performed on 19 pigs and a 20-mm LeVeen needle electrode was inserted into the liver for RFA. Intrahepatic pressure was monitored by placing a 21-G percutaneous ethanol injection therapy (PEIT) needle near the LeVeen needle and using an invasive blood pressure monitor. RFA was performed as follows: 1) Single-step method (standard ablation); After fully deploying the electrode, the power was initially applied at 30 W, then increased in increments of 10 W/min until power roll-off. 2) Multi-step method; The array was gradually deployed in 8 steps. At each step, the power was fixed at 30 W until power roll-off. B. Clinical study: Subjects were 32 patients with HCC. Mean tumor size was 14.7±1.1 mm. Under local anaesthesia, after inserting a 20-mm LeVeen needle percutaneously into the liver, a 21-G PEIT needle was placed near the LeVeen needle, and intratumoral pressure was monitored using an invasive blood pressure monitor. Based on the results of the basic study, RFA was performed using the multi-step method (30 W 8-step deployment). CT was performed postoperatively to ensure that ablation was performed sufficiently. [Results] A. Basic study: Mean time to reach power roll-off and hepatic parenchymal pressure were 163.1±40.4 s and 154±30.9 mmHg for the single-step method and 116.4±30.8 s and 24.1±18.2 mmHg for the multi-step method, respectively. Maximum diameter of ablated area for the single- and multi-step methods was 25.1±5 and 23.0±2.8 mm, respectively. B. Clinical study: Mean time for roll-off was 372.7±175.8 s and mean increase in intratumoral pressure was 39.1±28.7 mmHg. Postoperative CT showed that all HCCs were sufficiently ablated. Also, with the multi-step method, pain could be alleviated during treatment. [Conclusions] With the single-step method, hepatic parenchymal pressure rapidly increased just before power roll-off. Conversely, the multi-step method allowed suppression of increases in intrahepatic pressure in both basic and clinical studies. Because the patients did not experience much pain during RFA, the multi-step method should be used for RFA.

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410 TREATMENT OF RECURRENT HEPATOCELLULAR CARCINOMA AFTER CURATIVE RADIOFREQUENCY ABLATION

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BACKGROUND: Radiofrequency ablation (RFA) is widely applied as an initial treatment for unresectable hepatocellular carcinoma (HCC). AIMS: To elucidate the characteristics of recurrence after curative RFA, treatment modalities for recurrence and the prognosis. METHODS: We treated 667 naïve patients with HCC by RFA between 1999 and 2004. Patients were followed up according to our follow-up strategy after RFA with dynamic CT/MRI and tumor markers (alphafetoprotein, des-gamma-carboxy prothrombin and AFP-L3 fraction) every 4 months. Tumor recurrence was identified in 423 patients during the follow-up period until December 31, 2005. We enrolled 420 patients of them excluding 3 in whom recurrence was identified at other institutes to assess the following variables at the diagnosis of the recurrence: the size and number of recurrent nodules, presence of extrahepatic metastasis and portal venous invasion, and treatment modalities for recurrence. Survival analysis was performed according to the Kaplan Meier methods by following up patients until the end of 2006. Univariate and multivariate Cox proportional hazard model was applied to assess the risk factors for survival. Age, gender, the size and number of original and recurrent nodules, tumor markers at recurrence, etiology of background liver diseases and Child-Pugh class were adopted for the analysis. RESULTS: There were 264 men and 156 women, with a median age at recurrence, 70 years (range, 44-91). HCV antibody was positive in 343 and HBs antigen was positive in 47. Mean ± SD of Child-Pugh score was 6.3 ± 1.3, which increased by 0.3 as compared with that at initial treatment. Size of nodules at recurrence were < 2 cm in 268, 2.1-3 cm in 108, 3.1-5 cm in 36 and > 5 cm in 7. Extrahepatic metastasis and portal venous invasion were identified in 112 (2.6%) and 92 (2.1%) respectively. Patients were treated by RFA in 371 (88%), TACE in 49, radiotherapy in 4, systemic chemotherapy in 3, arterial infusion chemotherapy in 2, resection in 3 and best supportive care in 5 (including overlap). Cumulative survival rates at 1, 3 and 5 years after recurrence were 90.3%, 62.9% and 36.3%, respectively. Multivariate analysis with Cox proportional hazard model revealed that size of original tumor, number of recurrent nodules, presence of extrahepatic metastasis and portal venous invasion were independent risk factors for poor prognosis. Conclusion: Our strategy could identify recurrent nodules small in size and RFA was indicated 88% of patients. The change in liver function was minimal and unfavorable mode of recurrence was rare. RFA can be the first choice for recurrent HCC as well as naïve HCC.

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411 POLYMORPHISM OF INTERLEUKIN-18 IS A RISK FACTOR FOR THE CLEARANCE OF HEPATITIS B VIRUS INFECTION AND HEPATOCELLULAR CARCINOMA DEVELOPMENT

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Background/Aim: The outcomes of hepatitis B virus (HBV) infection are probably related to host immune factors. Interleukin-18 (IL-18) plays significant roles in immune defense. This study was undertaken to investigate the association between persistence of HBV infection or HCC occurrence and single nucleotide polymorphisms (SNPs) of IL-18 gene. Methods: Between March 2002 and December 2002, a total of 1,050 Korean patients were enrolled in three different groups; 'HBV clearance (n=320)' and 'chronic hepatitis (CH) / liver cirrhosis (LC) (n=637)' and 'hepatocellular carcinoma (n=93)'. We assessed polymorphisms at four polymorphic sites in IL-18 gene at position -9731G>T, -9212G>C, -140G>C, +4861A>C in subject studies. Results: IL-18 -9212C allele (OR = 0.25, P = 0.01), -140G allele (OR = 0.36, P = 0.02) and +4861C allele (OR = 0.25, P = 0.01) were significantly associated with HBV clearance in a recessive model. Additionally, IL-18 -9212G allele (OR = 2.18, P = 0.01) and 'dominant model, OR = 2.11, P = 0.03 in dominant model), -140C allele (OR = 1.97, P = 0.03 in dominant model, OR = 1.98, P = 0.04 in dominant model) and +4861A allele (OR = 1.91, P = 0.04 in co-dominant model) showed susceptible effect on the HCC development. Conclusions: This study suggested that SNPs at various sites of IL-18 gene were associated with HBV clearance and HCC occurrence.

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412 LONG TERM RESULTS OF RFA VS SURGICAL RESECTION FOR HCC IN PATIENTS WITH WELL PRESERVED LIVER FUNCTION

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Background/Aims: Surgical resection is considered optimal for potential treatment of hepatocellular carcinoma (HCC). However, only 20% of HCCs can be resectable due to the severity of cirrhosis or the multiple nodules. Radiofrequency ablation (RFA) has recently shown comparable results to surgical resection for the treatment of HCC. We compared the clinical results between surgical resection and RFA. Methods: From January 2000 to December 2002, 116 patients who had Child-Pugh class A and underwent surgical resection (n=61) or RFA (n=99) for HCC (single nodule < 5 cm in diameter, or less than 3 nodules with < 3 cm in diameter) were analyzed retrospectively. We compared the survival rates, the recurrence rates and the complication rates between two groups. Results: In Surgical resection group, the local and distant recurrence rates were 11.5% and 42.6%. Recurrence free survival rates at 1, 3 and 5-year were 73.1%, 45.1% and 39.2%, respectively and overall cumulative survival rates were 98.3%, 83.4% and 75.1%. In RFA group, the local and distant recurrence rates were 19.2% and 39.4%. Recurrence free survival rates at 1, 3 and 5-year were 72.4%, 40.4% and 33.7%, respectively and overall cumulative survival rates were 95.8%, 78.3% and 73.1%. There were no significant differences in the overall and
recurrence free survival rates between two groups (p=0.77 and p=0.58). The incidence of complication was similar between the two groups. Conclusions: RFA shows comparable results to surgical resection for the treatment of HCC and may be considered as an alternative to surgical resection for the treatment of HCC.

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A PROPOSAL OF NOVEL TREATMENT-ASSIST TECHNIQUE IN THE SONAZOID-ENHANCED ULTRASONOGRAPHY: VALUE OF DEFECT RE-PERFUSION IMAGING

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Background and Aim: Sonazoid (GE Healthcare) is a second generation ultrasound contrast agent, that has been approved for routine clinical use on January 10, 2007. Sonazoid-enhanced harmonic imaging (Sonazoid CEUS) provides extremely superb real-time vascular imaging as well as Kupffer imaging at 10 min. after intravenous injection of 0.015 mL/kg of Sonazoid. The local ablation therapy such as radiofrequency ablation (RFA) for hepatocellular carcinoma (HCC) is usually performed by ultrasound (US) guidance. However, RFA for sonography ill-defined HCC nodules or local recurring nodules after RFA is extremely difficult because of the difficulty of identification of the nodule on B-mode US. Aim of this study is to clarify the usefulness of newly innovated contrast US technique, “Defect Re-perfusion Imaging” by using Sonazoid re-injection method at the post-vascular (Kupffer) phase. Materials and Methods: From January to May, 2007, there were a total of 10 sonography ill-defined HCC nodules, all of which were not identified by B-mode US. A total of 0.015 mL/kg of Sonazoid were injected and Kupffer image was obtained at 10-20 min. after injection. Typical HCC nodules should show Kupffer defect since typical HCC has no Kupffer cells. Therefore, when defect area was observed at the post-vascular phase, 0.015 mL/kg of Sonazoid was re-injected and whether or not arterial flow is present within the Kupffer cell defective portion. Results: In all of 10 sonography ill-defined HCC nodules, positive enhancement was obtained within the Kupffer cell defective portion by Sonazoid re-injection, thereby, the diagnosis and localization of HCC was possible. Subsequently, RFA was successfully performed in all of 10 sonography ill-defined HCC nodules. Conclusion: Defect Re-perfusion Imaging is an extremely useful method to diagnose and identify the sonography ill-defined HCCs, which facilitates easy and correct insertion of RFA needle inside the HCC nodules, which were not possible by any other modalities before this technique was innovated.

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DISCOVER A NEW BIOMARKER FOR EARLY DETECTION OF HEPATOCELLULAR CARCINOMA USING MALDI-TOF MS AND CLINPROTOOLS

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Objectives: Detection of HCC at early stage is often difficult in chronic hepatitis C patients especially in cirrhosis. Once the diagnosis of HCC established, the overall 5-year survival rate remains less than 5%. The poor prognosis of HCC due to that the disease frequently diagnosed at an advanced stage, when the disease is too far for effective treatment. In order to reduce HCC mortality, early identification of hepatocellular carcinoma from is needed. MALDI-TOF (matrix assisted laser desorption/ionization time-of-flight) is a proteomic technique that enables the profiling of proteins present in any biological material. We used this approach to identify new biomarkers of hepatocellular carcinoma (HCC) in the sera of patients with HCV-associated cirrhosis. Patients and Method: Serum samples (5 uL) from the 16 of HCV-associated cirrhosis patients with hepatocellular carcinoma and the 15 of HCV-associated cirrhosis patients without HCC were prefractionated using magnetic Magnetic Beads based Weak Cation Exchange Chromatography resins (Bruker Daltonics, Germany) by ClinProTools automatic machine according to recommendatory protocol. We analyzed each spectrum obtained from MALDI-TOFMS with flexAnalysis™ software in automatic mode and ClinProTools(the data interpretation software of the mass spectrometry-based ClinProt solutions for biomarker analysis), the former to detect the peak intensities of interest and the latter to compare the peaks across the spectra obtained from all samples. The inter- and intra- reproducibility (IMAC Cu beads) assay of the MALDI spectra was subjected. Result After MALDI-TOF MS spectra and flexAnalysis™ analysis, there were 23 peaks increased 25 peaks decreased in group of HCV-associ- ciated cirrhosis with HCC. The representative spectra of peptide, it was of note that an 8568 Da peak, significantly increased in HCC group. 8568 Da peak increased in 11 samples in group of HCC in the context of HVC-related liver cirrhosis, on contrast, there are only 3 samples increased in group of HCV-associated cirrhosis without HCC. ClinProTools was used to check the difference between two groups of 8568 Da, the result of P values is: 1.08e-006. After purified on-chip, and cut out of gel followed trypsin digested, the peptide was identified by LC MS/MS, and confirmed that there is a significant difference indeed in LC and HCC groups by western blotting. We observed no associated level with AFP or PIVKAII and tumor size. Conclusion: These results demonstrated a novel approach to discover a new biomarker for early detection of hepatocellular carcinoma using MALDI-TOF MS and ClinProTools.

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415 GENETIC POLYMORPHISMS ASSOCIATED WITH CLEARANCE OF HEPATITIS B VIRUS INFECTION AND HEPATOCELLULAR CARCINOMA OCCURRENCE DETECTED BY SNP CHIPS

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Hepatitis B virus infection (HBV) associated chronic liver disease (CLD) and hepatocellular carcinoma (HCC) are major causes of death in HBV endemic areas. Expression of collagens is related progression and suppression in CLD and/or HCC. We investigated whether polymorphisms of collagen type III, alpha 1 (COL3A1) associated with genetic susceptibility of CLD and HCC. Three hundred and forty-two patients (230 CLD, 112 HCC) and 117 healthy controls were performed association study using Illumina’s SNP chip by allele specific extension method. Selected polymorphisms and haplotypes were analyzed by logistic regression controlling age and gender as covariables. In statistic analysis, SNP CHA-1 was high significantly association with control verse CLD, control verse HCC, and control verse CLD with HCC in allelic model, codominant, dominant (p=0.01 to 0.002). Haplotype 1 (ht1), ht 2, and ht 5 significant associated with to control verse CLD (p=0.021, p=0.030 p=0.0037, respectively), control vs HCC (ht 1: p=0.046), and control verse CLD with HCC (ht 1: p=0.013 and ht 5: p=0.046). In conclusion, we showed that SNP CHA-1 polymorphism and ht1 may increase the clearance of HBV infection, whereas ht2, ht5 may increase the susceptibility to disease progression, by using SNP chip analysis.

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416 LIVER TRANSPLANTATION IN PATIENTS WITH HEPATOCELLULAR CARCINOMA ACROSS MILAN CRITERIA

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Milan criteria are the most frequently used limits for liver transplantation (LT) in patients with hepatocellular carcinoma (HCC), but our previous experience with expanded criteria showed encouraging results. Aim: To investigate whether our expanded (CUN) criteria (one nodule up to 6 centimeters or 2-3 nodules up to 5 centimeters each) could be used to select patients with HCC for LT. Patients and methods. Eighty-five patients with HCC fulfilling CUN criteria were included as candidates for LT. We were excluded of the waiting list if they exceeded CUN criteria. Survival and recurrence rates were compared according to tumor staging. Results: Twenty-six out of 85 (30%) patients exceeded Milan criteria. Twelve patients had tumor progression on the waiting list. Four patients (7%) fulfilling Milan criteria and 8 patients (30%) exceeding them progressed exceeding CUN criteria (p=0.001). One, 3-, 5-, 7- and 10-year survival rates of the 73 transplanted HCC patients were 86%, 74%, 70%, 61% and 50%, respectively. According to radiological staging, 47 patients were within Milan criteria at the moment of transplantation and 26 (35%) exceeded them, but were within CUN criteria. Tumor recurrence and survival rates were similar for patients fulfilling Milan and CUN criteria. Tumor recurrence rates were significantly higher in patients whose pathological staging exceeded CUN criteria. Conclusion: CUN criteria (one nodule up to 6 centimeters or 2-3 nodules up to 5 centimeters each) increases the number of HCC patients who could benefit from LT, without worsening the results.

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The following people have nothing to disclose: I Ignacio Herrero, Mercedes Inarraiaegui, Bruno Sangro, Fernando Pardo, Jorge Quiroga, Felix Alegre, Fernando Rotellar, Custodia Montiel, Jesus Prieto

417 REGULATION OF THE NKG2D IMMUNORECEPTOR BY SOLUBLE MICA DURING TRANSCATHETER ARTERIAL EMBOLIZATION FOR HEPATOCELLULAR CARCINOMA

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Background & Aim: The immunoreceptor NKG2D is an activating molecules expressed on natural killer cells. MHC classI-related chain A (MICA) is the ligand of NKG2D and is over-expressed in injured cells or transformed cells. Recent studies indicate that MICA could be released as its soluble form (sMICA) from those cells and capable of downregulating NKG2D expression, leading to the attenuation of innate immune response to potentially malignant cells. Although sMICA has been shown to increase in sera of some cancer patients, the presence and significance of sMICA in liver diseases are not fully established. The present study examined soluble MICA in chronic liver disease and hepatocellular carcinoma (HCC) and their involvement in the immune cell expression of NKG2D during transcatheter arterial embolization (TAE) for HCC. Patients & Method: Serum levels of sMICA was differentially determined by ELISA from 581 individuals including 104 healthy volunteers (HV), 141 patients with chronic hepatitis (CH), 104 patients with cirrhosis (LC), and 232 patients with hepatocellular carcinoma (HCC). Peripheral blood mononuclear cells (PBMC) were collected before and 2 weeks after TAE therapy from 38 patients with HCC. Twenty-one untreated patients with HCC, matching the TAE group with respect to TNM stage and Child-Pugh stage were also enrolled as controls. NKG2D expression on NK cells and CD8-positive T cells were analyzed by flow cytometry, whereas sMICA was also evaluated by ELISA simontaneously. Difference between pretreatment and post-treatment values were tested by paired t test. Result: Levels of sMICA differed statistically (p<0.01) among each 4 groups (HV: median=50 pg/ml, CH: median 342 pg/ml, LC: median 586 pg/ml, HCC: median 648 pg/ml). The elevation of sMICA level was observed associated with the progression of fibrosis. In LC, significant correlation was observed between the Child-Pugh classification and the level of sMICA. In HCC the progression of liver disease and that of the tumor were independent determinants for sMICA level. The TAE therapy significantly decreased serum levels of soluble MICA, and increased the NKG2D expression on NK cells and CD8-positive T cells; there was an inverse correlation between changes in soluble MICA levels and in NKG2D expression. Conclusion: Although sMICA are produced from both HCC and premalignant cirrhotic livers, therapeutic intervention for HCC can reduce the levels of sMICA and thereby upregulate the expression of NKG2D. Cancer therapy may
have a beneficial effect on the NKG2D-mediated anti-tumor immunity.

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HIGHER EXPRESSION OF MICROSMAL PROSTAGLANDIN E SYNTHASE-1 IN NON-TUMOROUS LIVER TISSUES ASSOCIATES WITH SHORTER RECURRENCE-FREE SURVIVAL OF PATIENTS WITH HEPATOCELLULAR CARCINOMA

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Microsomal prostaglandin E synthase-1 (mPGES-1) is the terminal enzyme for the production of prostaglandin E2 from cyclooxygenase-derived prostaglandin H2. Microsomal PGES-1 is linked to the conditions that result in high PGES biosynthesis, which has been reported to be associated with various types of cancer. However, little is known about the prognostic value of mPGES-1 in non-tumor liver tissues after curative hepatectomy for HCC patients. We examined the expression of mPGES-1 protein by immunohistochemistry in 64 patients with HCCs who were underwent curative hepatectomy. Samples from seven histologically normal liver tissues were also examined. The level of mPGES-1 expression was higher in the hepatitic or cirrhotic liver tissues than in the normal liver tissues and the cirrhotic livers expressed more mPGES-1 than hepatitic livers (p < 0.05). All differentiated types of HCC expressed mPGES-1. Although there was no correlation between the mPGES-1 expression in HCC tumor tissues and prognosis, a close correlation was found between the mPGES-1 expression in non-tumor liver tissues and the shorter disease-free survival of the HCC patients (p < 0.01). Moreover, higher mPGES-1 expression in non-tumor liver tissues was associated with the presence of active inflammation (p < 0.05). In conclusion, the mPGES-1 expression in non-tumor tissues may play an important role in early recurrence of HCC after curative hepatectomy.

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MASS FORMING TYPE OF INTRAHEPATIC CHOLANGIOCELLULAR CARCINOMA: FAVOURING FACTORS AND HISTOLOGICAL CHANGES IN THE NON-TUMOROUS LIVER

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Microsomal prostaglandin E synthase-1 (mPGES-1) is the terminal enzyme for the production of prostaglandin E2 from cyclooxygenase-derived prostaglandin H2. Microsomal PGES-1 is linked to the conditions that result in high PGES biosynthesis, which has been reported to be associated with various types of cancer. However, little is known about the prognostic value of mPGES-1 in non-tumor liver tissues after curative hepatectomy for HCC patients. We examined the expression of mPGES-1 protein by immunohistochemistry in 64 patients with HCCs who were underwent curative hepatectomy. Samples from seven histologically normal liver tissues were also examined. The level of mPGES-1 expression was higher in the hepatitic or cirrhotic liver tissues than in the normal liver tissues and the cirrhotic livers expressed more mPGES-1 than hepatitic livers (p < 0.05). All differentiated types of HCC expressed mPGES-1. Although there was no correlation between the mPGES-1 expression in HCC tumor tissues and prognosis, a close correlation was found between the mPGES-1 expression in non-tumor liver tissues and the shorter disease-free survival of the HCC patients (p < 0.01). Moreover, higher mPGES-1 expression in non-tumor liver tissues was associated with the presence of active inflammation (p < 0.05). In conclusion, the mPGES-1 expression in non-tumor tissues may play an important role in early recurrence of HCC after curative hepatectomy.

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The following people have nothing to disclose: Gisèle Nkontchou, Jeanne Tran Van Nhieu, Adriana Andrea Luca, Valérie Bourcier, Nathalie Ganne Carrie, Véronique Grando Lemaire, Jean Claude Trinchet, Marianne Ziol, Daniel Cherqui, Michel Beaugrand

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SERUM ANTIBODY TO HEPATITIS B CORE ANTIGEN IS NOT ASSOCIATED WITH INCREASED RISK OF HEPATOCELLULAR CARCINOMA IN PATIENTS WITH HCV-RELATED CIRRHOSIS

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The impact of previous exposure to HBV as a risk factor associated with tumour occurrence in subjects with HCV-related cirrhosis has not been fully investigated. We assessed whether serum anti-HBc is associated with HCC development in HCV-RNA+ve, HBsAg-ve patients with cirrhosis. 693 patients (432 men, mean age 54.8 years) with HCV-related histologically-proven cirrhosis and anti-HBc testing, surveilled with ultrasound examinations every six months, was analysed. All patients were treated with Interferon (IFN) between January 1992 and December 1997. Independent predictors of HCC were assessed by Cox multiple regression analysis. Results. Mean follow-up was 96.2 months; 303 (43.8%) patients were anti-HBc positive. There was no difference in the incidence of HCC between anti-HBc+ve and anti-HBc-ve patients. In a subgroup with sustained viral response we observed a slight increase of incidence of HCC per 100 person-years in anti-HBc+ve patients as compared to anti-HBc-ve patients.
compared to negative ones (1.17, 95% C.I. 0.32-2.98 vs. 0.64, 95% C.I. 0.13-1.87). Conclusions. Serum anti-HBc+ve/HCV+ve cirrhosis treated with IFN irrespective to the achievement of response did not show an increased risk of HCC in comparison with anti-HBc+ve subjects. These results indicate that in everyday clinical practice patients with HCV-related cirrhosis and past exposure to HBV infection do not require a tailored surveillance for HCC.

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LEF TO LOBE LOCATION OF HEPATOCELLULAR CARCINOMA AS A RISK FACTOR OF COMPLICATIONS OF RADIOFREQUENCY ABLATION
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As the small volume of the left lobe might increase the risk of complication of radiofrequency ablation (RFA) in patients with hepatocellular carcinoma (HCC), we compared in a retrospective study the tolerance of RFA performed for the ablation of HCC located in the left vs the right lobe. Patients and Methods: Over a 5-year period, 197 consecutive patients with cirrhosis and HCCs located either in the left (n=38) or in the right lobe (n=159) underwent RFA for the treatment of 47 and 198 tumours, respectively. Patient's characteristics were similar between the two groups excepted a higher percentage of subcapsular tumours in the left lobe: 31% vs 17% (p=0.03) Results: Treatment of tumours located in the left and right lobes required a mean of 1.05±0.2 and 1.02±0.14 procedures (p=0.34), including a mean of 1.8±1.2 and 2±1.5 (p=0.43) applications, respectively. Complication rates per procedure performed in the left and right lobes were 7/38 (18%) vs 6/159 (4%) (p=0.01), including capsular haematoma (3 vs 2), transient ascites (1 vs 0), pseudoaneurysms (1 vs 1), portal thrombosis (1 vs 0), liver abscess (0 vs 1), stomach burn (1 vs 0), biliary tract stenosis (1 vs 1), pleural effusion (0 vs 1) respectively. In multivariate analysis, left lobe location OR=3.16 (1.05-9.53) p=0.04, multifocal HCCs, OR=3.45 (1.14-10.38) p=0.03 subcapsular location OR=3.45 (1.14-10.38) p=0.03 and platelet counts < 100000 (100000/ ml OR=3.45 (1.14-10.38) p=0.03 were independent risk factors of complications. Conclusion the left lobe location of HCC is a major risk factor of complication after percutaneous RFA.

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EFFECT OF INTRAPERITONEAL SALINE INFUSION FOR PERCUTANEOUS RADIOFREQUENCY ABLATION OF HEPATOCELLULAR CARCINOMA
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INTRODUCTION: Percutaneous radiofrequency ablation (RFA) is effective therapeutic modality for hepatocellular carcinoma. But the procedure is not always successful, because of tumor location, possibility of surrounding tissue damage, and poor ultrasonographic image. AIMS & METHODS: This study was performed to evaluate the efficiency and safety of saline infusion into peritoneal cavity for difficult RFA cases such as hepatic dome or surface located tumor and ill defined tumor on ultrasonogram because of covering omentum. Total of 71 patients were included from June 2001 to April 2007. The reason of intraperitoneal saline infusion is to secure approach route for hepatic dome located tumor (n=35), minimize the injury of adjacent organs for surface tumor (n=27), and to detect the tumor more clearly by moving aside of omentum (n=9). We assessed the success rate and complication of procedure. We also assessed improvement of ultrasonographic image in cases that the tumor was not sharply or clearly defined on ultrasonogram (n=48). RESULTS: The percutaneous RFA with intraperitoneal saline infusion was effective in 65 cases (91.5%). The success rate was 94.3% in hepatic dome tumor, 85.1% in surface tumor, and 100% in tumor with poor ultrasonographic image. Intraperitoneal saline infusion made tumors defined more clearly on ultrasonogram in 37 cases (77.1%), and the improvement of ultrasonographic image of tumors was significant (p<0.001). There was no adjacent tissue injury on spiral CT image after RFA and no significant hemoglobin change related to procedure. Procedure related complications were developed in 33 cases (46.5%). But no major complications were developed and most common complication was pleural effusion (38%). CONCLUSION: Intraperitoneal saline infusion is effective and safe method for percutaneous RFA of hepatocellular carcinoma located at hepatic dome or surface. In addition, the tumors can be detected more clearly on ultrasonogram after intraperitoneal saline infusion.

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ELUTING BEADS FOR HEPATOCELLULAR CARCINOMA - TRANSARTERIAL CHEMOEMBOLISATION USING DRUG ELUTING BEADS FOR HEPATOCELLULAR CARCINOMA - RESPONSE AND SIDE EFFECT PROFILE

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BACKGROUND: Transarterial chemoembolisation (TACE) prolongs survival in hepatocellular carcinoma (HCC) though still remains a palliative treatment. Drug eluting bead transarterial chemoembolisation (DEB TACE) utilises chemotherapy loaded beads to deliver chemotherapy and embolise the tumour arterial supply. This allows higher doses of chemotherapy to be retained in the tumour whilst minimising systemic side-effects. This is likely to cause a different side effect profile (post embolisation syndrome) to conventional TACE. AIMS: To evaluate the radiological and pathological response and side effect profile of HCC treated with DEB-TACE in our institution. METHODS: All patients were treated with a maximum of 4ml DC beads (Biocompatibles) loaded with epirubicin 37.5mg/ml until embolisation was judged to be complete. Follow up MRI or CT scan occurred 4 weeks post treatment. Patients with residual tumour vascularity were re-treated. Pathological response was assessed in patients who underwent HCC resection or transplant. Side effects and adverse events were recorded retrospectively at outpatient clinic reviews. RESULTS: 19 patients (17 males, mean age 65.0 +/-11.6 years) underwent 22 episodes of DEB-TACE. Underlying liver disease was alcohol (7), Hepatitis B (5), NASH (2) and hepatitis C (1). 11 patients were Childs A cirrhosis, 2 were Childs B cirrhotic and 6 were noncirrhotic. Of the 19 patients, 12 had no previous conventional TACE, radiofrequency ablation or percutaneous alcohol injection. Of these 8 had 100% tumour devascularization, 1 50-99% devascularization, 2 <50% devascularization and 1 defaulted follow-up. Histopathology of 4 specimens (2 tumour resection, 2 explanted livers) demonstrated beads causing vascular obstruction and variable tumour necrosis. 73% of patients experienced post embolisation syndrome (abdominal pain and fever) following TACE. This lasted for a median of 6 days. Of the three patients who had previously received Lipiodol TACE, all described the side effects of DEB-TACE to be more severe. Two patients required re-hospitalisation, one due to an abscess requiring drainage and prolonged IV antibiotics. CONCLUSIONS: Early radiological and pathological responses to DEB-TACE is promising. Post embolisation syndrome is common, more severe in patients with larger tumours and is more prolonged than that following standard TACE. This probably reflects increasing tumour necrosis.

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424 THERAPEUTIC OUTCOMES OF RADIOFREQUENCY ABLATION UNDER GENERAL ANESTHESIA FOR HEPATOCELLULAR CARCINOMA - PROPENSITY ANALYSIS OF RFA VERSUS SURGERY

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INTRODUCTION: In recent years, RFA has become the first choice among non-surgical treatments for HCC. However, there are few reports focusing on RFA as an alternative to hepatic resection. As randomized controlled trials are difficult to perform in Japan, we conducted a propensity analysis to compare the outcomes of RFA versus surgery. METHODS: From September 1999 through December 2005, 520 HCC cases with nodules ≤5cm underwent RFA (216 cases) or surgery (304) as the initial curative treatment. Overall and recurrence-free survivals were calculated for all cases, and the RFA and surgical groups were compared by Kaplan-Meier method and Proportional hazards analysis. The Propensity analysis was then performed. RFA was performed under general anesthesia, employing percutaneous ultrasound-guidance. A RITA 500PA was used during the first year, and thereafter a Cool-tip needle, as the electrodes for RFA. RESULTS: 1) In all patients, the overall survival rates at 5 years after RFA or hepatic resection were 66.4% and 72.4%, respectively (p=0.493). The respective recurrence-free survival rates at 5 years were 36.0% and 25.9% (p=0.017). However, multivariate analysis showed the absence of differences between the two groups. Surgery vs RFA: HR1.078 (95%CI 0.849-1.376) (p=0.540) for overall survival, 1.158 [0.997-1.347] (p=0.065) for recurrence-free survival. 2) Six factors (Age, HCC location, form, diameter, Prothrombin time and ICG-R15) determining treatment selection, RFA or surgery, were identified using multiple logistic regression analysis. Ninety pairs of RFA and surgery cases were selected (180 in total) as propensity-score-match cases. Propensity analysis confirmed that there were no significant differences in either overall or recurrence-free survival between the two groups. 3) Among the 220 RFA cases, only one experienced a major complication (bleeding into the abdominal cavity) and two developed refractory ascites. CONCLUSION: There was no difference in either overall or recurrence-free survival between patients undergoing RFA and those treated surgically. RFA under general anesthesia is a safe and effective method of treating HCC.

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425 ELEVATED SERUM HYALURONIC ACID MAY IDENTIFY VINYL CHLORIDE WORKERS AT HIGH RISK FOR THE SUBSEQUENT DEVELOPMENT OF HEPATIC ANGIOSARCOMA

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Purpose: Vinyl chloride (VC) exposure is a well-documented risk factor for angiosarcoma of the liver (ASL). This association was first made in 1974 at a Kentucky chemical plant. To date, 25...
employees from this plant have developed ASL, and this is the largest single site cluster in the United States. Nationally, 80,000 chemical workers have been exposed to VC. Due to a long latency period, many are still at risk for ASL. A biomarker is needed for the early detection of this deadly tumor. ASL arises from sinusoidal endothelial cells (SECs) which also metabolize hyaluronic acid (HA). We review our experience with ASL and test the hypothesis that serum HA is elevated in VC workers who have subsequently developed ASL. Materials and Methods: A cohort of 103 workers with high VC exposure has been followed clinically since 1974 for the development of ASL. Using a nested case control approach, serum HA levels were measured in stored samples from workers with ASL or from those who went on to develop ASL, and in control groups with either high or low VC exposure who did not develop ASL. Results: The mean age at time of diagnosis of ASL was 58.6 years. The mean cumulative vinyl chloride exposure was 17,500 PPM-Yr. Angiosarcoma presented following an average latency period of 22.2 years after reaching a threshold exposure of 7000 PPM-Yr. The average values of routine liver function tests were surprisingly normal at the time of diagnosis of ASL: ALT 24 U/L (±20, s.d.), AST 36 IU/L (±9), alkaline phosphatase 70.7 IU/L (±44.6), total bilirubin 1.2 mg/dL, (±0.15), albumin 4.3 g/dL (±0.21). All 25 patients died of hepatic angiosarcoma despite aggressive treatment in the majority of cases. One employee lived 14.5 years after surgical resection. The mean survival for the other 24 was only 1.24 years after diagnosis. The mean HA level in 2 patients after the hepatic resection. The mean HA level in 11 workers at 11.1+6.90 years prior to the diagnosis of ASL was 71.6 µg/L (±58.1). This was greater than HA levels in both the high VC exposure control group (30.0±24.9 µg/L, p = 5.57 x 10^-5) and the low VC exposure control group (32.0±26.7 µg/L, p = 0.00703). Conclusions: ASL develops after a long period of high occupational VC exposure and has been uniformly fatal. At the time of diagnosis of ASL, routine liver chemistries were normal while HA was markedly elevated. In workers with high VC exposures who went on to develop ASL one decade later, the average HA level was twice that of workers who did not develop ASL. Elevated HA may identify VC workers at risk for the subsequent development of ASL.

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426 COMPARISON OF FIBROSCAN, APRI, AST/ALT AND CLINICAL SIGNS AS NON INVASIVE PREDICTORS OF CIRRHOSIS
Raza Malik, Rory Farnan, Michelle Lai, Hannalisa Freitag, Detlef Schuppan, Nezam H. Afdhal - Grant/Research Support: Other

Background and Aims: Noninvasive identification of compensated cirrhosis is important. We therefore compared Transient Elastography (Fibroscan), APRI score, AST/ALT ratio, hyaluronic acid and clinical signs to determine which modality performs best in predicting compensated cirrhosis. Methods Patients were stratified into cirrhotic and non-cirrhotic. Fibroscan measurements were correlated with clinical (spider naevi & palmar erythema), serological, radiological, endoscopic and histology which served as gold standard. An intention to treat analysis was applied to compare the cohorts using the ANOVA test with differences reported if p<0.05. ROC curves were generated to assess the diagnostic value of each test. Results A total of 833 patients were recruited into the study of whom 73 patients were excluded as a Fibroscan measurement could not be obtained (8% drop out). Fibroscan >12 kPa was diagnostically superior to the other parameters (sensitivity, specificity, PPV and NPV > 80%) yielding an AUROC measurement of 0.87 when compared to HA>45 (0.74), APRI score>1 (0.74), AST/ALT ratio>1 (0.64) and clinical signs (0.63) (p<0.01). In sub group analysis, Fibroscan performed as well. In 524 Hepatitis C patients (272 cirrhotic and 252 non-cirrhotic), Fibroscan>12 kPa yielded an AUROC of 0.86 compared to APRI>1 (0.75), HA>45 (0.73), AST/ALT ratio>1 (0.62) and clinical signs (0.63) (p<0.01). In the 236 non-hepatitis C patients in the study of whom 106 had cirrhosis and 130 were non-cirrhotic Fibroscan was also diagnostically superior at identifying cirrhosis. Fibroscan yielded an AUROC of 0.89 compared to APRI>1 (0.70), HA>45 (0.66), AST/ALT ratio>1 (0.70) and clinical signs (0.62) (p<0.01). Importantly, Fibroscan performed best at identifying cirrhosis in 146 biopsy proven Childs Pugh A cirrhosis with no clinical, biochemical or radiological features of cirrhosis or portal hypertension. Here, Fibroscan >12 kPa yielded an AUROC of 0.86 compared to APRI>1 (0.74), HA>45 (0.72), AST/ALT>1 (0.60) (p<0.01). Conclusion Fibroscan accurately identified compensated cirrhosis using a liver stiffness cut off of 12 kPa, confirming it as the best non invasive method to identify cirrhosis.

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The following people have nothing to disclose: Raza Malik, Rory Farnan, Michelle Lai, Hannalisa Freitag, Detlef Schuppan

427 PROSPECTIVE STUDY OF NEOPLASTIC SEEDING IN PATIENTS UNDERGOING ULTRASOUND GUIDED PERCUTANEOUS DIAGNOSTIC OR THERAPEUTIC PROCEDURES OF HEPATIC TUMORS
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Neoplastic seeding along the needle track is a possible complication after percutaneous diagnostic and therapeutic procedures. In the retrospective studies the incidence of seeding of neoplastic cells after echoguided percutaneous biopsy ranges between 0.003 and 2%. Pancreatic neoplasms have a higher risk of neoplastic seeding. The risk increases using cutting needles. Percutaneous ablation treatments, like percutaneous ethanol injection (PEI) and radiofrequency thermal ablation (RFA), are safe and effective techniques to treat non resectable hepatic tumors. The referred risk of percutaneous seeding of neoplastic cells is 0.66-6.2% for PEI, 0.6% for RF. Aim of the study was to give a prospective evaluation of seeding occurrence in patients undergoing echoguided percutaneous diagnostic and therapeutic procedures. We included the patients undergone to fine needle biopsy (FNB) or ablation therapies for neoplastic lesions of liver; for each patient the needle entry point was marked on the skin with a tattoo. The seeding has been then searched with quarterly ultrasound examinations. From January 2001 to January 2005 we studied 270 patients, undergoing 574 procedures. On the whole 368 hepatic nodules were subjected to ultrasound-guided percutaneous procedures. During this period a total of 264 diagnostic fine needle biopsies have been performed: 164 in nodules detected in cirrhotic patients (97 cytological and 67 histological examinations), 100 in liver metastasis (64 cytological and 36 histological examinations). 310 ablation therapies have been
performed in 255 hepatic nodules (246 HCCs and 9 metastasis) detected in 149 patients. We have observed 3 cases of seeding in the studied population (1.1%). The first case was observed in a patient with HCC after FNB. The other 2 cases occurred in 2 patients with HCC after FNB and PEI. This first prospective longitudinal study about incidence of neoplastic seeding after hepatic echo-guided diagnostic or therapeutic procedures, has demonstrated a low rate of the complication. The contemporary emergence of the seeded nodules with recurrence of the neoplasms in other sites, doesn’t suggest an influence of this complication on the patients prognosis.

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428 CORRELATION OF LAPAROSCOPIC LIVER BIOPSY TO ELASTICITY MEASUREMENTS (FIBROSCAN®) IN PATIENTS WITH CHRONIC LIVER DISEASE

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Background: Transient elastography is a noninvasive method to assess liver fibrosis by measuring liver stiffness. Studies have compared elastography to percutaneous liver biopsy. Laparoscopic liver biopsy is associated with decreased sampling error since biopsies are obtained from both lobes and the presence of gross nodularity can be directly visualized. Aim: To assess the relationship between liver elasticity (E) measured with shear elasticity probe (FibroScan®) and liver fibrosis (F) and activity (A) assessed by histopathological and laparoscopic findings. Methods: Patients undergoing laparoscopic liver biopsy were enrolled. Gross appearance of the liver was assessed. Transient elastography (FibroScan®, Echosens) was used to measure liver stiffness. Spearman correlation analysis, multiple regression analysis and receiver operating characteristics (ROC) curve were used to analyze the data. Results: 95 patients were included, 50 +/− 9.4 years old (48% male). The indication for liver biopsy was HCV (64%), elevated enzymes (11%), NAFLD (9%), HBV (7%), AIH (4%), and other (5%). The distribution of fibrosis was F0 12.6%, F1 8.4%, F2 34%, F3 25%, F4 20% and activity G0 3%, G1 11.6%, G2 23.2%, G3 37%, G4 25.3%. E was normalized using a decimal logarithmic transformation (logE). In the correlation analysis, F was related to the E (r = 0.65; p < 0.0001), as was A (r = 0.46, p < 0.0001). A was also related to F. In a multiple regression analysis of logE, activity and fibrosis, E was strongly associated with advanced stages of F (stage 3 and 4) (r = 0.42; p < 0.001). The ROC curve for E to identify patients with F ≥ 3 and F4 had an area under the curve (AUC) of 0.84 and 0.86 respectively. For activity G2 ≥ 3 and G4 the AUC was 0.74 and 0.71. The table demonstrates the performance of various cutoffs of E. Conclusions: Shear Velocity (E) increases with liver fibrosis (F). The stage of fibrosis was associated with the grade of activity and must be considered in evaluation of elasticity to predict fibrosis. A cutoff of 11.9 kPa has moderate sensitivity and high specificity to identify patients with F ≥ 3. FibroScan® could be a useful noninvasive tool to detect advanced stages of fibrosis. Different models should be considered for different etiologies of liver disease.

<table>
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<th>Specificity(%)</th>
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429 COMPUTATIONAL FLOW DYNAMICS SIMULATION OF PORTAL BRANCHES IS USEFUL FOR ANALYZING THE MECHANISM OF NON-UNIFORM REGIONAL LIVER REGENERATION AFTER SURGICAL RESECTION

Yuju Iimuro, Shinichi Saito, Junichi Yamanaka, Tadamichi Hirano, Nobukazu Kuroda, Toshihiro Okada, Takaaki Sugimoto, Yasukane Asano, Naoki Uyama, Yugo Udo, Jiro Fujimoto; Surgery, Hyogo College of Medicine, Nishinomiya, Japan

Portal blood flow has been implicated in a critical factor regulating liver regeneration after surgical resection, while there has been no definitive evidence supporting this hypothesis. Whether the remnant liver regenerates uniformly or some specific region of the remnant liver preferentially regenerates is also unknown. We hypothesized that changes in blood flow in each portal branch regulate the regional regeneration, and tested this hypothesis employing computational simulation systems.

Methods: Out of 116 HCC patients who received hepatic resection during recent three years, 13 patients who underwent anterior or posterior segmentectomy and 7 patients who received lateral segmentectomy were analyzed. According to preoperative MD-CT, 3-D structure of portal branches was constructed, and each segment volume was accurately calculated using 3-D virtual hepatectomy simulation software (Hitachi Image Processing System) as we reported before (Hepatology 2005). Same procedure was performed 3 months after the operation, and regeneration volume in each segment was determined. Direct measurement of prompt changes in blood flow in each portal branch after surgery is technically difficult, so we used computational flow dynamics (CFD) software (Fluent 6.2, Fluent Inc.) for flow simulation. For the CFD analysis, mesh models of portal branches were constructed using a 3-D image processing and editing software (Mimics, Materialise). On the Fluent 6.2, changes in blood flow in the remnant portal branches were simulated by virtual cutting of an interested portal branch, and correlation between those changes and regeneration volume was investigated. Results: Actual hepatic resection volumes well correlated with the simulated volumes (r2 = 0.96), implying the accuracy of the virtual hepatectomy simulation software. In patients with right-side hepatectomy, degree of liver regeneration during 3 months was significantly greater in the right lobe compared with the left lobe (p < 0.05). On the other hand, liver regeneration was significantly greater in the left lobe when lateral segmentectomy was performed (p < 0.05). According to the CFD software, the increase in blood flow after virtual portal resection was significantly greater in a portal branch adjacent to the resected branch, and the regional regeneration volume tended to be greater in that portal branch area. These data possibly account for the greater regeneration volume in the resection-side of the remnant liver. Conclusions: Liver regeneration after surgical resection does not uniformly
According to genotype/phenotype criteria, hepatocellular adenomas (HCA) have been classified into four subgroups: HNF1α-mutated, inflammatory/telangiectatic, β-catenin mutated (half being also inflammatory/telangiectatic), and a group without any specificity. In this study we analysed retrospectively MRI of HCA before resection and classified using immunohistochemistry (IHC) [Hepatology 2007 in press]: LFABP negativity for HNF1α-mutated HCA (14 cases); SAA positivity for inflammatory/telangiectatic HCA (18 cases, 3 cases being also β-catenin mutated), β-catenin and glutamine synthetase overexpression for β-catenin activated HCA (1 case) and 5 unclassified cases. MRI were reviewed in consensus by two abdominal radiologists, without knowledge of IHC results. HNF1α HCA: most lesions had an homogeneous aspect on all sequences. The signal intensity on T1 weighted sequences was iso or slightly hyperintense with homogeneous signal dropout with fat-suppression sequences. The signal intensity was iso or slightly hyperintense on T2 weighted sequences and most lesions showed moderate enhancement on arterial, portal and delayed phases after gadolinium administration. Inflammatory/telangiectatic HCA: signal intensity on T1 weighted sequences was homogeneous with iso or slightly hyperintensity and no signal dropout with fat suppression sequence. Heterogeneous aspect was frequent on T2 weighted sequences but all lesions presented a markedly hyperintense component. All lesions showed a strong arterial enhancement with persistant enhancement on delayed phase. Good correlation was found between high signal area on T2 weighted sequence, delayed enhancement and peliotic zones on pathologic examination. The three lesions with both SAA expression and β-catenin activation showed the same MRI features. β-catenin mutated HCA: part of the lesion looked like inflammatory/telangiectatic lesion with high hyperintensity on T2 weighted sequence and delayed enhancement after gadolinium administration. Rest of the lesion was isointense on T2 weighted sequence and slightly hypointense on T1 weighted sequence with no signal dropout on fat suppression sequence. Unclassified HCA: inflammatory/telangiectatic MRI features were found in two lesions. The three others had characteristics of benign hepatocellular nodules with no specific MRI feature and among them, two had intratumoral hemorrhagic zones. Conclusion: The two main subgroups of HCA are associated with specific MRI patterns dependent on homogeneous steatosis for HNF1α-mutated HCA and peliotic component for inflammatory/telangiectatic HCA. No specific MRI feature was found to be associated with β-catenin mutated HCA.
The diagnosis of FNH is made using imaging techniques in 70% of the cases. A role for liver biopsy can become essential in FNH when imaging cannot establish firmly the diagnosis. However, in some cases of FNH histopathological diagnosis remains difficult, even in a resected specimen. Recently it has been shown that glutamine synthase (GS) was a useful marker in tumoral pathology including HCA (Hepatology, in press) and nodules from cirrhotic patients (Hepatology, 2007;45:725-34). The aim of the current study is to use GS as a marker in our practice to evaluate its ability to differentiate FNH from HCA or even hepatocellular carcinoma (HCC). FNH resected from normal or occasionally steatotic livers were retrieved from our collection. Seven groups were analysed as a: 15 cases with a firm preoperative diagnosis; b: 5 cases without a firm preoperative diagnosis (FNH or HCA or HCC); c: 2 cases with a preoperative diagnosis of HCA; d: 5 cases occurring in men with a suspicion of HCC; e: 10 cases associated with HCA (single, multiple or adenomatosis); f: 3 cases in patients with a past history of malignant tumors and g: 10 HCC on non cirrhotic liver, 5 expressing b-catenin. For all cases several blocks were available. Sections were stained using H&E, trichrome, reticuline, PAS, perls, and were immunolabelled for CK7 and 19, and GS. The diagnosis of FNH was established based on classical pathological standards. In case of uncertainty, additional immunostainings included IFABP, SAA and b-catenin. Controls included group e and g. In non tumoral liver, GS staining was strictly limited to 1 or 2 hepaticoportal plates around hepatic veins. In all FNH groups (a-f) GS staining was identical, intense, and included group e and g. In non tumoral liver, GS staining was never detected in all FNH samples tested. In b-catenin mutated HCA and HCC, GS was detected but its labelling was more diffuse, without the “map-like” pattern characteristic of FNH. In non b-catenin mutated HCA, GS was occasionally detected at the periphery and around some hepatic veins inside the tumor. Conclusion. In FNH, typical or not, independent of the size and its possible steatotic content, GS gave the same typical pattern and consequently represents the best marker to date which discriminates FNH from other hepatocellular nodules. The usefulness of this marker in liver biopsy needs however to be confirmed. The reason why GS immunostaining is increased in FNH remains presently unknown.

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IMPACT OF PREOPERATIVE VIRTUAL SEGMENTAL VOLUME ON LIVER RESECTION FOR HEPATOCELLULAR CARCINOMA IN PATIENTS WITH IMPAIRED LIVER FUNCTION

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BACKGROUND & AIM: Liver resection offers a potential cure for hepatic malignancies when the resection margin is negative. In cases of hepatocellular carcinoma (HCC), however, impaired hepatic function due to accompanying chronic liver disease restricts liver resection volume. Hence, accurate assessment of liver resection volume and resection margin is mandatory in preoperative planning for safe and curative hepatectomy. We have reported usefulness of virtual hepatic resection via three-dimensional (3D) image analysis software based on hepatic circulation (Hepatology, 2005). The aim of this study was to assess the feasibility and accuracy of multidetector CT scan based simulation in patients with impaired liver function undergoing hepatectomy for HCC. PATIENTS & METHODS: Hepatectomy simulation software was programmed to reconstruct detailed 3D vascular structure and to calculate liver volume based on portal vein perfusion. In 151 patients with HCC, liver resection volume was estimated preoperatively by both simulation and conventional planimetry. For validation, predicted resection volumes were compared with actual resected specimen weights. The resection margin as estimated by the simulation was compared with the margin in the specimen. RESULTS: Of the 151 patients enrolled in the study, 148 (98%) had complete simulation data. These patients showed impaired hepatic function with mean indocyanine green retention rate at 15 min (ICG R15) of 20%. Histology of the liver parenchyma showed bridging fibrosis or cirrhosis in 79%. Impaired hepatic function resulted in segmental or more limited resection in 52% of patients. No patients died within 30 days of resection. One patient deceased on postoperative day 67 due to postoperative liver failure and the mortality rate was 0.7%. There were no major complications requiring additional surgery. Minor complications such as ascites, pleural effusion, or bile leakage occurred transiently and were managed conservatively. Simulation showed higher correlation and smaller discrepancy (r=0.96; 9.3 mL) between predicted and actual liver resection volume than conventional planimetry (r=0.74; 174 mL). Simulation showed correlation (P < 0.01) between estimated and actual segmental volume, which was not measurable by planimetry. Simulation showed a correlation (r = 0.84) between predicted and actual margin, with a difference of 1.6 mm. CONCLUSIONS: Hepatectomy simulation in 3D predicted segmental liver volume and the resection margin accurately. This virtual method should contribute to preoperative planning to achieve safe and curative resection in HCC patients, whose hepatic function is compromised.

Disclosures: The following people have nothing to disclose: Junichi Yamanaka, Shinichi Saito, Yuji limuro, Tadamichi Hirano, Nobukazu Kuroda, Toshihiro Okada, Takaaki Sugimoto, Yasukane Asano, Naoki Uyama, Jiro Fujimoto
434 ASSESSMENT OF HEPATIC FUNCTIONAL RESERVE USING FUNCTIONAL LIVER MR IMAGING: CORRELATION WITH LABORATORY AND PATHOLOGIC FINDINGS IN ANIMAL MODEL

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Background and Aim: Surgical intervention in patients with abnormal liver function carries a higher morbidity and mortality. It is crucial to assess the risk before operation. However, there is no single reliable method for measuring hepatic functional reserve (HFR) until now. Thus, this study aimed to evaluate the reliability of functional liver imaging (FLI) in measuring HFR.

Materials and Methods: The FLI is a method of measuring HFR by calculating the percentage of functional volume that consisted of viable hepatocytes in a certain liver, compared with that in normal one using Mn-DPDP enhanced MR imaging and volumetry. We evaluated the change of HFR using serial FLI before and after acute hepatic necrosis (AHN) in the liver of 15 beagle dogs. AHN was induced by intraperitoneal injection of carbon tetrachloride. The MR images were obtained with 1.5T GE Signa Horizon system using EFGRE-3D sequence (TR/TE = 4.7/1.1 msec; FA, 20.0°) and torso-coil. The post-processing of the FLI was performed at a SUN Sparc workstation. All of the subjects were sacrificed immediately after the acquisition of second FLI, and percent of viable hepatocytes in pathologic specimens was determined using image analyzer. The correlation between the change of HFR measured with FLI (HFR-FLI) and the parameters of liver function tests (AST, ALT, albumin, bilirubin, prothrombin time), and the retention rate of indocyanine green 15 minutes after administration (ICG-R15) before and after induction of AHN were assessed. The correlation between the enhancement rate in the livers with AHN compared with that in corresponding normal ones (R(E AHN/ENL)) and the percent of viable hepatocytes in pathologic specimens was also determined. Results: The mean ± standard deviation of HFR-FLI in the livers with AHN was 59.7 ± 9.0 % in comparison with that of the normal ones. The change of HFR-FLI was well correlated with that of all the laboratory findings (serum level of AST, ALT, albumin, bilirubin, prothrombin time) and ICG-R15 (β = -0.858 or > 0.959, p < 0.05). The R(E AHN/ENL) showed strong positive correlation with percent of viable hepatocytes in pathologic specimens (β = 0.982, p < 0.05). Conclusion: The change of HFR-FLI was well correlated with that of the findings of laboratory liver function tests, and the R(E AHN/ENL) well represented the percent of viable hepatocytes validated by pathologic examination. Therefore, we suggest that the FLI could be used as a reliable method for measuring HFR.

Correlation (β) between HFR-FLI and Liver Function Profiles

<table>
<thead>
<tr>
<th>AST</th>
<th>ALT</th>
<th>Albumin</th>
<th>Bilirubin</th>
<th>Prothrombin time</th>
<th>ICG-R15</th>
</tr>
</thead>
<tbody>
<tr>
<td>HFR FLI</td>
<td>β = 0.963 *</td>
<td>β = 0.989 *</td>
<td>β = 0.949 *</td>
<td>β = 0.959 *</td>
<td>β = 0.858 *</td>
</tr>
</tbody>
</table>

* p < 0.05

Disclosures:
The following people have nothing to disclose: Jin-Woo Lee, Soon Gu Cho, Jung Il Lee, Seok Jeong, Kye Sook Kwon, Don Haeng Lee, Hyung Gil Kim, Yong Woon Shin, Young Soo Kim, Eun Joo Kim, Hyun-Joo Park, In-Sea Park

435 ULTRASONOGRAPHIC ANALYSIS OF HAVMS IN A LARGE COHORT OF HEREDITARY HEMORRHAGIC TELANGIECTASIA PATIENTS: DEFINITION OF A NEW HIGHLY-ACCURATE INTRAHEPATIC PARAMETER

Patrizia Suppressa1, Paolo Buonamico1, Giovanna Pasculli1, Gennaro M. Lenato1, Franco D'Ovidio2, Maurizio Memeo1, Luigi Castorani1, Carlo Sabbas1; 1Internal Medicine and Public Health, University of Bari, Italy, Bari, Italy; 2Statistics, University of Bari, Italy, Bari, Italy

Background: Hereditary hemorrhagic telangiectasia (HHT) is a rare genetic disorder (1:5000) whose diagnosis is based on 3/4 of the following criteria: familiar history, epistaxis, mucocutaneous telangiectases, visceral arteriovenous malformations. Hepatic arteriovenous malformations (HAVMs) have been found in more than 70% HHT patients by multislice CT (MSCT). Due to its noninvasivity and low cost, echo-color Doppler has recently been considered a valuable tool for long-term of HHT-related HAVMs; however, only open studies of selected patient cohorts using mainly extrahepatic ultrasonographic criteria are currently available. Aim of Study: To perform a blind controlled study with echo-color Doppler as a screening procedure for HAVMs in a large cohort of unselected HHT patients for investigation of the accuracy of “color spots” (a new intrahepatic echo-color Doppler parameter) and to compare its accuracy together with that of previously-reported extrahepatic ultrasound criteria (1) for diagnosis of HAVMs. Methods: 153 HHT patients (80 males and 73 females, mean age 47±15, range 15-75 yrs) were systematically screened with abdominal MSCT (considered as the gold standard) and echo-color Doppler (HDIL-9ATL System) by two independent operators to evaluate the presence of HAVMs. Both intrahepatic parameters (“color spots” with high velocity and low resistance index) and extrahepatic parameters (diameter, flow velocity and tortuosity of hepatic artery and portal vein) were utilized. The sensitivity and specificity for both classes of criteria were assessed (Cramer’s V index). Results: MSCT was positive in 128/153 (84%) HHT patients. Doppler color spots were found in 130/153 patients (see Table). The sensitivity, specificity and diagnostic accuracy of color spots compared to MSCT were 95%, 68% and 91%, respectively, and the “color spot criterion” showed a greater correlation to MSCT (Vindex = 0.655; p<0.0001) than the extrhepatic criteria (V = 0.39) (1). Conclusions: A new echo-color Doppler intrahepatic parameter (“color spot”) was identified showing an optimal accuracy for detecting HAVMs in HHT, thus permitting a non-invasive, ideal strategy for periodical screening of HHT patients. These data support the superiority of intrahepatic compared to extrahepatic criteria for identification of HAVMs. 1) Caselitz et al. Hepatology 2003;37:1139-46.

<table>
<thead>
<tr>
<th>Patients</th>
<th>Presence of Spots</th>
<th>Extrhepatic criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>Negative</td>
<td>Positive</td>
</tr>
<tr>
<td>MSCT+</td>
<td>128</td>
<td>122 (true positive)</td>
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<tr>
<td>MSCT-</td>
<td>25</td>
<td>8 (false positive)</td>
</tr>
<tr>
<td>Total</td>
<td>153</td>
<td>130</td>
</tr>
</tbody>
</table>

Disclosures:
The following people have nothing to disclose: Patrizia Suppressa, Paolo Buonamico, Giovanna Pasculli, Gennaro M. Lenato, Franco D’Ovidio, Maurizio Memeo, Luigi Castorani, Carlo Saba
Efficacy of Positron Emission Tomography CT for Metastatic Hepatocellular Carcinomas

Tomokazu Kawaoka, Hiroshi Aikata, Shoichi Takahashi, Kazuaki Chayama; Graduate School of Biomedical Science Hiroshima University, Hiroshima, Japan

Background/Aim: Since the patients suffering from hepatocellular carcinoma (HCC) live longer than before, thanks to improvement of diagnostic and therapeutic modality, the patients who possess remote metastasis of HCC are increasing. This study aimed to evaluate the efficacy of Positron Emission Tomography (PET) CT as a detection tool for metastatic HCCs compared with MDCT and bone scintigraphy. This included visual correlations of PET-CT and MDCT imaging in the detection of HCC using receiver operating characteristic (ROC) analysis. Materials and Methods: Thirty patients who have metastatic HCC at Hiroshima university hospital were enrolled in this study from Jan. 2005 to Jan. 2007. Those patients had 128 metastatic nodules, among which lung metastases were 80, bone metastasis were 29 and lymph node metastasis were 19 cases. In terms of tumor size, median size of lung metastasis was 6 mm (2-33 mm) in diameter, that of bone metastasis was 18mm (8-120 mm) and that of lymph node metastasis was 30mm (15-67mm). For the ROC analysis, 128 nodules were diagnosed as metastatic HCC by one experienced abdominal radiologists. And same numbers of segments that did not include HCC were prepared as negative image. Another three physicians independently reviewed both positive and negative images. Each physician read two or three sets of images such as MDCT, PET-CT and bone scintigraphy for bone metastasis. Each reader scored each image for the presence of metastatic HCC lesions and assigned confidence levels to his observations (1=definitely negative, 2=probably negative, 3=possibly positive, 4=probably positive, 5=definitely positive). Results: The mean sensitivity and the mean specificity were 94%, 81% with MDCT, 31%, 96% with PET-CT in lung metastasis, 41%, 93% with MDCT, 55%, 93% with PET-CT, 36%, 85% with bone scintigraphy in bone metastasis and 95%, 89% with MDCT, 89%, 68% with PET-CT in lymph node metastasis. The mean positive predictive values were 83%, 89% with MDCT or PET-CT in lung metastasis, 83%, 89% with MDCT, PET-CT or bone scintigraphy in bone metastasis and 90%, 74% with MDCT or PET-CT in lymph node metastasis, respectively. The mean Az values were 0.93, 0.49 with MDCT or PET-CT in lung metastasis, 0.72, 0.84, 0.68 with MDCT, PET-CT or bone scintigraphy in bone metastasis and 0.96, 0.65 with MDCT or PET-CT in lymph node metastasis, respectively. Conclusion: According to ROC analysis, MDCT was more useful for detection of lung and lymph node metastasis of HCC, while PET-CT was more useful for detection of bone metastasis of HCC. 

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Positron Emission Tomography (PET/CT) Using Fluoro-Choline Allows to Differentiate Between Adenoma and Focal Nodular Hyperplasia

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Background and aim: Despite the high performance of current imaging techniques, the differential diagnosis between hepatic adenoma and FNH could remain difficult in particular for nodules of small size, often inaccessible to the liver biopsy. PET/CT using F-18fluoro-2-D-glucose (FDG) is well established as a non-invasive diagnostic tool for the detection of a variety of malignant tumours. However, because of its low sensitivity, this technique is not used for the diagnosis of hepatocellular carcinoma (HCC). We have recently shown that F-18fluoro-methylcholine (FCH) was more sensitive than FDG for HCC detection. The aim of this study was to evaluate the uptake of FCH by hepatocellular benign tumours. Patients and Methods: 14 patients were prospectively explored for one or more nodules developed in a non cirrhotic liver and compatible with adenoma or focal nodular hyperplasia (FNH). The nodules were investigated by RMN and CT. An histological confirmation of the lesions was obtained in all patients except four because of the characteristic imaging features of FNH. Two PET/CT were carried out in each patient after injection of FDG and of FCH (4 MBq/kg of body weight) at four days of interval. The uptake of FCH and of FDG by the tumours was evaluated by the standardized uptake value (SUV). Results: 12 women and 2 men were included and presented with the following hepatic lesions: FNH (n=8), adenomas (n=4) including 2 adenomatosis, telangiectatic FNH (TFNH) (n=1) and association of a TPNF and a FNH (n=1). All FNH had an intense and early uptake of FCH (SUV: 7.86±1.4). This uptake gradually decreased at late time. In contrast, there was no FCH uptake by adenoma and TPNF. Concerning the patient presenting with TPNF and FNH, an intense uptake of FCH was only observed for the nodule having the features of FNH. None of these tumours (adenoma and FNH) showed an abnormal uptake of FDG. Conclusions: Our results show that the TEP/TDM using FCH is a sensitive and specific technique able to differentiate between FNH and adenoma. 

Disclosures: The following people have nothing to disclose: Franck Bumse, Virginie Huchet, Lionel Arrivé, Dominique Wendum, Francois Paye, Raoul Poupon, Jean-Noel Talbot, Olivier Rosmorduc

The Hepatic Transcriptome of HCV Infection and Interferon Treatment in Hepatocytes Recovered from Chimeric Mice

Donna Douglas, Gordon Broderick, Jamie Lewis, Yutaka Yasui, David Bond, Norman Kneteman; University of Alberta, Edmonton, AB, Canada

Differential viral loads and sensitivity to IFNα treatment occurs with HCV-1A and HCV-3A infections in chimeric mice (Kneteman, N.M., et al Hepatology, 2006. 43(6): p. 1346-53); an
outcome that parallels the clinical setting for the therapeutic administration of IFNα to chronically infected patients. The chimeric mouse model was used to study IFN-stimulated genes (ISGs) in the early innate immune response to HCV infection since it lacks an adaptive immune system. Human hepatocytes were recovered from HCV-1A and -3A infected mice (+/- IFNα treatment) and used for transcriptional profiling by microarray analysis. Hepatocytes from naive mice (+/- IFNα treatment) served as controls. IFNα treatment of naive mice was associated with a significant upregulation of the hepatic transcriptome (3.8% of 54,675 interrogations) and many of the upregulated transcripts were established ISGs but only 2 of these are known to have antiviral activities (GBP2 and IFNAR1). Also included were ISGs of unknown function (GIP3, IFI13, IFI27, IFI30, IFITM2). We observed very few genes associated with HCV infection, and none were found to be ISGs. Antecedent HCV infection had a negative impact on the induction of genes by administered IFN (from 3.8% to 0.2% and 3.3%, for 1A and 3A, respectively), including GBP2 and IFNAR1. Interestingly, antecedent HCV infection had no impact on unknown ISGs GIP3, IFI24 and IFI30. Thus far, we have identified the hepatic transcriptome induced by (i) by HCV-1A and -3A infection (ii) IFNα-2b monotherapy, and (iii) IFNα-2b monotherapy in the setting of HCV-1A and -3A infection.

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HEPATITIS C VIRUS CORE PROTEIN SELECTIVELY ACTIVATES CELL GROWTH FACTOR SIGNALING

Andrew Kim, Shigenobu Kawai, Waihong Chung, Eun Kim, Jack R. Wands, Jisu Li; Liver Research Center, Rhode Island Hospital and The Warren Alpert Medical School of Brown University, Providence, RI

Background and Aims: Primary hepatocellular carcinoma (HCC) is the fifth most common cancer worldwide. At least five growth factor signaling pathways have been implicated in the development and progression of HCC, including HGF, Wnt, IGF-II, TGFβ and TGFβ. Hepatitis C virus (HCV) is one of the principal etiological agents that dramatically increase the lifelong risk for HCC, and its core protein has been proposed to be a key contributing factor. To determine whether the HCV core protein modulated growth signaling pathways, we employed a cell culture system of inducible core protein expression (Huh-7/Tet-on) to investigate the transcriptional and functional changes of the signaling network. Methods: Gene transcriptional alterations in response to core protein expression were documented by microarray analysis. Activation of growth factor signaling was evaluated by reporter assay that monitored growth factors or downstream targets. The effect of growth factors on Huh-7 cell proliferation was examined by transient transfection of the cDNA constructs. Results: Microarray analysis of 39,000 human genes (Affymetrix U133) revealed that both Wnt and HGF signaling were activated as evidenced by marked up regulation of the ligands and downstream components of the pathway. In addition, the MAPK, Hedgehog, VEGF and PPAR signaling pathways were also found up regulated. In contrast, genes involved in the TGFβ, Notch, insulin signaling pathways were mostly down regulated. These data indicate that HCV core protein selectively up regulates certain growth signaling pathways. Since Wnt and HGF pathways have been implicated in >50% HCC cases, further experiments were performed to validate their contribution to cell growth. We found that overexpression of either Wnt-1 or HGF by transient transfection significantly increased Huh-7 cell proliferation, and the promoter activities of HGF and TCF (downstream target of the Wnt signaling) were marked increased by HCV core protein. Transfection with HGF or incubation of cells with HGF conditioned medium markedly increased TCF reporter gene expression, indicating crosstalk between HGF and Wnt signaling, with HGF signaling upstream of Wnt signaling. Conclusion: HCV core protein selectively up regulates the Wnt-1 and HGF growth signaling pathways. Interaction and crosstalk between these two pathways may contribute to the development of HCC.

Disclosures: The following people have nothing to disclose: Andrew Kim, Shigenobu Kawai, Waihong Chung, Eun Kim, Jack R. Wands, Jisu Li
441
APOLIPOPROTEIN C-IV EXPRESSION IS REGULATED BY KU ANTIGEN AND CORRELATES WITH TRIGLYCERIDE ACCUMULATION IN HCV INFECTED LIVER
Eun Kim, Jack R. Wands, Jisu Li; Liver Research Center, Rhode Island Hospital and The Warren Alpert Medical School of Brown University, Providence, RI

Background: Hepatic steatosis is frequently associated with persistent hepatitis C virus (HCV) infection. We recently found that HCV core protein upregulated transcription of genes related to fat/lipid metabolism, in particular apolipoprotein C-IV (ApoC-IV, ~10.7-fold increase). Sequence -122 to +41 (163bp) of the ApoC-IV promoter was defined as the core protein responsive element and Ku70/Ku80 complex (known as Ku antigen) was identified as the major nuclear proteins associated with the 5' end of this element. In the present study, we examined correlation between ApoC-IV expression and triglyceride accumulation in human liver of chronic HCV infection, and began to identify transcription factors important for regulation of ApoC-IV transcription. Methods: Nine HCV infected cirrhotic livers and four normal livers were compared for the levels of ApoC-IV mRNA using RT-real time PCR and triglyceride by enzymatic assay. The Ku70 coding sequence was amplified from Huh-7 cells by RT-PCR and cloned into the expression vector pcDNA3.1/Zeo+. The role of Ku70 overexpression in ApoC-IV promoter activity was examined by luciferase reporter assay. The transcription factors that bind to position -122 to -54 of the ApoC-IV promoter were predicted by TRANSFAC database search, and their role in ApoC-IV promoter activity was examined by site-directed mutagenesis of the 163-bp promoter. Results: The average ApoC-IV mRNA level was higher in HCV infected liver (6.2 ± 4.2) than in the control group (3.7 ± 2.6). Interestingly, the ApoC-IV transcript level strongly correlated with the concentration of intracellular triglycerides in the HCV infected liver (R² = 0.78), but not in the control group (R² = 0.57). Overexpression of Ku70 in Huh-7 cells increased steady-state level of Ku80 and also the ApoC-IV promoter activity, thus complementing our previous finding using Ku70 siRNA. Mutational analysis revealed that binding sites for transcription factors HNF4, C/EBPβ, and PPAR/RXR located at the 5' half [-122 to -54] of the 163-bp promoter were critical for core protein induced promoter activity. Conclusion: ApoC-IV may be the major regulator of triglyceride level in HCV infected cirrhotic liver, and it is controlled at the transcriptional level by Ku antigen, possibly through recruitment of other liver specific transcription factors. Further understanding of its regulation may help identify novel targets for intervention of hepatic steatosis.

Disclosures: The following people have nothing to disclose: Eun Kim, Jack R. Wands, Jisu Li

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ESCAPE IN AN HLA B8-RESTRICTED CD8 EPITOPE IN HCV NS3 IS ASSOCIATED WITH VIRAL FITNESS COSTS
Cesar Oniangue-Ndza1, Shadi Salloum1, Christoph Neumann-Haefelin2, Anna Eckart1, Robert Thimme2, Sergei Viazov1, Michael Roggendorf2, Joerg Timm1; 1Department of Virology and Lab Medicine, Tulane University of Health Sciences, New Orleans, LA; 2Virology II, National Institute of Infectious Diseases, Tokyo, Japan

Background: A convenient and reliable assay system is needed to be examined directly using fluorescence microscopy. Recently, a unique HCV clone derived from a Japanese FHF patient has been demonstrated to replicate efficiently in cell culture and produce infectious HCV particles. Aim: We sought to develop chimeric clones between green fluorescence protein (GFP) with full-length and subgenomic JFH-1 clones such that HCV replication and infection can be examined directly using fluorescence microscopy. Methods: Chimeric full-length (JFH-1) and replicon (pSGR-JFH-1) clones were prepared by inserting the GFP coding sequences in frame with the coding sequences of HCV NS5A protein of JFH-1 clone. HCV RNA transcripts were prepared from each clone by T7 RNA polymerase and transfected to Huh-7.5 cells by the electroporation method. The success of HCV transfection and replication was examined by developing stable replicon cell lines and in vitro infectivity of fluorescent virus particles using Huh-7.5 cells. Results: Both the replicon and full-length chimeric clone showed a clearly visible GFP expression of NS5A/GFP fusion protein that visualized by fluorescence microscopy and fusion protein can be detected by Western blot analysis. The replication of chimeric clones was confirmed by detecting positive strand HCV RNA by ribonuclease protein assay and the ability...
to form G418 cell colonies. We have now established several stable cell lines that support high-level replication of GFP-tagged sub-genomic HCV RNA. The level of viral replication between the wild type and chimeric clone is comparable. Huh-7.5 cells transfected with GFP tagged viral genomes produced infectious virus particles since expression of GFP was demonstrated in naive Huh-7.5 cells after virus infection. As a practical validation, we showed that replication and infectivity totally abolished by treatment with alpha interferon. Conclusion: We established a HCV cell culture system using GFP tagged viral genomic and sub-genomic clones that allow direct visualization of infected cells by fluorescence microscopy. These systems provide a powerful tool for the understanding of host-virus interactions and may provide a reliable model system to test varieties of anti-HCV substances. Acknowledgements: The work was supported by NIH, Louisiana Cancer Consortium and Tulane Cancer Center.

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444 ARREST OF NATURAL KILLER (NK) CELL DEVELOPMENT IN THE EARLY STAGES OF ACUTE HCV INFECTION MAY REPRESENT A DIRECT EFFECT OF HCV CORE PROTEIN

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Background: NKs recognize conserved structures that signal viral invasion, thus providing an important first line of defense. In humans, relative expression of CD56 identifies functionally distinct subsets (CD56bright CD56dim) of NKs. An increased proportion of immature CD56bright NKs has been reported in patients with chronic HCV infection, however, little is known of NKs in the acute setting. HCV proteins, including core, demonstrate immuno-modulatory properties, however, the effect of core on NK cells has not previously been investigated. Aim: To characterize NKs in the early and late stages of acute HCV infection and examine the effect of core protein on NK development and function. Methods: Treatment naïve acute HCV patients (n=22), including 10 with spontaneous recovery, were studied. Flow cytometry was used to phenotype NKs directly ex vivo and after culture. Functional readouts included natural cytotoxicity and cytokine production (IFN-γ). FACS-sorted CD56bright and CD56dim NK subsets from healthy controls (n = 5) were cultured with control βgal protein or in the presence of HCV core protein and stimulated with IL-15 to drive NK cell maturation. Results: Direct ex vivo analysis of patients with acute HCV demonstrated there was an increased proportion of CD56bright NKs early in acute HCV infection per se which returned to normal levels on resolution but remained elevated in chronically evolving infection (p=0.0037). Divergent NK CD16 expression in resolving compared to chronically evolving patients late in acute HCV infection (p=0.03) provides further evidence of maturational arrest. Natural cytotoxicity was reduced in both the early and late stages of infection and did not recover to normal levels after viral clearance (follow-up 6 months). HCV resolution was characterized by lower than normal TNF production (p=0.01) by NK cells whereas IFN-γ production was unaffected. In vitro culture with HCV core protein in normal subjects maintained a significant proportion of immature NKs in the CD56bright state, whereas cells cultured with control protein all achieved a CD56dim phenotype. Culture with core protein induced TNF production by effector NKs. Conclusions: Maturational arrest of NKs occurs early in acute HCV but is transient in patients who spontaneously resolve infection. HCV-core protein has an immunomodulatory effect on NK cells. The in vitro effect of core-mediated NK dysregulation closely correlates with NK characteristics measured directly ex vivo in the setting of acute infection. Our data suggest that arrest of NK development in HCV infection may be a direct effect of core protein and may affect the outcome of acute infection.

Disclosures:
The following people have nothing to disclose: Lucy Golden-Mason, Nicole Castelblanco, Young S Hahn, Hugo R. Rosen

445 HEPATITIS C VIRUS REPLICATION SENSITIZES HUMAN HEPATOMA CELLS TO TRAIL-INDUCED APOPTOSIS

Richard Fischer1, Sebastian Gorke1, Lin Lan1, Sibylle J. Rau1, Takaji Wakita2, Hubert E. Blum3, Mirjam Zeisel1, Thomas F. Baumert3; 1Department of Gastroenterology and Hepatology, University of Freiburg, Freiburg, Germany; 2Department of Virology II, National Institute of Infectious Diseases, Tokyo, Japan; 3Inserm U748, Université Louis Pasteur, Hopitaux Universitaires de Strasbourg, Strasbourg, France

Backgrounds and Aims: Several hepatitis C virus (HCV) proteins have been implicated to modulate apoptosis of HCV target cells. However, the molecular mechanisms leading to the death of hepatocytes during hepatitis C virus infection are only poorly understood. TRAIL, the TNF-related apoptosis-inducing ligand, has recently been implicated in the death of hepatocytes under inflammatory but not normal conditions. To determine the potential role of TRAIL in HCV-induced hepatitis and viral clearance, we studied the effect of HCV replication on TRAIL-induced apoptosis of human hepatoma cells. Methods: Huh7.5 cells were transfected with JFH-1, JFH-1/deltaE1E2, JFH-1/GND full-length and SGR/JFH-1 subgenomic HCV RNA. Apoptosis of Huh7.5 cells was studied by cleaved poly (ADP-ribose) polymerase-1 (PARP-1), caspase-8 and -9 activation and TUNEL assay. Results: Transfection of Huh7.5 cells with replication-competent JFH-1 but not with replication-deficient JFH-1/GND RNA resulted in apoptosis of Huh7.5 cells as shown by enhanced PARP cleavage, caspase activation and positive TUNEL staining. Following incubation with TRAIL, induction of apoptosis was markedly enhanced in Huh7.5 cells transfected with JFH-1 RNA compared to cells transfected with replication-deficient JFH-1/GND RNA or mock-transfected cells. A similar enhancement of TRAIL-mediated apoptosis was observed following transfection of hepatoma cells with JFH-1/deltaE1E2 and SGR/JFH-1 subgenomic HCV RNA suggesting that sensitization to TRAIL-induced apoptosis is independent of HCV structural protein expression but requires active viral replication. Conclusions: Our data suggest that HCV replication sensitizes hepatoma cells to TRAIL-induced apoptosis. These findings may have important implications for pathogenesis of hepatitis and mechanisms of viral clearance.

Disclosures:
The following people have nothing to disclose: Richard Fischer, Sebastian Gorke, Lin Lan, Sibylle J. Rau, Takaji Wakita, Hubert E. Blum, Mirjam Zeisel, Thomas F. Baumert
446 NEUTRALIZING ANTIBODY INDUCTION IN MONOTYPIC HEPATITIS C VIRUS INFECTIONS IN CHIMPANZEE DOES NOT CORRELATE WITH OUTCOME

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Recent publications have suggested a correlation between neutralizing antibody (NAb) to HCV and clearance of virus. In order to test NAb to HCV in the context of the complete viral particle we constructed a 1a/2a chimera virus containing the H77 (1a) coreE1E2 region and the JFH1 (2a) non-structural region. Following a lag phase we observed rapid spread of the virus in cells with CPE at d7 (MOI=0.1) or peak titers at d14, 3x10^4 ffu/mL (MOI=0.01). Using this chimera, we tested NAb using sequential acute phase plasma from monotypic H77-infected chimpanzees, rechallenged chimpanzees, and chimpanzees vaccinated with rE1E2 protein. The kinetics of NAb production showed weak increases during the acute phase (wks 6-10) mainly in animals that eventually developed persistent infections and substantial increases during the chronic phase (post wk 30, 90% neutralization) in these same animals, while the kinetics of NAb production fluctuated during the acute phase in cleared animals. In contrast, we observed decreases in neutralization using late chronic phase plasma (wk 150), this may indicate mutations in the circulating virus that no longer stimulate antibody able to recognize the original sequence. When we compared the average number of foci with untreated control in the acute phase by Hest, there was a significant neutralization of virus at a number of time points although inhibition was <50%. This was observed at an earlier time point in the cleared animals than in the persistently infected group, but there was an absence of higher levels of NAb production in the cleared animals during the acute phase. In addition, we found that NAb does not play a role in controlling HCV during re-exposure to the virus after clearance, as no substantial levels of NAb could be detected in plasma from 3/3 animals rechallenged with monotypic virus. Vaccination of chimpanzees with rE1E2 protein elicited NAb (titers >1:1000). However, although this antibody recognized a highly conserved neutralizing antibody epitope (amino acids 412-419) it did not exhibit substantial levels of cross neutralization using the 2a genotype virus indicating that reactivity to this epitope alone is not an indicator of broad neutralizing ability. In conclusion, our data suggest that these antibodies do not play an important role in determining the outcome of infection.

Disclosures:
The following people have nothing to disclose: Hisayoshi Watanabe, Frances Wells, Pei Zhang, Marian E. Major

447 SOCS-3 EXPRESSION AND HCV RELATED CHRONIC HEPATITIS: INSULIN RESISTANCE AND RESPONSE TO ANTIVIRAL THERAPY

Marcello Persico1, Mario Capasso2, Eliana Persico1, Monica Svelto1, Roberta Russo2, Daniela Spano2, Lori Crocè2, Vincenzo La Mura1, Francesco Maschella1, Flora Masutti1, Roberto Torella1, Claudio Tiribelli1, Achille Iolascon2

The response to antiviral therapy is lower in HCV patients with genotype 1 than in those with genotype 2. Overexpression of the suppressor cytokine signalling 3 (SOCS-3) gene in liver tissue was associated to a poorer treatment outcome in patients with chronic hepatitis C, viral genotype 1. Also insulin resistance has been implicated in non-response to anti-HCV treatment. To understand why HCV genotype 1 patients respond differently, we investigated SOCS-3 gene expression, the metabolic syndrome (MS) and the response to therapy in a cohort of patients with HCV-related hepatitis. A total of 198 patients (108 with genotype 1, and 90 with genotype 2) treated with pegylated-interferon plus ribavirin were consecutively enrolled in the study. We measured SOCS-3 expression in Epstein-Barr-virus-transformed lymphoblastoid cell lines derived from peripheral lymphocytes of a subset of 130 patients. The MS was more frequent in genotype-1 patients than in genotype-2 patients (p<0.01). Non responders (NR) (p<0.01), MS (p<0.001) and genotype 1 (p<0.001) were significantly related to SOCS-3 over-expression. However, SOCS-3 levels were higher in NR also irrespective of genotype (p<0.01). At univariate analysis, genotype (p<0.001), age (p<0.001), SOCS-3 (p<0.001) and MS (p<0.001) were significantly related to response to therapy. However, at multivariate analysis, SOCS-3 was the only independent predictor of response (OR: 6.7; p<0.005). Conclusions: We speculate that SOCS-3 expression per se may influence the response to antiviral therapy and that genotype 1b virus might induce its up-regulation. This may account for the different response to therapy between genotype-1 and genotype-2 infected patients.

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448 COMPREHENSIVE ANALYSES OF HCV-RELATED CELL SIGNALING PATHWAYS USING CELLS WITH OR WITHOUT EXCLUSION OF HCV REPLICON AND THEIR CURED CELLS

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Background: HCV replication is closely associated with several host factors. In this study, we have analyzed cellular effects on HCV replication and host genes that may regulate the virus replication by comprehensive gene expression analyses in two types of replicon cells, their cured cells and the parental HuH7 cells. Material and Method: Chimeric gene of YFP and neomycin phosphotransferase (Yeo) was inserted into HCV-1b and -2a replicon, and HuH7 cell lines that express the Yeorepli- con were established. YFP expression was detected by fluorescence microscopy and by FACS analysis. The cured cell lines, which are highly permissive for replicon expression, were established from the replicon cell lines by eliminating replicon by interferon-alpha treatment. The high YFP-replicon expressing cells were separated using FACS Vantage SE cell sorting system. 54,461 gene expression profiles of those cell populations were analyzed by DNA microarray (Gene Chip, Affimetrix). And we conducted a comprehensive molecular pathway analyses using our gene expression data by using a functional mapping tool, MetaCore (GeneGo). Results: Comparison of replicon cells with cured cells showed that 83 and 196 genes were up- and down-regulated in replicon cells (>2-fold and <0.5 fold). Comparison of cured cells with parental HuH7 cells showed that 335 and 181 genes were up- or down-regulated in cured cells. In molecular network analyses, replicon cells showed activation of NF-kappa B, AP-1, PPAR-alpha-related
449 INFECTION OF PRIMARY HUMAN HEPATOCYTES WITH RECOMBINANT HEPATITIS C VIRUS

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Attachment of the virus to the cell surface followed by viral entry is the first step in a cascade of events required for initiation of infection. Results obtained with surrogate model systems for HCV infection using human hepatoma cell lines suggest that HCV entry is most likely a multi-step process which requires a set of initial binding molecules such as glycosaminoglycans, as well as (co)-receptors for viral entry such as CD81, SR-BI and claudin-1. However, it still remains elusive which host cell factors are required for HCV entry into its natural target cell, the human hepatocyte. In this study, we used the recently established HCV/JFH-1 based-infectious tissue culture model system to generate high titer virion preparations (HCVcc) for infection of primary human hepatocytes (PHH). Cells were incubated with cell culture adapted JFH-1 with proven in vivo viability in the presence or absence of an NS3 protease inhibitor. Infection was assessed by measuring HCV RNA by quantitative RT-PCR at different time-points post infection. PHH could be reproducibly infected with HCVcc as shown by a time-dependent increase of HCV RNA in infected hepatocytes which was not observed when cells were treated with the NS3 protease inhibitor. Studies to assess the impact of CD81, SR-BI and claudin-1 as host entry factor for HCV infection of primary human hepatocytes are ongoing. In conclusion, our results suggest that PHH can be infected with cell culture grown HCV. This model system will allow further dissecting molecular mechanisms of HCV infection of its natural target cell - the human hepatocyte.

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450 AN ASSESSMENT OF THE ROLE OF SREBP1C IN THE PATHOGENESIS OF HCV-RELATED STEATOSIS

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Background: Hepatic steatosis occurs in 50% of subjects with hepatitis C virus (HCV) infection and is a risk factor for more rapid disease progression. Both metabolic and viral factors are involved in the pathogenesis of HCV-associated steatosis, which remains incompletely understood. Sterol regulatory element binding protein 1c (SREBP1c) is a transcription factor that regulates the expression of lipogenic genes, increasing fatty acid and triglyceride synthesis. SREBP1c is elevated in the fatty liver of obese, insulin resistant mice and has been implicated in the pathogenesis of hepatic steatosis. The role of SREBP1c in the pathogenesis of HCV-related steatosis is unknown. Aim: To investigate the role of SREBP1c in the pathogenesis of HCV-related steatosis. Methods: Expression of SREBP1c mRNA was measured by real-time (RT)-PCR in liver biopsy samples from 123 patients with chronic HCV (80 male), 12 with chronic Hepatitis B (HBV) (3 male) and 14 HCV/HBV-negative subjects with histologically normal liver (NDL). Fasting serum was collected at the time of liver biopsy and assessed for levels of insulin and glucose. Results: Increased hepatic expression of SREBP1c was seen in patients with chronic HCV (p=0.007) and chronic HBV (p=0.002) compared with NDL. There was no difference in SREBP1c expression between subjects with HCV and HBV (p=0.11). In the patients with HCV, a statistically significant negative correlation was seen between hepatic SREBP1c expression and stage of fibrosis (r=-0.375, p<0.001), severity of hepatic inflammation (r=-0.313, p<0.001) and grade of steatosis (r=-0.28, p=0.002). There was a trend towards increased hepatic SREBP1c expression in subjects with HCV genotype 1 compared to genotype 3 (p=0.06). Overall in subjects with HCV, no relationship was seen between hepatic SREBP1c expression and BMI, HOMA score, or alcohol intake. Following multivariate analysis (adjusting for age, gender, viral genotype, BMI, HOMA, fibrosis, steatosis and hepatic inflammatory score), hepatic SREBP1c expression remained independently associated with fibrosis (p=0.003), viral genotype (p=0.006) and hepatic inflammation (p=0.01). Conclusions: Hepatic expression of SREBP1c was elevated in subjects with chronic HCV and chronic HBV compared with HCV/HBV-negative control subjects. However, unexpectedly, increasing grade of steatosis and stage of fibrosis was associated with a decrease in the expression of SREBP1c, suggesting that it may not play a prominent role in the pathogenesis of hepatic steatosis in HCV.

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IN Volvement OF IL-7 AND Thymic Stromal Lymphopoietin IN Functional Impairment OF Myeloid Dendritic Cells IN Chronic Hepatitis C Virus Infection

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BACKGROUND AND AIMS: It has been reported that impaired functions of CD4 or CD8 T cells or dendritic cells (DC) are involved in HCV persistence, however, the precise mechanisms remains unclear. IL-7 is a lymphopoietic cytokine that acts on differentiation and maintenance of activity of lymphocytes or DC. Lesser expression of IL-7R on T cells has been reported in acute hepatitis C patients who eventually progressed to chronicity, suggesting the critical role of IL-7/IL-7R system in HCV infection. Thymic stromal lymphopoietin (TSLP), an IL-7-like hematopoietic cytokine, is a potent activator of myeloid DC (MDC) and activated CD4 T cells. The involvement of TSLP in allergic diseases has been reported, however, its role in HCV infection is poorly understood. To elucidate the role of IL-7/TSLP in chronic hepatitis C (CHC), we examined serum levels of these cytokines and their influences on the function of blood MDC. SUBJECTS AND METHODS: Sixty CHC patients and age-matched 60 healthy volunteers (HV) were enrolled in this study. Plasma levels of IL-7 and TSLP were measured by ELISA. MDC were magnetically separated from PBMC and were cultured in the presence of IL-7 (IL-7-DC), TSLP (TSLP-DC), or medium alone (med-DC). After 24h, we compared their phenotypes and stimulatory capacity of naive CD4 T cells between the groups. The ability of MDC to induce Th1 or Th2 was analyzed by cytokines released from DC-primed T cells. RESULTS: The levels of IL-7 and TSLP are lower in CHC compared with those in HV (p<0.05, respectively). In some CHC patients who cleared HCV by 48 weeks of peg-IFN-α and ribavirin combination therapy, TSLP levels elevated to the normal range after the completion of therapy, suggesting that HCV is involved in lower level of TSLP. In contrast, such restoration by HCV clearance was not observed in IL-7 levels. Both in CHC patients and HV, IL-7- and TSLP-DC displayed higher expression of CD86 and CD80 compared with med-DC (p<0.05, respectively). In HV group, IL-7- or TSLP-DC were more capable of CD4 T cell proliferation than med-DC. In CHC patients, IL-7-DC ability to stimulate T cell proliferation was less than that of IL-7-DC from HV or TSLP-DC either from CHC or HV (p<0.05), suggesting that MDC from CHC have lesser capacity of responding to IL-7. In DC ability to induce Th1/Th2 response, IL-7-DC from CHC primed T cells to produce more IL-13 than those from HV (p<0.05), while there was no difference in IL-13 release from TSLP-DC-primed T cells between the groups. CONCLUSION: Low level of IL-7/TSLP and lesser Th2-prone ability of MDC to stimulate CD4 T cells in response to IL-7 may be involved in functional impairment of MDC in HCV infection.

INSULIN RESISTANCE IN CHRONIC HEPATITIS C: A PROSPECTIVE STUDY SHOWING AN ASSOCIATION OF INSULIN RESISTANCE WITH GENOTYPES 1 AND 4, VIRAL REPLICATION AND LIVER FIBROSIS

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Background and Aims: There is growing evidence of an association between chronic hepatitis C and insulin resistance. However, the influence of viral factors such as HCV genotypes and viral replication level has not been well assessed. Another major issue is the relationship between liver fibrosis and insulin resistance. While this association was well demonstrated in non-alcoholic fatty liver disease, it remains controversial in chronic hepatitis C. The aim of this study was to analyze the association between insulin resistance, and HCV genotypes, viral replication level and liver fibrosis stage in a large prospective cohort of patients with chronic hepatitis C. Patients and Methods: 600 consecutive patients [chronic hepatitis C (n=500); chronic hepatitis B (n=100)] were evaluated on the day of liver biopsy. Insulin resistance (Homeostasis Model) and all components of the metabolic syndrome were assessed. Independent factors associated with insulin resistance (HOMA-IR >3) and significant fibrosis (META VIR F2-F4) were analyzed. Parameters of insulin resistance were compared between matched chronic hepatitis B and chronic hepatitis C patients. Results: The characteristics of chronic hepatitis C patients were: male gender (57%), mean age 47±10 years, mean BMI 24.8±4 kg/m2, metabolic syndrome (12%), diabetes mellitus (7.6%) and insulin resistance (35.6%). With multivariate analysis, insulin resistance was associated in non-diabetic patients with the metabolic syndrome, genotypes 1 and 4, significant fibrosis and severe steatosis. Insulin resistance was diagnosed in 15% of 145 patients without metabolic disorders or significant fibrosis, and associated with genotypes 1 and 4, high serum HCV RNA level and moderate-severe liver necroinflammation. Insulin resistance was less frequent in chronic hepatitis B than in matched chronic hepatitis C patients (5% vs. 35%; p<0.001). With multivariate analysis, significant fibrosis was associated in chronic hepatitis C patients with: male gender, age>40 years, insulin resistance, moderate-severe liver necroinflammation, and severe steatosis. Interestingly, HOMA-IR increased significantly and progressively with the fibrosis stages, whereas the C-peptide/insulin ratio was not different. Conclusion: Insulin resistance is a specific feature of chronic hepatitis C, especially with genotypes 1 or 4. Insulin resistance is significantly associated with serum HCV RNA level and fibrosis stage. Therefore, insulin resistance should be systematically assessed and its management could have a major impact on the outcome of chronic hepatitis C patients.

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The influence of adipose tissue and its cytokines on the regulation of metabolic and immunological processes is an area of considerable research. We hypothesize that adiponectin plays an important role in regulating the immune response in chronic HCV. The aims of the study are to: 1) Determine the relationship between serum adiponectin levels and the HCV specific immune responses; 2) Examine the direct effect of adiponectin on the peripheral immune response in chronic HCV; 3) Elucidate the molecular pathways whereby adiponectin mediates its regulatory effects on the immune response in chronic HCV. Methods: The serum concentration of adiponectin from 26 HCV infected patients was determined by a multiplex cytokine assay. T-cell specific immune response to HCV was determined by the IFN-γ ELISPOT and a Multiplex cytokine assay after stimulation of PBMCs from patients with pools of overlapping HCV peptides representing the entire HCV polyprotein. Assays were conducted in the presence of either HCV peptides, recombinant adiponectin or PBMCs treated with adiponectin and HCV peptides. Depletion of CD4 and CD8 cells from PBMCs was achieved by utilizing antibody bound microbeads. Activation of P38MAPK in response to adiponectin was determined by Western blotting. Results: There was a strong positive association between the concentration of serum adiponectin levels and the presence of an anti-HCV specific T cell response (p=0.005). Adiponectin serum levels were significantly decreased in HCV infected patients with increased BMI. Pre-treatment of PBMCs with recombinant adiponectin prior to HCV peptide stimulation significantly enhanced IFN-γ production (p=0.03). Further experiments confirmed T cells (CD4 and CD8 T cells) as the cellular source of the IFN-γ response to adiponectin. Cytokine assays showed that pre-treatment of PBMCs with adiponectin prior to HCV peptide stimulation resulted in increased expression of IFN-γ (p=0.03) while reducing the expression of HCV-induced IL-6 production (p=0.02). In addition, adiponectin treatment resulted in activation of the P38MAPK pathway. Selective inhibition of P38MAPK activity by SB203580 blocked the IFN-γ response by PBMCs to adiponectin. Conclusions: The data indicate that adiponectin plays a key role in regulating the immune response in patients with chronic HCV infection. Thus, low adiponectin levels in obese subjects with HCV infection may alter T cell function and shift the cytokine responses towards a pro-viral state. Further, the effect of adiponectin on the immune response appears to be in part mediated by the P38MAPK signaling pathway.

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Functional polymorphisms in adrenergic receptors and in adiponectin and its receptors have been reported to be associated with various phenotypes of the metabolic syndrome such as diabetes and obesity and also impact on the course of chronic HCV infection. In this study, we aimed to evaluate in HCV-infected subjects whether liver injury and response to antiviral therapy are influenced by polymorphisms in genes for the adrenergic receptors (ADRs) [alpha 2A, beta 2, beta 3] and genes for adiponectin and its receptors (1 and 2). Methods: 26 single nucleotide polymorphisms (SNPs) were tested across 12 genes for an association with liver fibrosis and response to antiviral therapy. Examined genes included ADRB2-related allelic variants [Arg16Gly, Gln27Glu, Thr164Ile], ADRB3 (Trp64Arg), ADR-alpha2B (Glu del 301-101), adiponectin (G276T) and adiponectin receptors 1 and 2. Gene polymorphisms were determined by direct sequencing of the polymerase chain reaction products. Comparisons within each group were performed with the Chi-squared tests. Categorical variables were compared using Fisher’s exact test. Results: The majority of the cohort were Caucasian 67%, and 60% were male, the mean age was 38 ± 15 yr. A significant association was present between the ADRB2 Gln27Glu allele and the presence of advanced fibrosis (OR 12.3, p=0.04). Failure to respond to antiviral therapy was strongly detectable in subjects with the variant alleles ADRB2 Gln27Glu (OR 2, p=0.02) or ADRB3 Trp64Arg (OR 2.2, p=0.04). Subjects with adiponectin G276T showed a trend towards a poor response to antiviral therapy (p=0.06). Further, adiponectin G276T and ADRB3 polymorphisms synergistically influenced the response to antiviral therapy (OR 2, p=0.01). In multivariate analysis including age, BMI, gender and alcohol intake, the association between ADRB2 Gln27Glu and fibrosis as well as response to therapy was still evident (p=0.04, & 0.03 respectively). The ADRB2 Gln27Glu polymorphism was present in 30% of the examined cohort, was more frequent in males than females (p=0.04) and Caucasians than African Americans (p=0.02). A correlation was present between the ADRB2 Gln27Glu polymorphism and increased BMI (p=0.005). Conclusions: These novel findings confirm in HCV infected subjects, that polymorphisms for the adrenergic receptor genes (in particular ADRB2 Gln27Glu) are associated with increased hepatic fibrosis and poorer responses to antiviral therapy. Similarly, adiponectin polymorphisms effect responses to antiviral therapy.

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INVESTIGATION OF RESIDUAL HEPATITIS C VIRUS (HCV) IN PRESUMED RECOVERED SUBJECTS

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Background: Published studies have suggested that peripheral blood mononuclear cells (PBMCs) serve as a reservoir of residual HCV even in persons persistently HCV RNA negative in plasma. Aims: 1) To measure HCV RNA in PBMC and specific mononuclear cells of chronically infected and recovered subjects; 2) To determine if PBMC-associated HCV RNA is in a replicative form; 3) to determine if HCV might bind to PBMC without infecting them. Methods: 29 patients with chronic hepatitis C and 23 presumed recovered subjects (repeatedly anti-HCV+, HCV RNA-) were studied. Viral load was determined by Cobas Amplicor (Roche Diagnostics) and an in-house nested real-time detection PCR (n-RTD PCR). Negative strand HCV RNA was detected using strand-specific n-RTD PCR. Subsets of PBMC were separated on a magnetic cell separator. Results: 28/29 chronic carrier PBMC tested HCV RNA+. In 8 patients where PBMC subpopulations were tested, the viral load (log copies/106 cells) in the B cell subset (4.14 ± 0.71) was higher than in total PBMC (3.62 ± 0.71, p< 0.05), T cells (1.67 ± 0.88, p< 0.05), and non-B/T cells (2.48 ± 1.15, p< 0.05). HCV negative-strand (replicative intermediate) RNA was not detected in any of 25 PBMC samples, or 8 B, T, or non-B/T subsets. Further, healthy donor PBMC, when mixed in-vitro with HCV RNA+ plasma, incubated at RT for 2 hours and extensively washed, became HCV RNA+ and mimicked cells recovered from chronic carriers (total PBMC: 3.46 ± 0.14 log copies/106 cells; B cells 4.35 ± 0.07, p<0.001). Based on these findings in chronic carriers, residual HCV was sought in presumed recovered patients; no residual virus was detected in PBMC, the B cell subset, or in plasma of any of 23 presumed recovered patients. Conclusions: 1) HCV can be detected in PBMCs in patients with chronic hepatitis C and is recovered predominantly in B cell subpopulations; 2) HCV detected in PBMC does not contain negative strand RNA and is presumed non-replicative; 3) PBMC from normals can take up HCV viral particles in vitro when mixed with HCV+ plasma and then mimic cells recovered from HCV-infected patients suggesting that HCV binds to, but does not infect or replicate in PBMC; 4) Residual HCV was not detected in any of 23 recovered patients suggesting that PBMC do not generally serve as a reservoir of persistent HCV infection in patients otherwise presumed to have recovered.

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UPREGULATION OF PROGRAMMED DEATH-1 (PD-1) ON HCV-SPECIFIC AND CMV-SPECIFIC CD8+ T CELLS IN CHRONIC HCV INFECTION ASSOCIATED WITH REVERSIBLE IMMUNE EXHAUSTION

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The inhibitory molecule programmed death-1 (PD-1) is up-regulated on exhausted virus-specific CD8+ T cells (CTLs) in mice with LCMV and humans with HIV; emerging data indicate a significant role for this immunoreceptor in HCV. Here, we examined PD-1 expression on peripheral and intrahepatic (IH) CTLs and assessed the effect of PD-1 blockade on the functional competence of HCV-specific CTLs. Methods: Four groups were studied: chronic HCV (n=31), including 13 patients from whom IH lymphocytes were studied directly ex vivo; HCV spontaneous resolvers (n=11); non-HCV liver disease (n=12); ten healthy subjects served as controls. Different pentamers were used to screen viral-specific CTLs. CD4+ T cell responses were assessed using overlapping peptides spanning the entire HCV polyprotein in an IFN-g ELISPOT. Results: HCV-specific CTLs displayed levels of PD-1 that were markedly elevated in patients with chronic HCV relative to resolved HCV infection (figure below). PD-1 MFI, which correlates directly with the number of molecules expressed on a per cell basis, was higher on HCV-specific CTLs within the IH compartment compared to peripheral blood. CD57+ level was greater in chronic than resolved infection on both PD-1 high and PD-1 low fractions. The effect of HCV on PD-1 expression extended to non-HCV-specific cells: CMV-specific CTLs exhibited higher PD-1 in patients with chronic HCV compared to non-HCV liver disease patients and normals (p=.007). Next, CFSE was used to monitor proliferation of HCV CTLs following in vitro blockade with monoclonal Abs specific to its ligands. Blockade with either anti-PDL-1 or anti-PDL-2 antibodies enhanced proliferation 2-fold whereas dual blockade of PD-1 and LFA-3 with Abs specific to its ligands enhanced proliferation 4-fold.

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HEPATITIS C VIRUS-INDUCED REACTIVE OXYGEN SPECIES CAUSE IRON ACCUMULATION IN MICE BY REDUCING HEPcidIN TRANSCRIPTION

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Background and Aims: Despite abundant clinical evidence, the mechanisms by which hepatic iron overload develops in patients with hepatitis C virus (HCV)-associated chronic liver diseases remain unknown. The aim of this study was to investigate how hepatic iron overload develops in the presence of hepatitis C virus (HCV) proteins. Methods: Male C57BL/6 transgenic mice expressing the HCV polyprotein and nontransgenic littermates were assessed for iron concentrations in the liver, spleen and serum, and iron regulatory molecules in vivo. Reporter gene assays were performed for primary mouse hepatocytes to study promoter activity of hepcidin. We also performed electrophoretic mobility shift assay using mouse nuclear extracts to examine DNA binding activity of transcriptional factors. Results: Transgenic mice had lower hepcidin expression in the liver accompanied by higher expression of ferroportin in the duodenum and spleen, which led to increased hepatic and serum iron concentrations, and a decreased splenic iron concentration. The expression level of divalent metal transporter 1 in the duodenum was not different between transgenic and nontransgenic mice. In response to hepatocellular iron excess, transferrin receptor 1 expression decreased and ferritin expression increased in the transgenic liver. Transgenic mice showed no inflammation in the liver, but preserved the ability to induce hepcidin in response to proinflammatory cytokines induced by lipopolysaccharide. Hepcidin promoter activity and the DNA binding activity of CCAAT/enhancer-binding protein alpha (C/EBPα) were down-regulated concomitantly with increased expression of C/EBPα homology protein (CHOP), an inhibitor of C/EBP DNA binding activity, and the abundance of reactive oxygen species (ROS) in transgenic mice at the ages of 8 and 14 months. The expression level of C/EBPα in the liver was not different between transgenic and nontransgenic mice. In response to hepatocellular iron excess, transferrin receptor 1 expression decreased and ferritin expression increased in the transgenic liver. Transgenic mice showed no inflammation in the liver, but preserved the ability to induce hepcidin in response to proinflammatory cytokines induced by lipopolysaccharide. Hepcidin promoter activity and the DNA binding activity of CCAAT/enhancer-binding protein alpha (C/EBPα) were down-regulated concomitantly with increased expression of C/EBPα homology protein (CHOP), an inhibitor of C/EBP DNA binding activity, and the abundance of reactive oxygen species (ROS) in transgenic mice at the ages of 8 and 14 months. The expression level of C/EBPα in the liver was not different between transgenic and nontransgenic mice. Conclusions: HCV-induced ROS down-regulate hepcidin transcription through inhibition of C/EBPα DNA binding activity by CHOP, which in turn leads to increased duodenal iron transport and macrophage iron release, causing hepatic iron accumulation. This finding provides a potential mechanistic pathway for HCV-induced iron accumulation in the liver, even though the present model is different to what is observed in patients with chronic hepatitis C with respect to a lack of inflammation.

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NATURAL HCV CORE VARIANTS REDUCE CELL GROWTH INHIBITION AND FACILITATE INDUCTION OF EPITHELIAL TO MESENCHYMAL TRANSITION (EMT) MEDIATED BY TGF-BETA

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The HCV core protein is a pleiotropic modulator of cell growth and viability which likely participates in HCV-related HCC. Although the precise mechanisms involved are still unclear, the TGF-β plays a major role in fibrosis and exerts a biphasic action during tumor progression, thereby acting as a tumor suppressor at early stages and as an enhancer of invasiveness at later stages. We have previously demonstrated that HCV core variants isolated from tumor and non-tumor nodules differentially bind Smad3, a transcriptional factor mediating the TGF-β specific signalling pathway but the biological relevance of this interaction remains to be established. We here present three major findings that shed light into the functional consequences of HCV core protein modulation of TGF-β signalling. First, the tumor-suppressive effects of TGF-β, in term of cell growth inhibition and apoptosis are highly reduced in Huh7 cell lines stably expressing these natural HCV core variants. Second, the same inhibitory effects on TGF-β cytostatic effects were obtained in both primary human hepatocytes expressing these HCV core variants after lentivirus transduction and primary hepatocytes isolated from transgenic mice expressing the same core sequences. Third, core variants increased the TGF-β-mediated EMT (epithelium to mesenchymal transition), a process involved in tumor spreading and invasiveness. These results define a new concept where HCV could participate to fibrosis by increasing TGF-β levels and later contribute to HCC development by emergence of new variants that are able to switch TGF-β responses from tumor suppression to tumor promotion functions.

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A CIRRHOSIS RISK SCORE IDENTIFIES THOSE CHRONIC HEPATITIS C INFECTED PATIENTS PRESENTING WITH NO LIVER FIBROSIS THAT ARE AT HIGH RISK FOR FIBROSIS PROGRESSION

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Background and Aims: Chronic hepatitis C (CHC) infection can promote liver fibrosis, yet the time course for fibrosis progression is highly variable and can be influenced by host factors including gender, age at infection, alcohol consumption and genetic variants. Recently, we described seven genetic variants that identify CHC patients at risk for developing cirrhosis and created a Cirrhosis Risk Score (CRS) that is calculated based on these seven SNPs and the patient’s gender. In this study, we asked whether the CRS could identify CHC patients at high risk for fibrosis progression. Methods: We examined Caucasian patients with CHC infection enrolled from the University of Padova, Italy. These patients received two liver biopsies spon-
ing an average interval of 80 months and had their biopsies scored according to the META VIR units (F0-F4) prior to receiving any treatment. For each patient, genomic DNA was isolated from whole blood and genotypes for the CRS genetic markers determined using a multiplex PCR and Oligonucleotide Ligation Assay based on the LumineX® 200TM System. Association of the proportion of F0 patients remaining at F0 at the second biopsy across low (0 to 0.5), intermediate (0.5 to 0.7) and high (0.7 to 1.0) CRS scores was assessed using the Armitage trend test. Results: Among 50 CHC patients with F0 at their first biopsy, 57% (9 of 16) with a low CRS remained at F0 at the second biopsy compared to 42% (8 of 19) with intermediate CRS and 20% (3 of 15) with a high CRS, trend test p value of 0.04. Since gender is a component of the CRS score, we also analyzed the data separately by gender although power was limited. Among females, we found no significant difference (p value = 0.73) in the proportion of subjects remaining at F0 at the second biopsy. However, in males, there remained a significant trend (p value = 0.02; age adjusted p value = 0.04) with 57% (4 of 7) of low CRS males remaining at F0 compared to 22% (2 of 9) with intermediate CRS and 14% (1 of 12) with high CRS. Conclusion: In this study, increasing CRS was associated with fibrosis progression in CHC infected patients presenting with no liver fibrosis. Previously, we described the ability of CRS to identify CHC patients at high risk for cirrhosis in patients enrolled from 5 US centers. This current study suggests that the CRS may identify CHC patients at high risk for fibrosis progression and the potential generalizability of the CRS to other non-US populations. Collectively, these studies suggest that the CRS genetic signature could potentially be a useful prognostic indicator of those patients with CHC infection most likely to develop fibrosis progression and/or cirrhosis.

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460 EFFECTS OF HEME OXYGENASE-1 (HO-1) OVEREXPRESSION ON HEPATITIS C VIRUS (HCV) REPLICATION AND CELLULAR INJURY IN VITRO
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Introduction. Oxidative injury to hepatocytes occurs as a consequence of HCV infection, replication, and viral protein expression. Modulation of host cell antioxidant enzymes is an attractive, yet unexplored strategy that potentially may minimize cellular injury, reduce viral replication and ultimately attenuate liver disease. We have previously described interactions of HCV viral proteins with HO-1, an important antioxidant enzyme that is induced in hepatocytes in response to oxidative stress. The focus of this work was to evaluate the effects of HO-1 overexpression on HCV replication and hepatocellular injury.

Methods. Full length (FL) or Non-structural (NS) replicons (I 389 NS3-3') were transfected with plasmid containing complete human HO-1 sequences or empty vector for control. Clonal cell lines overexpressing HO-1 (2.5 fold above basal values) or empty vector were isolated and quantified for HCV RNA synthesis, viral protein production and resistance to oxidative injury. Results. HO-1 overexpression markedly decreased HCV RNA synthesis in both FL and NS replicons (2- and 4-fold respectively) without affecting cellular growth or DNA synthesis. Both FL and NS replicons overexpressing HO-1 showed reduced prooxidant production as measured by dichlorofluorescein assay and increased resistance to peroxide toxicity. HO-1 overexpression also led to differences in levels of viral proteins with an increase in HCV core protein, but a decrease in NS5A levels in Huh7.5 FL replicon cells transfected with HO-1. Conversely, knock-down of HO-1 mRNA by siRNA in control FL or NS replicons with basal levels of HO-1 did not alter HCV replication but reduced expression of HCV core protein by greater than 50% with no change in NS5A level. Conclusions. Overexpression of HO-1 decreases HCV replication and protects from oxidative injury. Overexpression of HO-1 or knock-down of HO-1 mRNA leads to differential expression of HCV viral proteins which may, in part, mediate the observed changes in HCV replication and susceptibility to cellular injury. These findings suggest a potential role for HO-1 in antiviral therapy and protection against hepatocellular injury in hepatocytes infected with HCV.

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The following people have nothing to disclose: Zhaowen Zhu, Meleah Mathahs, Feng Wen, Kyle Brown, Bruce A. Luxon, Warren N. Schmidt

461 COMPREHENSIVE GENE EXPRESSION ANALYSIS OF IRON METABOLISM-RELATED GENES IN PATIENTS WITH CHRONIC VIRAL HEPATITIS AND HEPATOCELLULAR CARCINOMA
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Objective: Abnormal iron metabolism generates oxidative stress, DNA damage and cellular injuries that accelerate the progression of various liver diseases. We have reported gene expression profiling of chronic hepatitis (CH)-B, CH-C and hepatocellular carcinoma (HCC) using a cDNA microarray and serial analysis of gene expression (SAGE) (Hepatology. 2006, 44, 1122). To elucidate the functional role of iron metabolism in these liver diseases, we examined iron metabolism-related gene expression and analyzed the patho-physiological roles of these genes in liver disease. Material and Methods: Liver tissue samples were obtained from 36 patients with CH-B, 34 patients with CH-C, 17 patients with HBV-related HCC (HCC-B), 17 patients with HCV-related HCC (HCC-C) and 10 patients with normal livers. Gene expression profiling was obtained from these samples using an in-house cDNA microarray comprising 9614 clones selected from 256,550 tags of hepatic SAGE libraries. Results: We selected and evaluated the expression of 47 iron metabolism-related genes. These genes included iron-binding proteins, iron transport proteins, mitochondrial electron transport enzymes and oxidative-related enzymes. The comparison of CH-C and normal liver showed that genes related to iron storage, such as transferring receptor, DMT-1, sideroflexin 1 and transferrin were upregulated in CH-C. These genes are involved in iron uptake, iron transport from endosome to cytosol, iron transport to mitochondria and iron storage. In contrast, hepcidin, an inhibitor of iron uptake from the intestine, was significantly downregulated in CH-C. Interestingly, when gene expression was compared between CH-C and CH-B, hepcidin was significantly downregulated in CH-C and ferroportin-1, which is repressed by hepcidin, was significantly upregulated in CH-C. Iron metabolism is thus more activated in CH-C than in normal liver or CH-B. Consistent with these findings, genes related to electron transport enzyme
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AN ASSESSMENT OF THE ROLE OF ENDOPLASMIC RETICULUM (ER) STRESS IN THE PATHOGENESIS OF HCV  
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Background: Viruses are dependent on the endoplasmic reticulum (ER) to correctly fold and process viral proteins. If the ER becomes overloaded, unfolded or misfolded proteins accumulate causing ER stress, which triggers downstream intracellular pathways that globally attenuate the translation of mRNA, increase the production of molecular chaperones to aid protein folding, and promote cellular apoptosis. ER stress may also cause mitochondrial dysfunction, oxidative stress and cellular injury. A number of hepatitis C virus (HCV) proteins have been shown to induce ER stress in experimental models, however, it remains to be determined whether ER stress has a role in the pathogenesis of HCV-induced liver injury. Aims: To investigate the role of ER stress in the pathogenesis of HCV Methods: Liver biopsy samples from 123 patients with chronic HCV, 12 with chronic hepatitis B (HBV) and 14 HCV/HBV-negative subjects with histologically normal liver (NDL) were assessed. The mRNA expression of markers of ER stress (PERK, ATF6 and IRE1 pathways) were measured by real-time (RT)-PCR. Results: A comparison of the hepatic expression of ER stress markers between groups is shown in the Table. In comparison to NDL, subjects with HCV have significant upregulation of the PERK pathway and downregulation of the ATF6 pathway. Subjects with HBV have upregulation of the PERK and IRE1 pathway compared to NDL. There was significant upregulation of the PERK and IRE1 pathways in subjects with HBV compared with HCV. Among the patients with HCV, the expression of ER stress markers was higher in subjects with genotype 3 compared with genotype 1 (CHOP (p=0.02), GADD34 (p=0.01), EDEM (p=0.02), sXBP1 (p=0.02)). There were no relationships between the expression of ER stress markers and liver histology (inflammation, fibrosis or steatosis), BMI, HOMA score or alcohol intake in subjects with HCV. Conclusions: We observed differential expression of the hepatic ER stress pathways in subjects with HCV and HBV compared to NDL. In particular there was increased expression of CHOP which is known to increase the susceptibility to cell death.

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PROTECTION BY HLA-B27 IN HCV INFECTION: ROLE OF VIROLOGICAL AND IMMUNOLOGICAL FACTORS  
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B27 is a highly protective HLA allele in HCV infection. Indeed, 80% of B27+ Irish women with accidental HCV genotype 1b infection resolved the infection spontaneously while only 20% developed chronic infection (McKiernan et al., Hepatology 2004). We recently linked this protective effect of HLA-B27 as well as viral evolution within the few B27+ individuals who developed chronic infection to a single, immunodominant HLA-B27 restricted CD8+ T cell epitope within NS5B (Neumann-Haefelin et al., Hepatology 2006). In order to further define the mechanisms of protection mediated by this epitope, we analyzed the virological and immunological characteristics in more detail. To determine the replication capacity of the variants observed in vivo, we cloned these into a subgenomic Con1 replicon harbouring a luciferase reporter gene. All variants were still able to replicate, however a significant reduction in viral replication was observed for most mutants indicating impaired fitness. Interestingly, some clustered mutations seem to represent compensatory rather than escape mutations that restore viral fitness partially. The epitope has an enormous binding affinity to HLA-B27 molecules and cross-reacts with peptides from clinically common microorganisms, indicating that heterologous immunity contributes to the dominant effect of this epitope. In conclusion, our results show that both, immunological factors (high binding affinity, cross-reactivity) as well as virological factors (high viral fitness cost) contribute to the protective effect of HLA-B27 observed in vivo. We hypothesize that viral escape within this epitope is constrained, giving the strong CD8+ T cell response enough time to clear the virus in most acutely infected patients before viral evolution can lead to the establishment of persistent infection.

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The following people have nothing to disclose: Christoph Neumann-Haefelin, Eva Dazert, Susan McKiernan, Dermot Kelleher, Hubert E. Blum, Ralf Bartenschlager, Robert Thimme
464 IMPAIRED TLR/RIG-I-MEDIATED INNATE IMMUNITY IN MYELOID DENDRITIC CELLS IN HCV-INFECTED INDIVIDUALS
Masanori Miyazaki, Tatsuya Kanto, Naruyasu Kakita, Michiyao Inoue, Ichiyio Iose, Hideki Miyatake, Mitsuru Sakakibara, Takayuki Yakushijin, Naoki Hiramatsu, Tetsuo Takehara, Akinori Kasahara, Norio Hayashi; Osaka University Graduate School of Medicine, Suita, Japan

BACKGROUND and AIMS: Inhibition of innate immunity is one of the strategies of HCV escaping from immune surveillance system. HCV NS3/4A was reported to inhibit signaling pathways downstream of Toll-like receptor (TLR) 3 and RIG-I/MDA5 by cleavage of adaptor molecules TRIF or IPS-1, respectively. Dendritic cells (DCs) presumably sense HCV by TLR or RIG-I/MDA5 and produce type-I IFN and inflammatory cytokines, involving in the regulation of innate and adaptive immunity. Numerical and functional impairment of blood myeloid DC (MDC) in HCV infection was reported as a crucial mechanism leading to HCV persistence. However, little is known whether TLR or RIG-I system is impaired or not in MDC in HCV infection, as shown in HCV-replicating hepatocytes. We thus aimed to clarify this issue and its mechanism, in order to search for therapeutic targets contributing to immune restoration. METHODS: Thirty-nine patients with chronic hepatitis C (CH) and 52 healthy volunteers (HV) were enrolled. Blood MDCs were magnetically isolated from PBMCs. The expressions of TLR2, TLR3, TLR4, RIG-I and MDA5 in freshly isolated MDCs were quantified by real-time RT-PCR. For functional analyses, we stimulated MDCs with specific TLR agonists (TLR2: Pam3CSK4, TLR3/RIG-I: polyI:C, TLR4: LPS) and examined their cytokine induction by real-time PCR. To compare the signaling pathways downstream of TLR3/RIG-I in MDC between CH and HV, we stimulated MDCs with poly I:C and subjected them for PCR Array analysis. Then we compared the expression of adaptor molecules of TLR or RIG-I in MDC, such as MyD88, IPS-1, TRIF, and TRAF6. RESULTS: The relative expressions of TLR2, TLR3, TLR4 and RIG-I in MDCs from CH were significantly higher than those from HV (p<0.005, others: p<0.005), whereas TLR3 and MDA5 expressions did not differ between the groups. LPS or polyI:C-stimulated induction of IFN-α and TNF-α in MDC from CH were significantly lower than those from HV (p<0.005, p<0.01), suggesting that inhibitory mechanisms exist in the downstream of TLR3, TLR4 or RIG-I. PCR Array analysis demonstrated that some molecules categorized in TLR/RIG-I adaptors, MAPK and NF-κB pathways exhibited lesser degree of expression in MDC from CH. More precisely, the expression of TRIF and TRAF6 was lower in CH (P<0.05, P<0.001), whereas IPS-1 and Myd88 expression was higher in CH (P<0.05, P<0.005). CONCLUSIONS: In MDCs from HCV-infected patients, TLR3, TLR4 or RIG-I-mediated cytokine responses were impaired regardless of their enhanced expressions. Lower expression of TRIF and TRAF6 is one of the plausible mechanisms of such impairment, suggesting their potential role as therapeutic targets for immune modulation.

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465 ERADICATION OF HCV BY INTERFERON TREATMENT LEADS TO IMPROVEMENTS IN WHOLE-BODY INSULIN SENSITIVITY
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Aim: It is well known that HCV infection causes insulin resistance (IR) and hepatic steatosis, following the occurrence of hepatoma, as shown in an HCV core transgenic mouse model. But little is known about the precise mechanism for IR in HCV-infected patients. We analyzed how eradication of HCV by interferon (IFN) therapy influences whole-body IR, consisting of hepatic IR and skeletal muscle IR, in patients with chronic hepatitis C Methods: In twenty HCV-infected patients with sustained virologic response (SVR) and sixteen with non-SVR, who were undergoing IFN treatment, we assessed the HOMA-IR value as an index of hepatic IR, the ISI composite, an index of whole-body insulin sensitivity, which was calculated as 10000/√FPG × FIRI × mean BS (0-120) × mean IRI (0-120) from a 75 g oral glucose tolerance test, and the HOMA-β and insulinogenic index values for the evaluation of insulin secretion from the pancreas. Indices were determined at the start and 6 months after IFN treatment. Results: In the SVR group, ISI composite values after 6 months treatment were significantly higher than at the start of therapy (p=.022), but there were no significant changes in HOMA-IR values. The insulinogenic index at 6 months was lower than at the start of treatment (p=.047), but there were no significant changes in HOMA-β values. In the non-SVR group, there were no significant changes in the ISI composite, HOMA-IR, HOMA-β or insulinogenic index between the start of treatment and at 6 months. Furthermore, there were no significant changes in body weight between the start and after 6 months treatment in both groups. Conclusions: Eradication of HCV by IFN therapy could lead to improvements in insulin sensitivity in patients with chronic hepatitis C, not only HCV-infected liver but also whole-body (primarily muscle) insulin sensitivity. These results indicate that HCV infection might cause systemic insulin resistance, probably via cytokines or other humoral factors.

(75g OGGT in SVR cases)

Disclosures: The following people have nothing to disclose: Yasunori Kawaguchi, Toshihiko Mizuta, Yuichiro Eguchi, Tsutomu Yasutake, Keisuke Ario, Hirokazu Takahashi, Shinni Iwane, Iwata Ozaki
466 INTRAHEPATIC ANGIOPOIETIN-2 PROTEIN EXPRESSION MODULATION BY HEPATITIS C VIRUS: MAPK, PI3K AND ACTIVE OXYGEN SPECIES (ROS) IMPLICATION

Paloma Sanz-Cameno, Samuel Martín-Vilchez, Yolanda Rodríguez-Munoz, Maria Jesús Borque, Jose A. Moreno-Montenegro, Pedro L. Majano, Francisca Molina-Jimenez, Manuel Lopez-Cabrera, Ricardo Moreno-Otero.

Chronic hepatic disease related to hepatitis C virus (HCV) is characterized by intrahepatic inflammatory infiltrates, fibrosis and angiogenesis, and it is associated with cirrhosis and hepatocellular carcinoma in a substantial proportion of infected individuals. During these events there is a vascular homeostasis imbalance, in which the Angiopoietin/Tie2 system plays a significant role. Recently, we have observed elevated expression of Angiopoietin2[Tie2] in both, chronic hepatitis B (CHB) patients, which is mediated by HBx viral transactivator in CHB (Sanz-Cameno et al., 2006), as well as in chronic hepatitis C (41st Annual Meeting of the EASL, 2006). The AIM of this work was to identify the HCV protein/s that could mediate the Ang2 induced expression and to study its modulation by diverse cellular signalising pathways: MAPK, PI3K and ROS. METHODS: Ang2 expression was evaluated by western blot in hepatic cell lines harbouring genomic (I389/Core-3') or subgenomic (I377/NS3-3') HCV replicons (Bartenschlager, 2002) and in the parental cells Huh7. All these cell lines were subjected to different treatments, PD98059, LY294002 or Ebselen at 25 µM for 48 hours in order to inhibit or modulate several cellular signalling pathways: MAPK, PI3K and ROS, respectively. RESULTS: There was a significant Ang2 up-regulation in cells containing HCV complete genome compared with parental or non-structural proteins expressing cells. The MAPK inhibitor and the antioxidant, Ebselen, reflected a differential influence in Ang2 isoforms expression (64 and 50 KDa), with a significant induction of inflammatory and tumoral related one (50 KDa), meanwhile the PI3K inhibition diminished the expression of the 64 KDa form. CONCLUSIONS: The prominent Ang2 expression in cells containing HCV complete genome indicates that its modulation is mostly mediated by structural viral proteins. Additionally, the differential Ang2 isoforms regulation by diverse cellular signalling routes could account for an important point of vascular quiescence control in response of tissular microenvironment and could contribute to the clarification of the molecular pathogenetic mechanisms involved in the onset and progression of this and others chronic inflammatory diseases.

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467 LACK OF FUNCTIONAL RESTORATION BY PD1/PD-L1 BLOCKADE IN INTRAHEPATIC HCV-SPECIFIC CD8 T CELLS FROM CHRONICALLY HCV-INFECTED PATIENTS

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Programmed Death 1 (PD1) is negative immune costimulatory molecule. Increased PD1 expression in antigen-specific CD8 T cells during chronic viral infections is associated with functional impairment that may be reversed by inhibiting the interaction between PD1 and its ligand (PD-L1). While the role of PD-L1 blockade has been explored in peripheral HCV-specific CD8 T cells, we asked if HCV-specific CD8 T cells in the liver share similar responsiveness to PD-L1 blockade. Methods: The frequency, phenotype and antiviral effector function of CD8 T cells specific for HLA-A2-restricted HCV and control influenza or EBV epitopes were examined in peripheral blood of patients with acute, chronic and resolved HCV infection by FACS using class I tetramers directly ex-vivo and following in-vitro stimulation. Virus-specific CD8 T cells in blood and liver were examined concurrently in HCV-infected patients undergoing liver transplantation. In a subset, antiviral effector functions in peripheral blood and liver were compared before and after antigenic stimulation with and without PD-L1 blockade by anti-PD-L1. Results: PD1 was highly expressed ex-vivo in circulating CD8 T cells specific for HCV (but not Flu) from patients with acute and chronic HCV infection but not in those with resolved infection. The level of PD1 expression ex-vivo in virus-specific CD8 T cells correlated negatively with their effector function (e.g. perforin, granzyme B, IFNg and CD107α expression) following antigenic stimulation in-vitro. HCV-specific CD8 T cells were more highly PD1-positive in the liver than blood (84% vs. 28%, p<0.0001). Furthermore, intrahepatic HCV-specific CD8 T-cells showed markedly reduced capacity for antiviral effector function compared with peripheral HCV-specific CD8 T cells. These compartmental differences were HCV-specific since Flu-specific CD8 T cells from the same patients showed similar PD1 expression between liver and blood (Median 17% vs. 19%, p=0.44) with highly efficient effector function. Finally, in-vitro PD-L1 blockade improved in-vitro expansion and effector function of HCV-specific CD8 T cells from peripheral blood, but not the liver. Conclusion: While these findings confirm the association between virus-specific CD8 T cell dysfunction and PD1 expression in HCV persistence, we also show that PD-L1 blockade promotes functional restoration of HCV-specific CD8 T cells from peripheral blood but not the highly PD1-positive HCV-specific CD8 T cells from the liver. These results suggest a profound and potentially irreversible impairment in intrahepatic HCV-specific CD8 T cells with pathogenetic and therapeutic relevance.

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CRYOGBLOBULINEMIA IN CHRONIC HEPATITIS C A NEW MARKER OF ADVANCED FIBROSIS AND SEVERE STEATOSIS

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Background and Aim Hepatitis C virus infection is associated with a spectrum of extrahepatic manifestations, mainly mixed Cryoglobulinemia. The positive association between cryoglobulin and severity of liver disease in chronic hepatitis C (CHC) remains a controversial issue. The aim of this prospective study was to assess the association of asymptomatic Cryoglobulinemia with steatosis and liver fibrosis stage in a large cohort of patients with CHC. Patients and Methods 400 consecutive patients with CHC and no signs of vasculitis were evaluated on the day of liver biopsy. Insulin resistance was assessed using the homeostasis model and defined as HOMA-IR >3. Cryoglobulins were detected by precipitation at 4°C in sera adequately collected and centrifuged at 37°C. Serum HCV RNA was quantified (bDNA, Bayer) and genotype determined for all patients. Liver histology was assessed using the METAVIR score. Results The baseline characteristics of patients were: male gender (58%), mean age (48±10 years), mean BMI (25±4 kg/m2). 35% of patients had insulin resistance. Genotype distribution was: 1 (55%), 2 (9%), 3 (14%) and 4 (20%). Mean serum HCV RNA level was 1.05±1.1 106 IU/ml, > 600000 IU/ml in 46% of patients. Cryoglobulins were detected in the serum of 53% of patients. Liver necroinflammation was significant (METAVIR A2-A3) in 27%, and fibrosis significant (METAVIR F2-F4) in 58% of patients. Steatosis was present in 52% of patients, moderate-severe (>30% of hepatocytes) in 31% of patients. At univariate analyses, the presence of cryoglobulin was associated with serum HCV RNA level < 600,000 IU/ml, significant necroinflammation, significant fibrosis, and moderate-severe steatosis. Patients with compared to those without cryoglobulin had similar age and duration of disease. At multivariate analysis, the presence of cryoglobulin was associated with serum HCV RNA level < 600000 IU/ml (p=0.01, OR=1.7), significant fibrosis (p=0.001, OR=2.5), and moderate-severe steatosis (p=0.002, OR=2.2). At multivariate analysis, significant fibrosis was associated with male gender (p=0.001, OR=2.2), age > 45 years old (p=0.006, OR=2.0), insulin resistance (p=0.008, OR=2.0), Cryoglobulinemia (p=0.001, OR=2.3), significant necroinflammation (p=0.001, OR=3.36) and moderate-severe steatosis (p=0.004, OR=2.2). Conclusion In this prospective study including 400 consecutive patients with CHC, asymptomatic cryoglobulinemia was detected in 53%. Cryoglobulinemia was an independent factor associated with more severe liver disease (fibrosis and steatosis). Further studies are ongoing to investigate the relationship between cryoglobulinemia and HCV RNA level, steatosis and fibrosis.

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HIV AND GP120 ENHANCE HCV REPLICATION AND UPRREGULATE TGF-β1

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Background/Aims: Due to their shared routes of transmission, HIV/HCV coinfection is prevalent in the U.S. HIV increases HCV RNA levels, TGF-β1 expression, hepatic fibrosis progression, HCV persistence, decreases response to interferon-based anti-HCV therapy. However, the mechanism by which HIV and TGF-β1 contribute to HCV pathogenesis has not been well studied. We sought to explore whether inactivated HIV and gp120 increase HCV through TGF-β1 regulation by using HCV replicon and infectious JFH1 models. Methods: Inactivated HIV and recombinant HIV proteins (gp120, Gag, Tat, Pol, Rev) were incubated for 48 h in OR6 replicon (Huh7 cell stably harboring a genotype 1b full-length HCV RNA and co-expresses Renilla luciferase) or infectious HCV JFH1 cells. HCV replication was monitored by measuring Renilla luciferase unit (RLU) in OR6 cells and by measuring HCV core in JFH1 cells. To test the effect of HIV and gp120 on TGF-β1 expression in hepatocytes, we performed the assay in serum-free medium, which has undetectable levels of TGF-β1. TGF-β1 levels were measured using a human TGF-β1 ELISA. Recombinant TGF-β1 and neutralizing antibody to TGF-β1 were introduced to assess the effect of TGF-β1 on HCV replication. Neutralizing antibodies to the CXCR4 and CCR5 receptors were added to the HCV-infected cells to verify that gp120 and inactivated HIV were signaling through these receptors. Results: We found a greater than 2-fold increase in HCV replication levels from baseline in the presence of gp120 (45305± 3375 vs 20997± 1428 RLU in OR6 cells, 2014± 101.07 vs 1043± 66.9 pg/ml HCV core in JFH1 cells). In contrast, there were no changes in HCV RNA or core protein in the presence of any of the other HIV proteins. The increased HCV levels were specifically abrogated by preincubation with neutralizing antibody to CCR5 or CXCR4. HIV gp120 protein significantly increased TGF-β1 expression in serum-free medium (3721± 266.1 vs 1814.4± 114.3 pg/ml in OR6 cells, 2996± 203.8 vs 1593.2± 105.2 pg/ml in JFH1 cells.). Recombinant TGF-β1 (4 ng/ml) enhances HCV replication in OR6 cells (41151± 259.2 vs 21427± 1466.1 RLU) in serum free medium. Neutralizing antibody to TGF-β1 blocked this effect (p< 0.003 for all comparisons). Conclusions: Our data indicate that inactivated HIV and gp120 have a proximal effect on HCV replication that is dependent on co-receptor engagement. We speculate that HIV and gp120 promote HCV replication through upregulating TGF-β1 in HCV-infected hepatocytes. These results implicate an effect of circulating HIV on innate antiviral immunity to HCV, and suggest a novel mechanism by which HIV enhances HCV replication and hepatic fibrosis progression.

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470 INCREASED IRF-1 AND IFN-α GENE EXPRESSION IN CHRONIC HEPATITIS C G-300A IRF-1 PROMOTER POLYMORPHISM A ALLELE CARRIERS

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Interferon regulatory factor-1 (IRF-1) is a type I and type II interferon (IFN) inducible transcription factor which is involved in the regulation of the IFN response. Recently, we were able to report on an association of the G-300A IRF-1 promoter polymorphism genotype and the outcome of an hepatitis C virus (HCV) infection. We now analyzed IRF-1 and IFN-α transcript expression with regard to G-300A IRF-1 genotype in peripheral blood mononuclear cells (PBMC) from healthy individuals (n=11) and from chronic hepatitis C patients (n=17) ex vivo and after overnight incubation. Both, basal IRF-1 and IFN-α mRNA levels were found to be significantly higher in chronic hepatitis C patients than in healthy volunteers. Whereas IRF-1 transcript expression was not observed to be significantly different with regard to promoter genotype distribution in ex vivo isolated PBMC, an overnight incubation in the presence of autologous HCV containing sera revealed an increase of IRF-1 transcripts preferentially in hepatitis C A allele carriers, whereas such an increase was not observed for patients homozygous for the wildtype allele G nor for healthy individuals. Moreover, IFN-α transcript expression in freshly isolated PBMC from hepatitis C A allele carriers was found to be 5-fold higher compared to homozygous G patients. Our findings support the assumption that the genetic background in the IRF-1 promoter may be related to type I IFN response in hepatitis C infection. Allele specific transcript quantification (ASTQ) studies are running to evaluate the functional impact of the two alleles.

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471 IMPAIRMENT OF CHEMOKINE RECEPTOR CXCR4-EXPRESSING IN HCV INFECTION AS POTENTIAL IMMUNE ESCAPE MECHANISM

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The Hepatitis C Virus (HCV) causes chronic infection in about 85% of patients. Thus, the virus is able to combat the immune system very efficiently. The mechanisms of immune evasion and the role of the early immune response in chronic infection caused by HCV are still unclear. In order to address this issue, we studied the expression of diverse surface molecules on liver cells in HCV infected versus non-infected liver tissue and noticed a considerable difference in surface expression of the chemokine receptor CXCR4. While ‘healthy’ cells express CXCR4 on their surface, HCV infected cells show a rather perinuclear stain for CXCR4 in immunohistochemistry. Using HCV replicon cells, we could confirm these findings in vitro employing immunofluorescence staining of Huh7-replicon-cells, which display a cytoplasmatic localisation of CXCR4, versus ‘native’ Huh7 hepatoma cells, with CXCR4 expression on the cell surface. Interestingly, upon HCV core transfection of Huh7 cells, the receptor showed the same perinuclear localisation as in replicon-containing cells or HCV infected liver cells. Moreover, CXCR4 colocalises with HCV core in perinuclear compartments. In addition, HCV infected cells and HCV core transfected cell lines show diminished CXCR4 mRNA expression compared to uninfected liver tissue and untransfected cell lines. The mechanism of receptor internalisation is matter of ongoing investigations. Previous results demonstrate that HCV-infection causes induction of the CXCR4-ligand SDF-1a. So far, our data suggest the following sequence of events: HCV causes induction of SDF-1a secretion which is followed by internalisation of CXCR4 via ligand binding. Inside the cell CXCR4 accumulates in the cytoplasm/ perinuclear compartments, most likely in complex with HCV core. In addition, CXCR4-expression is directly impaired on the transcriptional level by HCV core. Considering that CXCR4 was initially described to regulate the homing of lymphocytes in inflammatory tissues and, thus, the established role of CXCR4 and its ligand for attraction of immune cells to sites of inflammation, the downregulation of CXCR4 by HCV may be a newly identified strategy by HCV to efficiently hide from immune recognition.

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472 CONTROLLING B-CELL ACTIVATING FACTOR (BAFF/BLYS) LEVELS IN CHRONIC HEPATITIS C VIRUS INFECTION

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B-cell activating factor (BAFF) is predominantly expressed in myeloid cells including macrophages and dendritic cells and is primarily responsible for B cell maturation and survival. The factors that determine BAFF levels in man are unclear. Possible feedback mechanisms might would allow control of B cell numbers. Induction by direct hepatitis C virus (HCV) activation of macrophages through TLR dependent or independent pathways could begin to explain the lymphoproliferative nature of chronic HCV infection. We measured Human BAFF in serum using a solid phase ELISA (Quantikine, R&D Systems, MN) designed to eliminate interference by biological factors, including cryoglobulins. We compared BAFF levels in patients with HCV-related mixed cryoglobulinemia HCV-MC (n=11) with a similar group of patients with HCV-MC, eight weeks after treatment with rituximab (n=3) to deplete the mature B cell pool. We also measured BAFF levels at short intervals immediately after an initial dose of pegylated interferon and ribavirin and correlated these with HCV RNA levels using bDNA technology (Bioreference Labs, Elmwood Park, NJ) in a single interferon naive patient with HCV-MC. BAFF levels in HCV-MC patients after rituximab therapy were significantly higher, 998±1734 pg/ml than the control group of HCV-MC patients that had not received rituximab, 2927±3464, p<0.0009. Both BAFF and HCV RNA levels rose after an initial decline. The changes in HCV RNA after interferon correlated extremely closely with BAFF, r = 0.93, p=0.006 (fig 1). These data suggest an important role for BAFF in the control of the mature B cell pool in man. The very close relationship between HCV RNA and BAFF raises the interesting possibility that the cytokine might be induced by direct viral activation and could begin to explain the lymphoproliferative effects of this virus.
INVolvEMENT OF REGULATORY T CELL DYNAMICS IN THE ACHIEVEMENT OF BIOCHEMICAL RESPONSE IN 48-WEEK PEG-INFNα2b AND RIBAVIRIN COMBINATION THERAPY FOR CHRONIC HEPATITIS C PATIENTS

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Aim: In Peg-INFNa and ribavirin combination therapy for chronic hepatitis C, the therapy might be discontinued in patients who fail to become negative HCVRNA by week 24 of the therapy, since the probability of SVR in them is extremely low. It is reported that the progression of fibrosis is slow and the incidence of hepatocellular carcinoma is low in patients who achieved biochemical response (BR), even if they failed to eradicate HCV. Therefore, in order to prevent hepatocarcinogenesis in patients who fail to eradicate HCV, it is clinically important to continue the therapy in patients who would achieve BR. Regulatory T cells (Treg) are capable of suppressing antigen-specific and non-specific T cell responses, which is reported to be increased in chronic hepatitis C. We reported that Treg function is enhanced in patients with persistently normal ALT levels (PNALT) than in patients with active hepatitis, suggesting that Tregs possess minor roles in virological response. In parallel with such increase of Treg, plasma TGF-β level in BR patients was higher than those in non-BR, suggesting its possible role in Treg induction. Conclusion: Increase of Tregs may be involved in biochemical response in PEG-INFNα and ribavirin combination therapy, being independent of virological response.

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474 ADIPONECTIN: A NEW INDEPENDENT PREDICTOR OF LIVER STEATOSIS AND RESPONSE TO IFN-A TREATMENT IN CHRONIC HEPATITIS C?

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Background/Aims: Adiponectin is an insulin-sensitizing adipokine with anti-inflammatory properties. In the past year, 3 research groups have investigated serum adiponectin in chronic hepatitis C (CHC) with respect to the patients’ histological and biochemical characteristics. However, the relationship between IFN-a therapy and serum adiponectin has not been investigated in detail. We conducted a study in order to: compare serum adiponectin levels between CHC and chronic hepatitis B (CHB) patients; investigate for associations between adiponectin and histological or viral characteristics of CHC; investigate adiponectin alterations during IFN-a treatment and to assess the relationship between adiponectin and response rates to treatment. Methods: Adiponectin concentrations were determined from serial samples (before, the middle, the end of treatment and 6 months after end of treatment) from 83 CHC and 59 CHB patients. 43 donors served as healthy controls. Patients treated with IFN-a (4.5 MU/tiw) for 12 months in CHB and IFN-a (3 MU/tiw) plus ribavirin for 6-12 months according to HCV-genotype in CHC. Results: After adjustment for body mass index (BMI) and gender, CHC patients infected with HCV-genotype 3 had significantly lower adiponectin levels at baseline compared to those patients with non-3 HCV-genotypes (p<0.05). Lower serum adiponectin at baseline identified as an independent predictor of liver steatosis (HCV-genotype 3; p=0.02 and HCV-genotype 1; p=0.025) and for no virologic response at the end of treatment (OR 0.76; 95% CI: 0.66-0.87; p<0.001). At the end of IFN-a, HCV-genotype 3 patients had significantly higher serum adiponectin compared with their levels before the initiation of treatment (p<0.05). Conclusions: This study suggests that HCV-genotype 3 may directly affect adiponectin. This is further supported by the significant increase of adiponectin in HCV-genotype 3 patients at the end of treatment compared to their pretreatment levels. The direct implication of IFN-a seems unlikely, as similar increase of adiponectin was not observed in CHB and CHC patients infected with non-3 HCV-genotypes. Serum adiponectin at baseline appears to be an independent predictor of liver steatosis and for the achievement of end of treatment virologic response irrespective of the HCV-genotype. We believe that further efforts should be done in an attempt to corroborate our findings regarding SVR.
and to evaluate the possible usefulness of a potential increase of serum adiponectin using novel molecules or treatments leading to an increase of adiponectin (e.g. thiazolidinediones) before the initiation of antiviral treatment in CHC patients.

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475 ABERRATIONS OF CELL CYCLE MACHINERY IN CHRONIC HEPATITIS C INFECTION
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Introduction: Increasing scientific evidence suggests that Hepatitis C Virus (HCV) manipulates the cell cycle machinery during the course of HCV-associated pathogenesis. Considering that the cell cycle proteins play a fundamental role in the regulation of cell growth, HCV-associated disruption in their function contributes to disease progression and, subsequently, hepatocellular carcinoma. Objective: To identify cell cycle disruptions during the course of chronic HCV disease. Methods: Liver biopsy samples classified on histological basis as early (Stage 1-2) or advanced (Stage 3-4) fibrosis were used to extract RNA. Pooled RNA samples from normal liver (NL), HCV-early fibrosis (EF) and HCV-advanced fibrosis (AF) groups were analyzed by RT-PCR cell cycle array (Superarray). Additionally, protein expressions of differentially expressed cell cycle genes (TP53, CDKN1B and CDKN2B) were studied in same groups by immunohistochemistry. Results: PCR array analysis of cell cycle genes showed more than two fold change in 36% of the transcripts in EF as compared to normal liver sample. 10% of these were significantly differentially expressed including positive regulators of cell cycle [Mcm-2, cyclin E1] and G1, G2/M phase checkpoint genes [CDKN1B, GADD45A and KNTC1]. In EF versus AF comparison 23% showed more than 2-fold change, 50% of which were significantly differentially expressed including G1 inhibitor CDKN2B and tumor suppressor p53. Evaluation of protein expression shows decreased immunopositivity for p53 in AF as compared to EF, however, in case of p15 and p27 mRNA and protein levels are concordant. Conclusion: Based on the observations in the expression pattern in EF, it can be suggested that the up-regulation of cell cycle activators along with G1, G2 and M checkpoint regulators are seen as an early event in HCV-associated liver injury, however, in AF a different set of inhibitors was found up-regulated and tumor suppressor p53 is down-regulated that might predisposes cells to malignant transformation.

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476 SELECTION, EXPANSION AND FUNCTIONAL RESTORATION OF NS3-SPECIFIC CD4 T CELLS FROM HCV-INFECTED PATIENTS
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Introduction: Tolerance of T cells to non-structural antigens of hepatitis C virus may explain persistent infection. We hypothe-
E6AP-mediated ubiquitin-proteasome pathway plays some roles in HCV life cycle and viral pathogenesis.

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479 HUMAN MONOCLONAL ANTIBODIES RECOGNIZING A CONSERVED HCV E1 DOMAIN EFFECTIVELY NEUTRALIZE HCV IN THE CELL CULTURE (HCVCC) SYSTEM

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Background: Until recently, neutralizing antibodies for HCV have been shown to be directed against the E2 envelope protein. In this study we explored the properties of an E1 epitope region recognized by three different human monoclonal antibodies (humAbs) with strong neutralizing capacity against HCV pseudoparticles. Methods: Epitope mapping of neutralizing and non-neutralizing antibodies directed against E1 was initially performed with overlapping peptides. Detailed analysis of the neutralizable epitope region was done by alanine scan, analysis of natural variants, and affinity measurements. For the neutralization assays, the HCVcc genotype 2a (JFH1) system was used. Results: Neutralizing humAbs against E1 were mapped to an extremely well-conserved E1 domain (amino acid 313-326), which was bound with high affinity (≈10^{-10} M). Several non-neutralizing anti-E1 humAbs also recognized this domain, but detailed epitope mapping revealed recognition patterns that were different for non-neutralizing and neutralizing antibodies. The alanine scan revealed a remarkable flexibility of the neutralizing antibodies in recognition of variant peptides. This was confirmed by investigating reactivity with natural variants representing 94% of known E1 sequences, and is in line with the previously observed broad cross-genotype neutralization in the HCVpp system (Meunier et al., Hepatology 2006,44, suppl 1, 344A). Since the HCVcc system is expected to mimic HCV more closely than the pseudoparticle system, two anti-E1 humAbs were further tested for their neutralizing capacity against HCVcc. Both humAbs could effectively neutralize genotype 2a infectious HCV in vitro. The different properties of the neutralizing humAbs allowed selection of one antibody for development of a passive immunotherapy candidate for application in HCV-related liver transplantation. Conclusion: The E1 protein contains a neutralizable domain consisting of several epitopes, which resides in the most conserved region of the entire HCV envelope. Several humAbs were identified that recognize this domain and which display unique properties with respect to cross-genotype recognition and neutralization.

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CELL CULTURE-PRODUCED HEPATITIS C VIRUS IMPAIRS PLASMACYTOID DENDRITIC CELL MATURATION AND FUNCTION VIA DIRECT INTERACTION, BUT NOT INFECTION

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Previous studies suggested a functional impairment of dendritic cells (DCs) in patients with chronic hepatitis C. To investigate whether this effect was mediated by a direct interaction of HCV with DCs we studied the effects of cell culture-derived, infectious HCV (HCVcc) on ex vivo isolated plasmacytoid and myeloid DCs and on in vitro generated monocyte-derived DCs of healthy blood donors. HCVcc inhibited maturation and TLR9-mediated IFN-α production by plasmacytoid DC. This inhibitory effect did not require HCV infection of plasmacytoid DCs, because it was also observed in response to UV-inactivated, non-infectious HCVcc, and it was not abrogated by neutralizing antibodies. Infection of plasmacytoid DCs with influenza A virus restored maturation and TLR9-mediated IFN-α production. In contrast to its effect on plasmacytoid DCs, HCVcc inhibited neither TLR3- and TLR4-mediated maturation nor IL-12, IL-6, IL-10, IFN-γ and TNF-α production by myeloid DCs and monocyte-derived DCs. Likewise, HCVcc did not alter the capacity of myeloid DCs and monocyte-derived DCs to induce CD4 T cell proliferation. While phagocytosis of apoptotic hepatoma cells by monocyte-derived DCs resulted in DC maturation, this effect was independent of whether the phagocytosed hepatoma cells were infected with HCV or not. In conclusion, HCVcc inhibited maturation and IFN-α production of plasmacytoid DCs, but not myeloid and monocyte-derived DCs via a direct interaction that did not require infection. The response of plasmacytoid DCs to influenza A virus infection was not impaired, consistent with the observation that HCV-infected patients are not impaired in their immune response to influenza A virus.

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HEPATITIS C P7 PROTEIN ALTERS THE PROTON CONDUCTANCE OF INTRACELLULAR MEMBRANE VESICLES

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The hepatitis C virus (HCV) p7 protein functions as a virus-encoded ion channel, but its role in the virus life cycle is not known. A similar viroporin, the m2 protein of influenza virus, provides a proton conductance that prevents acidification during viral particle exocytosis and an analogous function has been proposed for HCV p7. The AIM of this study was to determine whether p7 mediates proton movement and pH changes in intracellular vesicles. METHODS: Flag-tagged HCV p7 and a channel defective mutant (p7KR) were expressed in 293T cells. Subcellular distribution was examined by immunofluorescent confocal microscopy and membrane biotinylation. Intracellular vesicles were isolated by density gradient centrifugation. Proton movement in isolated vesicle fractions was assessed using the fluorescent indicators acridine orange (AO), which quenches in acidic compartments, and the pH sensitive fluorophore, carboxyfluorescein. RESULTS: When expressed in 293T cells, p7 displayed a diffuse intracellular distribution that overlapped with ER. Plasma membrane localization could not be detected by cell surface biotinylation. To assess proton movement, isolated vesicles were added to a pH 7.4 AO solution and pH changes were measured. When vesicles had been pre-equilibrated at pH 7.4, addition resulted in a rapid fluorescence increase as AO associated with the lipid membrane. There were no subsequent fluorescence changes. The situation differed when vesicles were pre-equilibrated at pH 5.5. In this case, both mock transfected and, mutant p7KR transfected vesicles behaved as before causing a rapid initial increase and no subsequent change. However, the wild-type p7 containing vesicles caused a transient decrease in fluorescence compared to control which persisted for approx. 90 sec. This fluorescence quenching event thus required the presence of both p7 and a transversable pH gradient. To confirm that this change reflected proton movement across the vesicle membrane, we performed preliminary experiments after incorporating the pH indicator, carboxyfluorescein, into vesicles. Vesicles were treated with the K ionophore, valinomycin, and exposed to an inwardly directed K gradient to create an electrochemically positive vesicle interior. When the vesicles contained p7, this maneuver alkalized the vesicle interior. In mock transfected vesicles, the inward K gradient did not alter intravesicular pH. CONCLUSIONS: The HCV p7 protein allowed transvesicular proton fluxes markedly greater than those seen in vesicles from either mock transfected or p7KR mutant transfected cells. This indicates that p7 could serve as a pH equilibrating agent in the HCV lifecycle.

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INTERFERON (IFN) SENSITIVITY OF NATURAL RECOMBINANT RF1-2K/1B HCV STRAIN IN CHIMERIC UPA/SCID MICE

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Background: Transplanted with human liver, urokinase-type plasminogen activator-transgenic SCID mice (chimeric mice) became recently available for studying in vivo replication of the hepatitis C virus (HCV), and its sensitivity for antiviral drugs. HCV genotype is an important determinant of the virological response to antiviral therapies. A series of recent reports described natural inter-genotypic recombinants isolated from HCV-infected individuals. Aim: To assess usefulness of the chimeric mice in maintaining replication of the natural recombinant HCV RF1 RF1_2k/1b (RF1) strain, and to test the effect of the recombinant on the interferon sensitivity of the virus. Methods: A cohort of chimeric mice was infected with human sera obtained from RF1 HCV-carrier. Two cohorts used as control were; [1b-SVR] infected by a stored “base-line” serum from HCV-1b-infected patient, who had sustained virological response, and [1b-NR] infected by an “in-treatment” serum from another 1b-infected patient who had null response to Peg-IFN & Ribavirin treatment. The mice were treated by 3 intraperitoneal injections of pegylated interferon alpha-2a using two different dose-regimens; 3 and 30 mg/kg. Results: Mean HCV titer in the
1b-NR and RF1 groups reached plateau at 7.6 and 7.0 log copies/ml, respectively by the day 14 after infection, whereas titer of the 1b-SVR rose to 8.5 log until day 21 and then reached plateau at 8.0 log. At day 35 after infection, the interferon was administrated in dose of 3 mg/kg three times (day 0, 2, 5). Relative trends were seen when HCV titer at Day 0 was taken as 100% and titers at consecutive Day 2, 5 and 7 were calculated respectively; by the day 7, HCV-RNA levels of 1b-SVR and RF1 were 22-23%, whereas in 1b-NR it remained unchanged. Restoration of the baseline levels for 1b-SVR and RF1 observed on day 9 and 12 after injection, respectively. After confirmed stabilization of the HCV titers, second course was launched with dose of 30 mg/kg (day 0, 2, 5). This resulted in significant reduction of the viral titers at log scale; compared to the baseline (day 0 of the second course), more than 2.0 log copies/ml reduction was observed by the day 7 in HCV titer of the 1b-SVR (2.3) and RF1-infected mice (2.7) that was lower than in 1b-NR (1.4 log, p<0.04). Interestingly, sequencing of the previously reported interferon sensitivity determinant region revealed wild-type in 1b-NR, mutant in 1b-SVR and intermediate in RF1. Conclusion: Chimeric mice model is useful for a natural recombinant HCV replication. The HCV-2k/1b is sensitive to the Peg-IFN, suggesting that the variant is useful for a natural recombinant HCV replication. The HCV-2k/1b is sensitive to the Peg-IFN, suggesting that the variant is useful for a natural recombinant HCV replication.

C. Coito, S. Kota, D. Willoughby, T. Tellinghuisen, D. Strosberg

**483 PRODUCTION OF INFECTIONOUS GENOTYPE 1B HEPATITIS C VIRUS IN HUMAN HEPATOMA CELLS**

Carlos Coito, Smitha Kota, David Willoughby, Timothy Tellinghuisen, Donny Strosberg

Chronic infection with Hepatitis C virus (HCV) afflicts over 170 million people world-wide. The only effective treatment, pegylated interferon combined with ribavirin, displays considerable differences in efficacy. Notably, strains of genotypes 2 and 3 are cleared in up to 80% of infected patients, while strains of genotype 1 are cleared in only about 40% of patients. To gain a better understanding of these genotype-specific differences, considerable efforts have gone into the development of an in vitro mammalian monolayer cell culture system to assess mechanisms of action of treatment regimens on infectious HCV. Infectious HCV cell culture systems have been described for genotypes 2a, (ref.1-3), and more recently genotype 1a (4), but an infectious cell culture system, combined with previous 2a systems, should significantly improve our understanding of how HCV genotype relates to susceptibility to anti-HCV therapy.


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**484 THE CHOLESTEROL AND SPHINGOLIPIDS OF HEPATITIS C VIRUS PARTICLES PLAY CRITICAL ROLES IN THE VIRAL INFECTIVITY**

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Background and aims: It is known that enveloped viruses are highly dependent on viral lipids for infection of the host cells. Although association of lipids with HCV particles has been suggested, its role in the viral assembly and infection is poorly characterized. The aim of this study is to assess the requirement for lipids within the viral envelopes in HCV infection. Methods: Infectious HCV particles (HCVpp) derived from JFH-1 are used for lipid composition analysis, cholesterol-depletion assay, sphingomyelin-hydrolysis assay, inhibition analysis of sphingolipid biosynthesis, and viral binding assay. Results: Lipid composition analysis of HCVpp and cellular membranes demonstrated that the cholesterol to phospholipids molar ratio, which is known as a major parameter of membrane viscosity, is significantly higher in HCVpp compared to that in total cellular membrane fraction, suggesting that HCV virions are enriched with cholesterol through the course of their assembly and maturation. Upon depletion of cholesterol from HCVpp with methyl-beta-cyclodextrin (B-CD), infectivity was almost completely abolished. Replenishment by adding exogenous cholesterol restored infectivity to that of untreated control. Sphingomyelinase (SMase)-mediated hydrolysis of viral surface sphingomyelin also reduced infectivity. Furthermore, we found that inhibitors for serine palmitoyltransferase (Myriocin) or for ceramide trafficking from ER to Golgi ((1R, 3R)-N-(3-hydroxy-1-hydroxymethyl-3-phenylpropyl) dodecanamide) suppress HCVpp production but not RNA replication of JFH-1 replicon. These data suggested that cholesterol and sphingomyelin are important for viral particle formation and/or secretion. Sphingolipids and cholesterol are known to be major components of lipid rafts. The insolubility in nonionic detergents suggested that subpopulations of HCV structural proteins are associated with lipid rafts in HCV-infected cells, and that transmembrane domains of envelope proteins are important for this association. B-CD- or SMase-treatment of HCVpp strongly inhibits the viral binding to cells. Conclusions: We suggest that cholesterol and sphingomyelin associated with HCV virions play critical roles in the early step of the infection. These studies provide important insights into the mechanism of assembly and budding of HCV and also suggest novel potential inhibitors targeting HCV particles.
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mura, Takaji Wakita, Tetsumo Suzuki

485 NEUTRALIZING HOST RESPONSES IN HEPATITIS C VIRUS INFECTION TARGET VIRAL ENTRY AT POST-BIND-
I NG STEPS AND MEMBRANE FUSION

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Hepatitis C virus (HCV) is a leading cause of chronic hepatitis worldwide. Viral attachment and entry - representing the first encounter of the virus with the host cell - are major targets of adaptive host cell defences. The mechanisms of antibody-mediated neutralization by host neutralizing responses in HCV infection are only poorly understood. Retroviral HCV pseudotypes (HCVpp) have been successfully used for the study of viral entry and antibody-mediated neutralization. In this study, we used this model system to study the mechanism of antibody-mediated neutralization by monoclonal anti-envelope antibodies and polyclonal anti-HCV immunoglobulin purified from HCV-infected patients. Using a panel of monoclonal anti-E1 and anti-E2 antibodies, we show that anti-envelope antibodies neutralize HCV entry during various steps of HCV entry including binding and post binding events. Interestingly, we observed that host neutralizing responses in the majority of HCV-infected individuals target HCV entry during an entry step occurring post binding of virus to the target cell membrane. Using a kinetic assay based on HCVpp entry, we demonstrate that purified antiviral immunoglobulins derived from defined HCV infected individuals appear to inhibit HCV infection during an entry step closely linked to CD81. Using a recently developed HCVpp-based membrane fusion assay, we identified one individual with neutralizing immunoglobulins able to inhibit HCV fusion. This inhibition could occur through steric or allosteric blocking of the HCV E1E2 glycoproteins and could prevent conformational rearrangements required to promote fusion with their target membrane. Our results indicate that host neutralizing responses in HCV-infected humans target HCV entry at a post binding step most likely related to HCV-CD81, SR-B1 or claudin-1 interaction or membrane fusion. These findings may have important implications not only for the understanding of the pathogenesis of HCV infection but also for the design of novel immunotherapeutic and preventive vaccine approaches.

Disclosures:
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486 HEPATITIS C VIRUS NSSA BINDING TO NUCLEOSOME ASSEMBLY PROTEIN 1 (NAP 1) ACCELERATED HCV REPLICATION

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Background & Aims: Hepatitis C virus (HCV) non-structural protein, NSSA, was reported to counteract host interferon-induced antiviral response. Recently, it was suggested that NSSA binds with various host factors, and plays an important role in HCV replication as well as modulates host intracellular signaling pathways. The aim of this study is to determine host NSSA binding protein and its effect on HCV replication. Methods: A HuH7 cell line stably expressing FLAG-tagged HCV NSSA was generated. Then, FLAG-tagged NSSA and its binding proteins were co-immunoprecipitated using anti-FLAG antibody. FLAG-tagged NSSA binding proteins were sequenced using 2D nano LC-MS/MS system. Binding of NSSA and target protein was determined using confocal fluorescence microscopy. Then, the target gene was knocked down by RNA interference technique in HuH7 cells. The effect of knocking down target gene on HCV replication was observed using a recently developed genotype 2a full-length HCV (JFH1) replication and infection system. Results: Nucleosome assembly protein 1 (Nap1) was identified as an NSSA binding protein. Nap1 is a histone chaperone and a chromatin-assembly factor having additional roles in transcriptional regulation, apoptosis, histone shuffling, and cell-cycle regulation. NSSA and Nap1 co-localized mainly in the cytoplasm. Replication of JFH1 was significantly reduced in Nap1 knocked-down HuH7 cells. Conclusions: HCV NSSA binds with Nap1, and this is crucial for HCV replication. Nap1 could be a target for anti-HCV drug development.

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**HLA CLASS I ASSOCIATED SEQUENCE POLYMORPHISMS IN HCV REVEAL REPRODUCIBLE PATTERNS OF IMMUNE ESCAPE – TOWARDS A COMPREHENSIVE MAP OF KEY RESIDUES FOR VACCINE DESIGN**


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**HEPATITIS C RESOLUTION CORRELATES WITH PATTERNED KIR RECEPTOR EXPRESSION ON KILLER LYMPHOCYTES**

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**Background:** Inhibitory killer immunoglobulin receptors (KIR) on lymphocytes bind to HLA class I ligands and downmodulate immune defense against infection. Antibodies to KIR can block inhibitory effects and they may augment antiviral responses to HCV. Genes for weakly inhibitory KIR-ligand pairs are associated with HCV resolution, while strongly inhibitory KIR-ligand pairs are associated with viral persistence. Reported effects of KIR genes on HCV are modest, perhaps due to confounders that cannot be controlled by genotyping alone. Individuals with similar KIR-HLA genotypes exhibit great variability in KIR expression among their lymphocyte subsets. Also, individual allelic polymorphisms of a single KIR gene may affect receptor expression and function. Aim: To correlate KIR expression patterns on lymphocytes with HCV resolution. Methods: Flow cytometric analyses of KIR were performed on natural killer (NK) and T cell subsets from injection drug users (IDU) with chronic or resolved HCV. NK (CD56+/CD3-) cells and CD3+ T cells were stained with antibodies directed against KIR2DL3, KIR2DL1, KIR3DL1/3DS1, and KIR3DL2. The percentage of NK cells and T cells staining for individual and multiple KIR receptors was quantified. Phenotypic factors associated with HCV resolution were analyzed using Student’s t-Tests, univariate and logistic regression analyses. Results: Among 46 patients, 18 were HCV resolvers and 28 were chronically infected. The percentage of NK cells expressing the weakly inhibitory KIR2DL3 was higher in resolvers than in chronic patients (p=0.06). Conversely, there was a trend toward a greater percentage of NK cells expressing the strong inhibitor KIR2DL1 in chronically infected HCV patients compared with resolvers. The ratio of KIR2DL3/KIR2DL1+ NK cells was greater than unity in 16 of 18 (89%) resolvers, compared with only 14 of 28 (50%) chronically infected patients (p=0.06). NK cell staining with an antibody reactive against both KIR3DL1 and KIR3DS1 was highly variable, with a correlation toward higher levels in chronic patients (p=0.07). T cell expression of KIR was low, but ranged up to 5% in some chronic patients. Linear regression analyses show that the expression of KIR2DL3 and the KIR2DL3/KIR2DL1 >1 were independently associated with resolution (R=0.43, p=0.055). Conclusion: These preliminary data support the hypothesis that NK cell KIR phenotypes for KIR2DL3 and KIR2DL1 play a primary role in the regulation of HCV immunity. Further phenotypic and correlating genotypic analyses will further elucidate the contribution of KIR to HCV immunity.

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**MAP OF KEY RESIDUES FOR VACCINE DESIGN**
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INTERACTIONS OF CXCL-8 WITH INNATE ANTIVIRAL DEFENSES DURING HCV INFECTION

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Background: The alpha chemokine, CXCL-8 (interleukin 8) has been previously shown to be induced by HCV infection and inhibit the antiviral actions of IFN alpha. In the current study, we investigated induction of CXCL-8 by HCV, the effects of CXCL-8 on HCV replication, and whether CXCL-8 blocks IFN signaling. Methods: Genomic or subgenomic replicon cell lines, or direct JFH-1 infection of Huh7 and Huh7.5.1 cells, were used to study the effects of CXCL-8 on HCV replication. Human dendritic cells (DC) were exposed to apoptotic cells expressing HCV proteins and tested for changes in gene and protein expression, and IFN signaling. Results: HCV dsRNA induction of the RIG-I pathway-activated CXCL-8 transcription via recruitment of IFR-3 to the CXCL-8 promoter. The half-life of CXCL-8 mRNA increased in 3 of 4 HCV replicon cell lines, particularly after treatment with TNF-alpha. CXCL-8 protein levels correlated positively with HCV RNA levels in 4 replication lines (R = 0.41, P = 0.0013). In contrast, CXCL-8 mRNA levels correlated inversely with CXCL-8 protein and HCV RNA levels in all cells. HCV RNA synthesis in BB7 replicon cells was inhibited by CXCL-8 silencing. Huh7 and replicon cells expressed CXCR1 but not CXCR2, yet CXCL-8 did not inhibit IFN alpha signaling in these cells, even when both receptors were over-expressed. In contrast, when exposed to NSSA intracellularly, DCs expressed CXCL8, which was associated with inhibition of basal and IFN-induced Stat1 and Stat2 phosphorylation. Conclusions: The data indicate that: 1) HCV infection triggers dsRNA signaling pathways that induce CXCL-8 via transcriptional activation and mRNA stabilization, 2) CXCL-8 protein levels are positively associated with HCV replication, 3) CXCL-8 does not block IFN signaling in Huh7 cells, and 4) DCs exposed to NSSA increase CXCL8 expression which is associated with blockade of IFN signaling. The results suggest that HCV protein-induced CXCL-8 can block the IFN-alpha pathway at multiple levels, depending on the cell type and the manner by which the HCV proteins are encountered by the cell.

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A NOVEL IRES IN THE CORE-ENCODING REGION STIMULATES PRODUCTION OF MINI-CORE, A SMALL PROTEIN COMPRISED OF THE C-TERMINAL PORTION OF THE CORE PROTEIN

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The HCV core gene is a complex genetic element whose full coding and regulatory functions remain unknown. 293T cells transfected with in vitro transcribed RNA containing HCV core gene sequences were analyzed by Western blot. A unique monoclonal antibody directed against core amino acids 104-110 detected a p9 protein designated “mini-core”. Mini-core had not been detected previously because it contains only the C-terminal portion of core and does not react with the more widely used anti-core antibodies that recognize the N-terminal portion. While mini-core is produced from both 1a and 1b expression constructs that extend from the 5’UTR through 2/3 of E1; genotype 1b constructs produce more. Cellular stress (Na arsenite) increases the amount of mini-core. Mini-core is also expressed from a full length1a/2a (H77/JFH) replication-competent virus in Huh7.5 cells. Investigations into mini-core production revealed that the core-encoding region may contain a novel IRES that is able to drive mini-core expression. Deletion

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GENOMIC ANALYSIS OF HCV BREAKTHROUGHS OCCURRING DURING THE HALT-C TRIAL LEAD-IN TREATMENT PHASE

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Background: The various hepatitis C virus (HCV) genotypes have different rates of clearance following interferon (IFN) and ribavirin (RBV), suggesting that the virus itself determines therapy response. In the HALT-C study lead-in phase, patients received 48 weeks of therapy if virus was undetectable at wk 20. Viral breakthrough (BT) was observed when HCV RNA reappeared at wk 36 or 48. We analyzed NSSA sequence changes over time in BT patients since this protein has been considered important to IFN resistance. Methods: Of 1,045 patients, 43 patients had breakthrough but, of these, only 12 patients had taken full dose (>80%) of peg-IFN and RBV. The entire NSSA region was amplified with RT-PCR and the amplification products sequenced from sera from baseline (BL), wk12 and at the time of BT (either wk36 or 48) from the 12 BT patients. Sera from BL, wk12 and wk24 from 2 matched non-responders (NR) for each BT patient were also sequenced. The number of synonymous and non-synonymous substitutions, total number of amino acid (aa) substitution and error rate were calculated by comparing BL to wk12 and BT time points. For NR patients we compared BL to wk12 and wk24. Results: BT HCV genotypes were 1a in 6 patients, 1b in 4 and 3a in 2; thus far, we have analyzed all 1a time points only. The 1a BT patients [median(range): 4(2-5) vs. 1(0-5), P=0.01], but BT patients and NR patients had comparable number of synonymous substitutions [median(range): 22(3-38) vs. 4(3-31), P=0.09]. At the protein level, BT patients also demonstrated significantly higher numbers of aa substitutions compared with NR patients [median(range): 3.5(2-5) vs 1(0-5), P=0.03]. When the later BT time point sequences (or wk24 sequences from NR patients) were analyzed, both groups had comparable median number of synonymous substitutions and non-synonymous substitutions and total number of aa substitution (P=NS). The error rates of nucleotide (nt) and aa at the time of breakthrough are 2.8X10-2 nt/site/ys and 1.5X10-2 aa/site/ys respectively in BT patients and 1.72X10-2 nt/site/ys and 9.7X10-3 aa/site/ys at wk24 in NR patients (both P=NS). Conclusions: The higher number of non-synonymous and aa substitutions at wk12 compared with NR patients [median(range): 4(2-5) vs 1(0-5).150, P=0.01], but BT patients and NR patients had comparable number of synonymous substitutions [median(range): 22(3-38) vs. 4(3-31), P=0.09]. The following people have nothing to disclose: Hejun Yuan, Mamta K. Jain, Michael Gale, William M. Lee
mutagenesis mapped this second IRES to the region between codons 56 and 154. Transfected DNA lacking the first 55 codons of the core gene not only retains the ability to express mini-core, but is greatly enhanced for mini-core production, as expected if core and mini-core compete for translational initiation. Constructs with codons 1-154 fused to a 3XFLAG-tag express (truncated) mini-core, whereas constructs with codons 1-125 do not. RNA transcripts lacking both a 5’ cap and the conventional HCV IRES express both mature core and mini-core, providing further evidence that a novel IRES activity is embedded within the core gene. (NIH DAO16156, DK066939 and ALF Scholar’s Award).

492 DEVELOPMENT OF JFH1-BASED INTERGENOTYPIC CELL CULTURE SYSTEMS OF HEPATITIS C VIRUS GENO-TYPES 1-5 AND THEIR USE IN STUDIES OF VIRAL ENTRY AND NEUTRALIZATION

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Recently the first full hepatitis C virus (HCV) life cycle culture systems were developed for the genotype 2a strain JFH1, as well as for the intragenotypic recombinant J6/JFH in which Core through NS2 of JFH1 were replaced by the corresponding genes from the genotype 2a strain J6CF. It has been reported that efficient growth of JFH1 in the hepatoma cell line Huh7.5 depends on adaptive mutations. However, in the present study we found that J6/JFH could be cultured efficiently without the need for adaptive mutations. We aimed at establishing cell culture systems for the different major genotypes of HCV. Utilizing the unique replication characteristics of JFH1 we developed a panel of intergenotypic recombinant HCV cDNA clones by insertion of the structural genes (Core, E1, E2), p7, and NS2 of genotype 1a, 1b, 1b, 2a, 3a, 4a, and 5a prototype isolates, respectively, into the JFH1 backbone. After transfection of RNA transcripts into Huh7.5 cells, viral spread occurred after an eclipse phase for all recombinants, except 2b/JFH1, which spread immediately. However, after serial passage the released intergenotypic viruses showed growth kinetics, genome titers and infectivity titers comparable to the intragenotypic recombinant J6/JFH control virus. The HCV RNA and infectivity titers in culture supernatant peaked at approximately 7 log10 IU/mL and 5 log10 50% tissue culture infectious doses (TCID50) per mL, respectively. By sequence analysis of the complete open reading frame of recovered viral genomes, we identified putative adaptive mutations for the intergenotypic recombinants and analyzed their role by testing them singly and in combination in reverse genetic studies. Thus, we identified adaptive mutations in p7 and NS3 for 1a/JFH1 and 3a/JFH1, in NS2 and NS3 for 1b/JFH1, and in NS2 for 4a/JFH1 and 5a/JFH1 recombinants that promoted efficient growth in Huh7.5 cells. Others have found that JFH1 infection depends on the putative co-receptor CD81. In initial studies we have demonstrated anti-CD81 dependent inhibition of 3a/JFH1, 4a/JFH1 and 5a/JFH1 infection in Huh7.5 cells. Thus, CD81 appears to be important for entry of all genotype viruses. Furthermore, we have demonstrated homologues serum neutralization of 100 TCID50 of 4a/JFH1 (100% neutralization) and 5a/JFH1 (>98% neutralization) viruses. The 4a/JFH1 and 5a/JFH1 viruses were furthermore cross-neutralized using genotype 1a serum. In summary, we developed intergenotypic recombinant cell culture systems for 5 of the 6 major HCV genotypes, which will be an important research tool for genotype specific functional studies of Core, E1, E2, p7 and NS2, and for related therapeutics.

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493 PLAQUE-FORMING ASSAYS FOR HEPATITIS C VIRUS AND ISOLATION OF HCV-JFH1 MUTANTS WITH ENHANCED CYTOPATHOGENICITY AND REPLICATION CAPACITY

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Background and Aim: The mechanisms of hepatitis C virus-induced liver damage are not understood completely. HCV-JFH1 culture in-vitro (Wakita, Nature Medicine 2005) results in massive cell death, which suggests the involvement of HCV-induced cytopathic effects. Therefore, we investigated the mechanisms and viral nucleotide sequences involved in this effect using HCV-JFH1 cell culture and a newly developed cytopathic plaque-forming assay. Method: In-vitro synthesized HCV-JFH1 RNA was transfected into Huh-7.5.1 cells (Zhong, PNAS 2005) and the virus-containing culture supernatant was inoculated serially onto naive cells. Virus replication was measured by HCV core antigen assay and real-time RT-PCR. Viral cytopathogenicity was measured using the plaque-forming assay. Results: The HCV plaque-forming assay showed the appearance of cytopathic plaques, depending on the titer of the inocula. In the virus-infected cells, the ER stress markers, GRP 78 and phosphorylated eIF2-alpha, were overexpressed. Cells in the plaques were strongly positive for the apoptosis marker, annexin V. Isolated virus subclones from individual cytopathic plaques had acquired substantially greater replication efficiency and cytopathogenicity than the parental virus strain. The cytopathic JFH1 subclone, Pl #1, had 9 amino acid substitutions, of which 5 were clustered in the C terminal of NS5B region. The 3 amino acid substitutions of NS5B region were redundantly appeared in the 4 plaque-isolated clones and in all three Pl #1-derived subclones. Conclusion: HCV infection and replication showed cytopathic effects that were characterized by ER-stress-induced apoptotic cell death. HCV nucleotide sequences in certain regions might determine the viral replication and cytopathogenicity.

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494 ANTI-MALARIAL DRUG CHLOROQUINE SUPPRESSES THE REPLICATION OF HCV REPLICON VIA PKR-INDEPENDENT MECHANISMS

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Background: It has been reported that autophagy plays a pivotal role in the replication of some RNA viruses. We previously reported that chloroquine treatment, which inhibits lysosomal and autophagic acidification, prevents replication of HCV replicon. However, it is unclear whether continuous chloroquine treatment keeps an antiviral effect. The mechanisms by which chloroquine inhibits the replication of HCV replicon has not been characterized. Here, we confirmed an antiviral effect of continuous chloroquine treatment and determined if the treatment of chloroquine activates interferon signaling and blunts autophagic protein degradation.

Methods: Huh-7 cells transfected with HCV replicon expressing firefly luciferase (Huh7/Rep-Feo) were used for this study. Cells were cultured with chloroquine (10^{-5} M) and/or IFNα (100 U/ml) for 7 days, then continued to incubate without drugs for another 21 days. Replication levels of HCV replicon were determined by luciferase assay at 7th and 21th day from cessation of drugs. Expression of phospho-PKR (p-PKR) and microtubule-associated protein-1 light chain 3 (LC3) were detected by western blot analysis. Alteration of autophagic proteolysis was evaluated by protein degradation assay. The number of autolysosome in cytoplasm was counted by using transmission electron microscopy.

Results: Luciferase activity was undetectable in cells treated with IFNα for 7 days; however, it reappeared when cells were subsequently cultured without IFNα for 21 days. In sharp contrast, co-incubation with IFNα and chloroquine for 7 days suppressed HCV replication for the extensive period up to 21 days even in the absence of these drugs. On the other hand, P-PKR was detected in cells treated with IFNα for 48 hours, but it was not observed in cells treated with chloroquine. Marked accumulation of autolysosome was observed in cytoplasm after 18 hours chloroquine treatment. Chloroquine treatment induced the expression of LC3-II to 3-fold over control after 4 hours. Moreover, chloroquine treatment for 4 hours significantly decreased degradation of leucine to about 76% of control value.

Conclusions: These findings indicate that chloroquine treatment possesses a powerful antiviral effect to prevent re-propagation of HCV replicon in combination with IFNα through the unique mechanism independent on interferon signaling pathway. It was hypothesized that suppression of autophagic protein degradation contributes to antiviral effect of chloroquine. The efficacy of chloroquine against HCV replication might provide a new therapeutic option for the patients with chronic hepatitis C.

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495 CROSS-LINKING OF CD81 WITH HCV E2 REGULATES MIGRATION OF NATURAL KILLER (NK) CELLS IN A ROCK-DEPENDENT FASHION

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Background: Natural killer (NK) cells are crucial for the establishment of an efficient antiviral immune response but are also suggested to contribute to the HCV-induced hepatic injury. Previous data demonstrated that cytotoxic functions of NK cells is affected by interactions of the HCV E2 protein with CD81, a member of the tetraspanin family. Tetraspanins are organized in a cell-surface network containing several different tetraspanins which interact specifically with each other and their associated proteins (including integrins), called the tetraspanin web. Based on in vitro studies CD81 has been proposed to be part of the cellular receptor mechanism for HCV entry via binding of the HCV envelope protein E2. In addition, tetraspanins are involved in many cellular responses such as cell adhesion, activation, differentiation, and migration. In the present study we analyzed the effects of HCV E2/CD81 interactions on the migratory capacity of NK cells. Methods: NK cells were immunomagnetically isolated and stimulated with anti-CD81 or immobilized HCV E2 protein. NK migration towards RANTES (CCL5) was analyzed using a nitrocellulose filter micro chamber system. Surface expression of adhesion molecules were analyzed by flowcytometry and laser scanning microscopy. Activation of the proteins Ezrin, Moesin and Radizin (ERM), which are involved in lymphocyte migration, was studied by Western blot. Results: We found that cross-linking of CD81 by HCV E2 dramatically enhanced migration of NK cells towards the inflammatory chemokine CCL5. This effect could be blocked by pre-incubating NK cells with anti-CD81 mAb. HCV E2/CD81 interactions did not affect expression of chemokine receptors CCR1, CCR3 or CCR5. However, HCV E2-induced NK migration was associated with down regulation of surface expression of the adhesion molecules CD29, CD49d, and CD54 which are critically involved in cell migration. Western blot analysis confirmed that cross-linking of CD81 induced a ERM phosphorylation which could be blocked by pre-incubation with the ROCK-inhibitor Y27632. Conclusion: We found that cross-linking of CD81 by HCV E2 dramatically enhances the migratory responses of NK cells to CCL5 in a Rock-dependent fashion. As chronic hepatitis C is associated with enhanced intrahepatic expression of CCL5 this mechanism might be facilitate NK cell migration towards the liver potentially contributing to NK cell-mediated liver damage.

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SCAVENGER RECEPTOR BI IS REQUIRED FOR AN ENTRY STEP CLOSELY LINKED TO CD81
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Hepatitis C virus (HCV) is a major cause of chronic hepatitis worldwide. Scavenger receptor class B type I (SR-BI) has been shown to bind HCV envelope glycoprotein E2, participate in entry of HCV pseudotype particles and modulate HCV infection. We have previously demonstrated that anti-SR-BI antibodies directed against epitopes of the human SR-BI extracellular loop and down-regulation of SR-BI expression by SR-BI-specific siRNAs markedly inhibited HCVcc infection. These data suggest that SR-BI plays an important role in productive HCV infection of human hepatoma cells. In this study, we mapped the HCV entry step targeted by SR-BI and addressed whether SR-BI and CD81 are involved in the same HCV entry pathways. Kinetic studies demonstrated that anti-SR-BI antibodies were able to inhibit HCVcc infection when added both during and after binding of the virus to the target cell. These data indicate that SR-BI is involved in an entry step occurring after HCV binding. Kinetic studies using anti-SR-BI and CD81 antibodies in side-by-side experiments demonstrated that SR-BI is required for an entry step occurring at a similar time point as CD81-HCV interaction. Both anti-SR-BI and anti-CD81 antibodies were able to inhibit HCV infection when added up to 60 min post binding and lost its ability to inhibit HCV infection when added 80 min post binding. In conclusion, our data suggest that SR-BI (i) represents a key host factor for HCV entry, (ii) is implicated in the same HCV entry pathway as CD81 and (iii) targets an entry step closely linked to HCV-CD81 interaction.

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EVIDENCE FOR HIV AND HCV REPLICATION IN THE PERITONEAL MACROPHAGE
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Background: HCV replication has been reported in extra-hepatic cells, including white blood cells. Peritoneal macrophage are related to circulating monocytes, but have unique properties. To determine whether they harbor HCV and to measure their susceptibility to HIV, we isolated macrophage from the ascitic fluid (AF) of cirrhotic HCV patients and controls and maintained these powerful immunomodulatory cells in culture.

Methods: AF was collected from cirrhotic patients with HCV (n=9), HCV/HIV (n=4), alcoholic liver disease (n=5), and HBV (n=1), with IRB approval. Ascitic mononuclear cells (AMCs) were isolated from ~5 liters of AF by Ficoll-Histopaque density gradient centrifugation and plated in RPMI 1640 with 10% fetal calf serum. Non-adherent cells were removed after 12 hours. Adherent AMCs were cultured without cytokine supplementation for up to 3 months. Cells were stained with anti-CD68 (KP1), anti-CCR5, and produced >50 ng/ml of p24 at 12 days after HIV exposure. HIV infection appeared to increase the level HCV RNA at 14 days after infection. Conclusions: The majority of adherent cells in AF are CD68-CCR5-positive, phagocytic peritoneal macrophage. When isolated from HCV patients, these cells contain HCV RNA and viral proteins which persist for up to 4 weeks in culture. Peritoneal macrophage support HIV replication and HCV RNA is increased following HIV infection. These cells may represent a valuable cell culture system capable of supporting the replication of clinical isolates of HCV and exploring interactions between HIV and HCV. (NIH DA016156/DK066939)

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ESTABLISHMENT OF INFECTIOUS GENOTYPE 1B HEPATITIS C VIRUS CLONE TO HUMAN HEPATOCYTE CHIMERIC MOUSE
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Background/Aims: Interferon (IFN) is the only useful drug to eradicate the hepatitis C virus (HCV). The therapeutic effects of IFN vary according to the HCV genotype; genotype 1b is the most resistant. However, the underlying mechanism of the vari- able IFN resistance among genotypes is yet to be solved. In this study, we attempted to establish a genotype 1b molecular clone which replicates in human hepatocyte chimeric mice. Methods: A full length HCV genome, HCVK19, was cloned from a serum
sample from a patient who developed severe acute hepatitis. Each of the chimeric mice was inoculated intrahepatically with 30 μg of in vitro-transcribed HCV RNA. Sera obtained from infected mice were subjected to passage experiments. HCV infected mice were treated with 1000 IU/g/day of IFN-alpha.

Results: The intrahepatic injection of transcribed HCV-KT9 RNA to chimeric mice resulted in measurable viremia at 2 weeks post-infection, which persisted for more than 6 weeks. Passage experiments indicated that the sera of these mice contained infectious HCV. Interestingly, a similar clone HCVKT1, a shorter poly(U/UC) tract version of KT9, showed poorer infectivity and replication ability. These results confirm the importance of poly(U/UC) tract length in the experimentally-induced viremia (HCV-KT1 clone has 86-nucleotide tract compared with HCV-KT9, which has a 115-nucleotide tract) and are consistent with a previous study that reported the high replication ability of HCV replicon with a long poly(U/UC) tract. A two-week IFN-treatment showed only 1.25 log reduction in HCV levels. These results are consistent with our previous study that showed a similar poor reduction of HCV RNA in mice infected with genotype 1a clone, and differ from our results in mice infected with HCV genotype 2a, which became negative for HCV RNA following daily treatment with 1000 IU/g of IFN for two weeks. These results are in agreement with our clinical experience that genotype 1 is more resistant to IFN therapy than genotype 2.

Conclusions: The established genotype 1b clone using human hepatocyte chimeric mice seems useful for the study of HCV virology, particularly the mechanism underlying the variable resistance of HCV genotypes against IFN therapy.

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HCV-specific T cell responses are essential for viral resistance of HCV genotypes against IFN therapy. In virology, particularly the mechanism underlying the variable resistance of HCV type 1 is more resistant to IFN therapy than genotype 2. Consistent with our previous study that showed a similar poor reduction of HCV RNA in mice infected with genotype 1a clone, and differ from our results in mice infected with HCV genotype 2a, which became negative for HCV RNA following daily treatment with 1000 IU/g of IFN for two weeks. These results are in agreement with our clinical experience that genotype 1 is more resistant to IFN therapy than genotype 2. Conclusions: The established genotype 1b clone using human hepatocyte chimeric mice seems useful for the study of HCV virology, particularly the mechanism underlying the variable resistance of HCV genotypes against IFN therapy.

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HYPERPROLACTINEMIA ASSOCIATED WITH CHRONIC HEPATITIS C AND INDUCTION OF PROLACTIN EXPRESSION IN HUMAN PERIPHERAL BLOOD MONONUCLEAR CELLS STIMULATED BY HEPATITIS C VIRUS

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Background: Prolactin (PRL) is a pituitary hormone associated with lactation and reproduction. It is also known as an immunoregulatory hormone secreted from lymphocytes, and noted to have influence on cell immunity in infectious disease. PRL stimulates the production of IFN-γ and increases the effect of IL-2 in lymphocytes. Elevation of serum PRL levels has been reported in patients with HIV or in those with some infectious diseases, and hyperprolactinemia has been demonstrated in 20% of HIV-infected men. Thus PRL influences cell immunity in infectious disads, however, PRL induction in relation to HCV infection has not been elucidated. Methods: We examined serum PRL levels and frequency of hyperprolactinemia in both 232 subjects of our HCV cohort study and 31 male patients of the hospital, who were chronically infected with HCV. Serum PRL levels in healthy individuals without HCV infection were used as controls. Furthermore, serum PRL levels were compared in 20 male patients before and after anti-viral therapy using interferon. We measured expression of PRL mRNA level in PBMCs in 12 male patients and 6 healthy controls, and also investigated PRL mRNA of PBMCs collected from 5 healthy men that stimulated by HCV produced by Huh7.5 cells in vitro. The levels of serum PRL and PRL mRNA in PBMCs were measured by chemiluminescence immunoassay and real-time PCR, respectively. Results: Serum PRL levels were significantly higher in the HCV-infected subjects than in the controls (p<0.01). They were significantly higher in HCV-infected male subjects than in the controls (p<0.001), however, the difference was not significant between HCV-infected female subjects and controls. Frequency of hyperprolactinemia was significantly higher in HCV-infected male subjects than in the controls (p<0.05). Serum PRL levels were significantly higher in male patients than in the controls (p<0.01). Frequency of hyperprolactinemia was significantly higher in male patients than in the controls (p<0.05). Serum PRL levels decreased significantly after anti-viral therapy using interferon in 10 patients with sustained virological response to therapy (p<0.05), but no changes were found in 10 patients
501 ANTIVIRAL EFFECTS OF INTERFERON-INDUCED PROTEINS, GBP-1, IFI-6-16 AND IFI-27 AND THEIR INTERACTIONS WITH HEPATITIS C VIRUS NONSTRUCTURAL PROTEINS

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Backgrounds: Interferons (IFNs) and the interferon-stimulated genes (ISGs) play a central role in antivirus responses against HCV infection. We have previously reported that ISGs including GBP-1, IFI-6-16 and IFI-27 directly and specifically inhibited HCV subgenomic replication (Itsui, JVH 2006). In the present study, we screened effects of ISGs against HCV cell culture and their direct mechanisms of antiviral actions. Materials and methods: The ISGs that were examined in this study were 18 genes, which expression levels were highly induced by the IFN-alpha treatment. Plasmids expressing individual ISG were constructed and were transfected into Huh7 cells that express selectable chimeric reporter protein of neomycin phosphotransferase and firefly luciferase (Huh7/Rep-Feo, Tanabe, J Infect Dis 2004). After transfection, replication level of HCV replicon was analyzed by luciferase assay. Plasmids expressing siRNA against GBP-1, IFI-6-16 and IFI-27 were transfected into Huh7/Rep-Feo. HCV-JFH1 cell culture system was used, and the replication levels were quantified by core protein detection in the culture medium. Plasmids expressing HCV-Nonstructural protein (NS3, NS4A, NS4B, NS5A and NS5B) and plasmids expressing ISG were cotransfected into HEK 293-T cells. Immunoprecipitation assays and mammalian two-hybrid assay were performed to analyze interaction of ISG with HCV proteins. Results: HCV subgenomic and genomic replication were significantly suppressed by overexpression of PKR (48.7±17.13%), MxA (46.8±5.60%), IRF9 (44.8±4.44%), GBP-1 (36.3±7.49%), IFI-6-16 (37.4±19.2%), IFI-27 (28.4±12.24%), 250AS (25.6±4.10%) and IRF1 (8.64±1.13%). The siRNA-knock down of GBP-1, IFI-6-16, and IFI-27 caused increase of HCV replication. The protein interaction assays showed that GBP-1 directly bound thumb epitope of HCV-NS5B- RNA-dependent RNA polymerase (RdRp). Conclusion: GBP-1, IFI-6-16 and IFI-27 negatively regulated HCV infection and replication. GBP-1 showed interaction with NS5B, suggesting direct effects on RdRp. Search for ISGs that regulate HCV replication may help elucidating the cellular defense mechanisms against HCV infection.

502 HCV-RELATED PROTEINS INDUCE PROINFLAMMATORY CYTOKINE PRODUCTION BY THE HUMAN KUPFFER CELL VIA THE MYD88-DEPENDENT SIGNALING CASCADE

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Previously, it was reported from this laboratory that oxidative stress and the number of the activated Kupffer cell increased in patients infected with hepatitis C virus (HCV); however the exact mechanism of activation of the Kupffer cells is still unclear. Accordingly, the specific purpose of this study was whether HCV-related proteins could activate the Kupffer cell isolated from human liver tissues. Kupffer cells were isolated from non-cancerous surgical specimen by collagenase digestion and differential centrifugation using Nycodenz. After 24 hours incubation, Kupffer cells were co-cultured with HCV-related proteins (Core, NS3, NS4, and NS5) for 24 hours, and production of cytokine (TNF-α, IL-1β and IL-10) was determined by ELISA. Furthermore, DCF-probed Kupffer cells were cultured with HCV-related proteins, and production of free radical (H2O2) was assessed using the fluorescence microplate reader for 24 hours. The Kupffer cells produced proinflammatory cytokines, TNF-α and IL-1β by stimulation of all HCV-related proteins studied in a dose dependent manner and values were as same as production by the Kupffer cells stimulated with LPS (10 µg/ml). On the other hand, production of anti-inflammatory cytokine IL-10 was minimal. Production of TNF-α was greatest by stimulation of NS3 protein. Importantly, this production was significantly blunted by 40% by a neutralization antibody of the Toll-like receptor (TLR) 4 or a homodimerization inhibitory peptide of the myeloid differentiation factor (MyD) 88. Since production of interferon β did not detected by stimulation of NS3 proteins, HCV-related proteins activated the Kupffer cell predominantly via the MyD88-dependent signaling cascades. On the other hand, cytoklasatin B, which is an inhibitor of phagocytosis, did not affect production of TNF-α by the Kupffer cells. Free radical production was not detected in Kupffer cells stimulated with core proteins. Although free radical production was detected in Kupffer cells incubated with NS3, NS4 and NS5 proteins, the greatest production was observed by stimulation of NS5 protein. This production was not affected by an antagonist of TLR4. Thus, HCV-related proteins may cause prolonged activation of the Kupffer cells, leading to accumulation of inflammatory cytokines and oxidant stress in the liver in patients infected with HCV.

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503 FULL-LENGTH SEQUENCE ANALYSIS OF HCV GENOTYPE-3A REVEALS NOVEL REGIONS OF HYPERVERSUS-ABILITY

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Background: Patients with HCV genotype-3a are very commonly encountered in the UK as this genotype dominates in UK intravenous drug users and is highly prevalent in some immigrant populations. Surprisingly only 4 full length genotype-3a
sequences have been published to date. This makes both peptide design for T-cell assays and primer design for assessment of viral evolution problematic. The NIH have recently produced a peptide set for T cell analysis based on a single genotype-3a strain. The relevance of this single strain is currently unclear. Aim: To obtain near full-length (aa 1-2929) genotype-3a sequence from 23 patients in our local Oxford cohort. Methods: Viral RNA was concentrated from patients’ plasma by high-speed centrifugation for 1 hour then extracted using Qia-gen vRNA Extraction Kit. RNA was converted to cDNA using RT-PCR One-step reaction to produce two first round products at 4kb and 7kb in length. Ten second round primer pairs were used to generate 700-1500bp overlapping PCR products. These products were sequenced using 17 additional primers to build a sequence for each patient. Results: Amino acids 1-2929 were successfully obtained in 18 of 23 patients. In genotype-3a, regions of polymorphism are completely distinct as compared to other genotypes. The hypervariable region at the N-terminal of E2 was observed, as previously described. However we report 2 novel regions of hypervariability in E2, one of these is contained within the 15-nucleotide genotype-3a insertion. Conclusion: Full-length sequence analysis of genotype-3a infection has enabled us to generate a consensus sequence essential for peptide design and has revealed novel areas of hypervariability and polymorphism.

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LOSS OF AUTOPHAGY PREVENTS APOPTOTIC CELL DEATH DURING ISCHEMIA-REPERFUSION IN THE MOUSE LIVER
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Background: The underlying mechanism by which apoptosis is activated during the early phase of reperfusion after liver ischemia remains poorly understood. In cerebral and cardiac ischemia, autophagy has been recognized as a protective response against nutrition deprivation and cellular damage. On the other hand, it has been suggested that autophagy can be activated by pro-death stimuli culminating in a programmed cell death. Here, we investigate if autophagy is directly linked to apoptosis in hepatic ischemia-reperfusion injury. Methods: Hepatic ischemia-reperfusion injury was induced in female C57BL/6 mice (wild type) and conditional Atg7-knockout mice, which have autophagy-deficient liver as described (J Cell Biol. 2005; 169, 425-34). Serum ALT value was measured to evaluate liver damage. The number of autophagosomes in cytoplasm was counted by using transmission electron microscopy. Moreover, expression of microtubule-associated protein-1 light chain 3 (LC3), cathepsin B and D were detected by Western blot analysis. TUNEL stain and immunofluorescent analysis of cytochrome c on the liver section were performed. Results: Serum ALT level at 2 hours post-reperfusion was elevated to about 50-fold as basal level in wild type mice, however, this elevation of ALT was blunted in mutant mice to about 60%. On the other hand, 2 hours after ischemia-reperfusion, the number of autophagosomes was increased to about 4-fold relative to basal level in wild type mice. Moreover, after ischemia-reperfusion, LC3-II expression gradually increased and reached a maximum at 2 hours after reperfusion and the elevated level was sustained up to 6 hours in wild type mice. The formation of autophagosome was not detected in Atg7 knockout mice liver during ischemia-reperfusion. Furthermore, deletion of Atg7 completely abolished apoptotic cell death in hepatocytes, whereas TUNEL-positive hepatocytes were induced by ischemia-reperfusion in wild type mice. Additionally, increases in the expression of both cathepsin B and D in the liver and the distribution of cytochrome c into cytosol of hepatocytes were observed after ischemia-reperfusion in the wild type mice, but not in the mutant mice. Conclusion: Autophagosome formation induced in hepatocytes during ischemia-reperfusion was correlated with the liver injury. Interestingly, loss of autophagy completely abolished apoptosis of hepatocytes due to ischemia-reperfusion. These results provide a direct evidence that autophagy is essential for induction of apoptosis in liver during ischemia-reperfusion.

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GP130, THE SIGNAL-TRANSDUCING IL-6 RECEPTOR SUBUNIT, IS A SUBSTRATE OF CASPASES
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Interleukin-6 (IL-6) is involved in acute phase reaction, liver regeneration, proliferation and anti-apoptosis. In this study we analysed the effects of bile acids and CD95 ligand on gp130 expression in rat hepatocytes, perfused rat liver and HepG2 cells, which were stably transfected with the sodium-taurocholate-cotransporting peptide (NTCP). Preincubation of rat hepatocytes with CD95 ligand or bile acids inhibited IL-6-induced tyrosine-phosphorylation of signal transducer and activator of transcription 3 (STAT-3) by down-regulation of gp130. CD95 ligand had no effect on gp130 mRNA levels or gp80 protein expression, but strongly decreased protein expression of the signal-transducing IL-6 receptor subunit gp130. Inhibition of proteasomal or lysosomal proteolysis by specific inhibitors did not prevent the CD95-induced gp130 loss. However, inhibition of caspase 3 (Z-DEVD-FMK) or 8 (Z-IETD-FMK) was able to recover CD95 ligand-induced inhibition of STAT3-phosphorylation on tyrosine 705 and gp130 down-regulation. The intracytoplasmic tail of gp130 contains an overlapping DXD motif at position 800-806 of its amino acid sequence, which might be an preferred cleavage motif of caspase 3. For confirming this hypothesis, we transfected NTCP-HepG2 cells with a chimeric receptor consisting of the extracellular domain of the erythropoietin (EPO) receptor and the intracytoplasmic domain of gp130. We mutated the potential cleavage motif by exchanging the aspartate at position 803 to glutamate. In vitro studies showed that addition of recombinant active caspase 3 to lysates of NTCP-HepG2 cells, which were cotransfected with EPO/gp130 wild type receptor, leads to down-regulation of this chimeric receptor, while a cleavage product simultaneously occurs with a molecular weight of nearly 18 kD. However, lysates of NTCP-HepG2 cells, which were transfected with an mutated EPO/gp130 (803E) as indicated above, showed no degradation in response to active caspase 3 in vitro. Cleavage resistance of gp130 in response to the hydrophobic bile acid glycochenodeoxycholate (GDC) was also obtained in NTCP-HepG2 cells, which were transfected with the mutated EPO/gp130 (803E). This down-regulation of gp130 may aggravate the hepatotoxicity in cholestatic liver disease by
blocking the known hepatoprotective effects, mediated by gp130 activation in response to IL-6.

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506 ENDOTOXIN INDUCES ER STRESS IN RAT PRIMARY HEPATOCYTES VIA STELLATE CELLS: ROLE OF JNK MAPK ACTIVATION

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Background and Aims: Endotoxin-stimulated hepatic stellate cells (HSCs) were found to stimulate nitric oxide synthesis, inhibit DNA synthesis and cause apoptosis of hepatocytes. However, mechanisms of the effects of LPS-stimulated HSCs on hepatocytes are not known. We investigated signaling mechanisms of the actions of LPS-stimulated culture-activated HSCs on hepatocytes. Methods: HSCs isolated from the rat liver were activated in primary culture and stimulated with gram-negative bacterial lipopolysaccharide (LPS) for 24 hours. The conditioned medium was transferred to rat hepatocytes in primary culture. For control, hepatocytes were incubated without or with LPS and medium conditioned by HSCs without LPS. Morphological characteristics and signaling pathways were determined at various time points. Results: LPS-conditioned HSC medium (LPS-HSCm) caused cytosolic vacuolization and ER dilation as evidenced phase-contrast and electron microscopy. The effects were maximal at 6 hours of incubation. Medium conditioned by HSCs in the absence of LPS also elicited similar responses but the magnitude was much lower. The effects of LPS-HSCm were associated with the activation of ER stress molecules ATF6, PERK and eIF2α as well as CHOP. Although LPS-HSCm also caused activation of p38, ERK- and JNK-mitogen-activated protein kinases (MAPK) in hepatocytes, blockade of only JNK-MAPK ameliorated the ER stress [and apoptosis] caused by LPS-HSCm. These effects of LPS-HSCm on hepatocytes were comparable to the established ER stress inducers thapsigargin and tunicamycin. Conclusions: The results demonstrate for the first time that LPS-stimulated HSCs release mediators that cause ER stress in hepatocytes and this effect, which is related both to survival and apoptotic death, may be an important mechanism of hepatocyte survival/death in hepatic pathophysiology.

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507 ARSENIC AT SUBHEPATOXIC DOSES SYNERGISTI-
CALLY ENHANCES LIPOPOLYSACCHARIDE-INDUCED LIVER INJURY IN MICE

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Background. The risk of developing chronic liver disease (e.g., NAFLD) depends on many factors, both environmental and genetic. Exposure to arsenic via drinking water is a serious health concern in the US. Whereas studies have identified arsenic alone as an independent risk factor for liver disease, concentrations of arsenic required to damage this organ are generally higher than found in the US water supply. The purpose of the current study was to test the hypothesis that arsenic (at subhepatotoxic doses) may also sensitize the liver to a second hepatotoxin. To test this hypothesis, the effect of chronic exposure to arsenic on liver damage caused by acute lipopolysaccharide (LPS) was determined in mice. Methods. Male C57Bl/6 mice (4-6 weeks) were exposed to arsenic (49 ppm as sodium arsenite in drinking water). After 7 months of exposure, animals were injected with LPS (10 mg/kg i.p.) and sacrificed 24 h later. Results. Arsenic alone caused no overt hepatotoxicity, as determined by plasma enzymes and histology. Whereas LPS significantly increased hepatic lipids, arsenic had no effect on these parameters, neither in the presence nor absence of LPS. In contrast, arsenic exposure dramatically enhanced liver damage caused by LPS, increasing the number and size of necroinflammatory foci. This effect of arsenic was coupled with increases in indices of oxidative stress (4-HNE adducts, depletion of GSH and methionine pools). The number of apoptotic (TUNEL) hepatocytes were similar in the LPS and arsenic/LPS groups. In contrast, arsenic preexposure blunted the increase in proliferating (PCNA) hepatocytes caused by LPS; this change in the balance between cell death and proliferation was coupled with a robust loss of liver weight in the arsenic/LPS compared to the LPS alone group. The impairment of proliferation after LPS caused by arsenic was also coupled with alterations in the expression of key mediators of cell cycle progression (p27, p21, CDK6 and Cyclin D1). Conclusions. Taken together, these results suggest that arsenic, at doses that are not overtly hepatotoxic per se, synergistically enhances LPS-induced liver injury. These results further suggest that arsenic levels in the drinking water may be a risk modifier for the development of chronic liver diseases.

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508 METFORMIN PROTECTS RAT HEPATOCYTES AGAINST APOPTOSIS VIA THE PI3-KINASE/AKT SURVIVAL PATHWAY, BUT HAS NO EFFECT ON NF-κB SIGNALING

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Background. Metformin improves glucose tolerance in Diabetes Mellitus type 2. Additionally, metformin ameliorates liver damage in patients with non-alcoholic-fatty-liver disease (NAFLD). The mechanism of this hepatoprotective effect has not been elucidated yet. Previously we have shown that metformin protects hepatocytes against bile acid-induced apoptosis. The mechanism is still unknown. Furthermore, the effects of metformin in acute liver injury have not been examined yet. Previous research indicated that the PI3-kinase/Akt survival pathway and the transcription factor NF-κB are key regulators of the balance between survival and death in hepatocytes. Aim of this study was to elucidate the mechanism of the anti-apoptotic properties of metformin and to investigate the effect of metformin in an in vitro model of acute liver failure. Methods Primary rat hepatocytes were exposed to the bile acid glycochenodeoxycholic acid (GCDCa, 50 µM, 4 hrs), or 16 hrs to TNFα (20 ng/ml) with or without actinomycin D (200 ng/ml) or 16 hrs to a cytokine mixture (CM, containing mTNFα, hIL-1β, rIFNγ and LPS). Metformin (1 mM) was added simultaneously with the apoptotic stimuli. The PI3-kinase/Akt signaling pathway was blocked using LY294002 (50 µM). The mRNA
expression of the NF-κB-regulated gene iNOS, and the anti-apoptotic gene bcl-xl were determined by qPCR. Caspase-3-like activity was measured in cell lysates with a fluorometric assay. Necrotic cell death was determined using the Sytox Green nuclear staining. Results Metformin reduced GCDCA-induced caspase-3 activity by 84%, without increasing necrosis. In contrast, metformin did not inhibit TNF/ActD-induced apoptosis. Inhibition of PI3-kinase completely abolished the protection of metformin against GCDCA-induced apoptosis. Metformin increased Bcl-xl mRNA expression, while co-treatment with GCDCA even further increased Bcl-xl mRNA levels. Metformin did not inhibit CM-induced iNOS expression, and did not sensitize hepatocytes to TNF-induced apoptosis, indicating that metformin does not affect TNF-induced NF-κB-activation. Conclusion Metformin protects hepatocytes against bile acid-induced apoptosis, without switching cell death to necrosis. The protective effect of metformin is dependent on the PI3-kinase/Akt pathway, and may be partly mediated via induction of the anti-apoptotic protein Bcl-xl. Metformin does not protect against TNF/ActD-induced hepatocyte apoptosis, an in vitro model of acute liver failure. Metformin has no effect on NF-κB signaling and does not sensitize hepatocytes to TNF-induced apoptosis. Our results suggest metformin may be used in cholestatic liver disorders accompanied by inflammation.

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509 ZINC SUPPLEMENTATION STIMULATES HEPATIC REGENERATION BY PRESERVING HEPATOCYTE NUCLEAR FACTOR-4α IN MICE SUBJECTED TO A LONG-TERM ETHANOL ADMINISTRATION

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Alcoholic liver disease is associated with sustained liver damage and impaired regeneration response. Our previous studies demonstrated that dietary zinc supplementation normalizes hepatic zinc level in association with inhibition of alcoholic liver injury. This study was undertaken to examine whether or not dietary zinc supplementation can improve liver regeneration in mice subjected to long-term ethanol exposure. 129SVE mice were paired-fed an ethanol-containing liquid diet for 6 months, and the effects of zinc supplementation on ethanol-induced liver injury and regeneration were analyzed. Zinc supplementation prevented ethanol-induced decrease in hepatic zinc concentration and liver injury as measured by serum alanine transferase activity and histopathological changes. Zinc supplementation also enhanced liver regeneration as indicated by increased number of PCNA-positive and BrdU-labeled hepatocytes in the ethanol-exposed mice. Real time RT-PCR demonstrated that zinc supplementation not only prevented ethanol-reduced suppression of gene expression of IGFBP1 and MT, but also upregulated HGF and cyclin D1. To determine the mechanism for zinc regulation of gene expression, hepatocyte nuclear factor-4α (HNF-4α), a zinc finger transcription factor, was evaluated. Zinc supplementation up-regulated the mRNA level of HNF-4α and preserved the protein level of HNF-4α. The link between zinc and HNF-4α in cell proliferation was examined in HepG2 cell cultures. Zinc deprivation by TPEN retarded cell growth in association with dysfunctions of HNF-4α. Gene silencing for HNF-4α retarded cell growth death in association with remarkable decrease in the protein levels of IGF-I, IGFBP1, MT and cyclin D1. HNF-4α siRNA transfection also attenuated zinc-induced expression of IGF-I, MT and cyclin D1. In conclusion, zinc supplementation stimulates liver regeneration through preservation of HNF-4α, through which gene involved in the regeneration were upregulated. (Supported in part by the National Institutes of Health grants and the Veterans Administration, Louisville)

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510 QUALIFICATION OF FOUR SERUM BIOMARKERS OF HEPATOTOXICITY BY THE PREDICTIVE SAFETY TESTING CONSORTIUM (PSTC)

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The Predictive Safety Testing Consortium (PSTC), a collaboration among 16 pharmaceutical companies with FDA participation, has the goal of qualifying preclinical and clinical safety biomarkers for regulatory acceptance. This consortium, administered by the C-Path Institute, is formally endorsed by the FDA as a “Critical Path Initiative” activity. Several working groups have been formed to advance safety biomarkers for hepatic, renal, and vascular injury as well as carcinogenicity. Member companies use the consortium mechanism to bring forward putative safety biomarkers that have been derived from the literature or discovered in their internal laboratories that have significant supporting data and biological rationale. Cross-validation studies are planned to collaboratively qualify biomarkers for preclinical and ultimately clinical use. Specifically, this has involved the exchange of assay protocols and archived serum samples from internal studies. This poster will focus on the advances the Hepatotoxicity Working Group has made towards the qualification of four serum biomarkers of hepatotoxicity: glutamate dehydrogenase, paraoxonase, purine nucleoside phosphorylase, and malate dehydrogenase. The overall value of this PSTC effort extends beyond sharing of costs and intellectual resources as it will also enable a more rapid safety biomarker qualification and direct engagement of FDA on qualification and acceptance criteria for safety biomarkers.

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The following people have nothing to disclose: Shelli J. Schomaker, Holly Jordan, Frances Clemo, Kyle Kolaja, Patrick Wier, William Mattes

511 OXIDATIVE STRESS MODULATES THE EXPRESSION OF KRÜPPEL-LIKE FACTOR-6 AND ITS SPLICE-VARIANTS

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Induction of ROS is one of the central mechanisms by which ethanol is hepatotoxic. Krüppel-like factor 6 (KLF6) is a transcription factor and a tumor-suppressor gene suggested to be mutated in several carcinomas. KLF6 is an immediate early responsive gene to cellular stress and injury, but the impact of ROS on its expression is unknown. Aim: To investigate the contribution of two different sources of ROS, cytochrome P450 2E1 (CYP2E1) and NADPH oxidoreductase (NQO1), on the modulation of KLF6 expression, alternative splicing to KLF6-full expression, and the effect on two downstream targets TNFRα and TGFβ. Results: 1) In a culture model of endogenous ROS production, CYP2E1-expressing HepG2 cells showed higher levels of KLF6-full mRNA and protein and its spliced variants compared to controls. Co-incubation with
arachidonic acid, arachidonic acid plus iron (inducers of lipid peroxidation-derived reactions), β-naphthoflavone (a CYP2E1 inducer), and H$_2$O$_2$, further enhanced KLF$_{6\text{full}}$ and its spliced forms. These effects were prevented by vitamin E, which blocks lipid peroxidation, and by CYP2E1 inhibitors. In primary hepatocytes isolated from rats fed the alcohol-containing Lieber-DeCarli diet for 4 months there was elevated lipid peroxidation-end products, H$_2$O$_2$, and CYP2E1, associated with a 2-fold increase in KLF$_{6\text{full}}$mRNA compared to hepatocytes isolated from rats fed a control diet. These results validated the role of alcohol and CYP2E1-derived ROS in the induction of KLF$_{6\text{full}}$ and the spliced variants. 2) Two powerful pro-oxidants such as menadione and paraquat which undergo two electron reduction via NQO1 induced KLF$_{6\text{full}}$ and its isoforms. Menadione also increased KLF$_{6\text{full}}$ in a colon cancer cell line. Addition of antioxidants such as catalase, SOD, glutathione ethyl ester, and vitamin E, and of dicumarol, a NQO1 inhibitor, all suppressed both the basal effect by CYP2E1 expression as well as the increase in KLF$_{6\text{full}}$mRNA by menadione and paraquat. Moreover GSH depletion by L-buthionine sulfoximine enhanced 2-fold the effect of menadione and paraquat suggesting that KLF$_{6\text{full}}$ expression may be also thiol-sensitive. 3) The CYP2E1-expressing cells and controls were transfected with siRNA for KLF$_{6\text{full}}$ and KLF$_{6\text{V1}}$. While levels of H$_2$O$_2$ and O$_2^-$ remained similar to those of the non-transfected cells, inhibition of KLF$_{6\text{V1}}$ caused an increase in both TNFα and TGFB mostly in the CYP2E1 cells. Conclusion: These results indicate that several sources of endogenous ROS increase KLF$_{6\text{full}}$ expression and its spliced variants, and that KLF$_{6\text{V1}}$ may play a key role in modulating the expression of critical cytokines involved in the development of liver disease.

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P38- AND ERK- MAPK MEDIATE SUPEROXIDE-INDUCED APOPTOSIS OF ACTIVATED RAT HEPATIC STELLATE CELLS, WHICH IS REVERSED BY RETINOIC ACID

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A PROTEOMIC APPROACH TO NUCLEOSIDE ANALOGUE ASSOCIATED MITOCHONDRIAL TOXICITY

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Introduction: Treatment with nucleoside inhibitors of reverse transcriptase (NRTI) may contribute to mitochondrial toxicity in chronic HIV-infection. Recent research has primarily focussed on mitochondrial DNA (mtDNA) encoded enzymes which are known to be affected by NRTI-associated inhibition of mtDNA replication. This approach ignores the majority of mitochondrial proteins which may be essential for manifestation of mitochondrial toxicity. Because of their limited protein content mitochondria are excellent candidates for proteomic analyses. We used this technique for examination of mitochondrial protein expression in an established in vitro model of NRTI-related hepatic mitochondrial toxicity. Methods: Human hepatocytes (HepG2) were exposed to steady state peak plasma levels of AZT and ddC for 14 days. Mitochondria were isolated by differential ultracentrifugation. Proteome analysis was performed by using a recently introduced DIGE (fluorescence 2-D difference gel electrophoresis) system. Differentially expressed proteins were subsequently identified by MALDI-PMF/PFF-MS analysis. Results: In preliminary image analysis we identified 18 differentially regulated mitochondrial proteins in NRTI exposed cells (t-test<0.05, expression change>1.5). Besides expected enzymes of the respiratory chain complex we identified several new proteins involved in cellular oxidative damage protection (e.g. peroxiredoxin-3, heat-shock protein 10) which were regulated in NRTI-exposed hepatocytes. Surprisingly protein expression pattern of AZT and ddC exposed cells showed distinct differences (only 3 identical proteins). Conclusion: Proteomic analysis may be useful for identification of yet unknown proteins beyond pol-γ-hypothesis and respiratory chain trail which are involved in manifestation of mitochondrial and cellular damage in NRTI-exposed cells and tissues. We identified several proteins which may play a crucial role in hepatocellular adaptation to NRTI-exposure. The distinctly different mitochondrial protein expression pattern in AZT- and ddC-exposed cells may explain the lower toxic potency of AZT. Future research may demonstrate the in vivo transferability of these in vitro results and will define the functional relevance of the identified proteins.
514 SUPPRESSIVE EFFECTS OF RETINOIDS ON IRON-INDUCED OXIDATIVE STRESS IN LIVER BY DOWNREGULATION OF HEMOJUVELIN

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[Background/Aim] We previously reported that dominant negative retinoic acid receptor alpha transgenic (RAR-E) mice exhibit steatohepatitis, possibly due to the abnormalities of lipid metabolism, leading to hepatocellular carcinoma (HCC). Recently, oxidative stress induced by hepatic iron overload has emerged as an important factor for the progression of HCV-related chronic hepatitis and non-alcoholic steatohepatitis (NASH). In the present study, we examined the effect of RA on the iron metabolism in vitro and in vivo. [Methods] In vitro studies, human hepatocellular carcinoma cell line HuH7 cells were treated with all-trans retinoic acid (ATRA). In in vivo studies, C57BL/6 mice were fed with retinoid-deficient, normal and retinoid-excessive diets for 1 month followed by intraperitoneal iron-dextrane administrations. The expression of iron metabolism-related genes was analysed by real-time RT-PCR. Promoter analyses were performed by chromatin immunoprecipitation (ChIP) assay. Non-heme iron concentrations and 59Fe-transferin uptake were determined by a colorimetric assay and a gamma counter, respectively. [Results] The overexpression of hemojuvelin (Hjv) gene and significantly higher iron contents were observed in RAR-E mice livers, compared to those in wild type mice. ATRA clearly inhibited HJV gene expression. ChIP assay demonstrated that RARalpha and RXRbeta2 were recruited to the 5'-region of HJV gene containing two RA response element half-sites. ATRA also significantly decreased the amount of iron in HuH7 cells. Furthermore, the iron-induced decrease in antioxidant activity and cytotoxicity were significantly prevented in the presence of ATRA. In accordance with in vitro observations, retinoid-excessive diet significantly decreased Hjv expression and iron contents in C57BL/6 mice, leading to decrease in iron-induced oxidative stress. [Conclusions] It was shown that retinoid has a protective effect on iron-related oxidative stress by regulating the HJV gene expression. Our findings suggest that the mechanism of hepatic carcinogenesis by loss of retinoid signals involves the abnormalities of iron metabolism as well as those of lipid metabolism.

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515 HEPATOCYTE GROWTH FACTOR PROTECTS AGAINST OXIDATIVE INJURY INDUCED BY ETHANOL METABOLISM

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Introduction: Hepatocyte growth factor and its receptor c-met, are involved in many cellular responses such as morphogenesis, mitogenesis, motility and apoptosis protection, however the effect against oxidative injury induced by ethanol (EtOH) metabolism is not well understood. Objective: The aim of the present work was to address the mechanism of HGF-induced protection against EtOH-generated oxidative stress damage in EtOH metabolizing cells. Material and Methods: VL-17A cells, HepG2 cells transfected with cytochrome P450 2E1 and alcohol dehydrogenase genes, were pretreated with 50 ng/ml HGF for 12 hr and then treated with 100 mM EtOH for 0 – 48 hr. Lipid peroxidation was determined by Buege and Aust method, protein oxidation was analyzed by Oxylab kit. Antioxidants enzymes and actin were determined by Western blot and cell proliferation was analyzed by BrdU DNA incorporation. Results: EtOH induced cellular damage and oxidative stress judge by a decrease in cell viability (28%) and cell proliferation (55%), elevation in lipid (4.7-fold) and protein oxidation, and reactive oxygen species production, effects that were prevented by HGF pretreatment. In order to explore if protection is dependent of an elevation of the antioxidant systems, we performed a Western blot analysis of catalase and superoxide dismutase1 (SOD1). We found that HGF induced the expression of both catalase (2.7-fold) and SOD1 (2.0-fold) in a time dependent manner. HGF/c-met can activate many signal transduction pathway which can promote survival. In order to explore the signaling pathway that regulates HGF-induced oxidative stress protection, VL-17A cells were pretreated with the PI3K inhibitor wortmannin, AKT inhibitor II, and sul-fasalazine an inhibitor NF-κB. We found that HGF-induced oxidative stress protection was abrogated by all inhibitors. HGF-induced catalase and SOD1 expression were suppressed. In conclusion our data sustains that HGF protects cells from damage induced by EtOH metabolism by a mechanism driven by NF-κB and PI3K/Akt signaling pathway. This work was supported in part by CONACyT: 45921.

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516 HEPATIC MITOCHONDRIAL α-TOCOPHEROL AND CAR-DIOLIPIN IN YOUNG RATS MAY AFFORD PROTECTION AGAINST BILE ACID-INDUCED MITOCHONDRIAL PATHWAYS OF CELL DEATH

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Retention of bile acids in cholestatic disorders causes liver injury by mechanisms involving mitochondrial (mito) pathways of cell death. Although the human neonate is particularly susceptible to cholestatic injury compared to adults, liver mitos from young rats were surprisingly resistant to bile acid-induced mito permeability transition (MPT) and cytochrome c release (Hepatology 2005;42 [Suppl. 1]: 501A). Levels of MPT pore proteins, Bcl-2 proteins and antioxidant enzymes did not account for this resistance. Mito membrane function is also sensitive to lipid composition. Consequently, the objective of the current study was to determine whether mito alpha tocopherol (α-TH) or cardiolipin (CL) content played a role in this MPT resistance in young rats. [Methods] Mitos were isolated from pooled livers of 10 day-old or adult (9 wk-old) male Sprague-Dawley rats. MPT was assessed by mito swelling at 540nm during 5 minutes exposure to 100μM glycochenodeoxycholate (GCDC). α-TH concentrations in mitochondria were determined by HPLC with electrochemical detection. Mito CL (total and sub-
ALTER CELL FATE FOLLOWING OXIDATIVE STRESS DOES NOT ALTER CELL FATE
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Background: Mitochondrial oxidative damage is a common feature of liver diseases such as NAFLD. Though formation of mixed disulfides between oxidized glutathione and protein sulfhydryl groups, known as protein glycation, temporarily inactivates key mitochondrial enzymes, it also generates reduced glutathione, which can protect protein sulfhydryl groups from irreversible oxidation by reactive oxygen species. Thus modulation of mitochondrial protein glycation following oxidative stress may impact upon cell fate. Aim: The aim of this study was to determine whether several known antioxidants modulate mitochondrial protein glycation and are cytoprotective. Methods: HEK cells, which express little endogenous β2-microglobulin, were either transfected with a vector encoding β2-microglobulin or transduced with adenovirus encoding cathepsin (Ad·cat). In other experiments HEK cells were preincubated with estradiol. Following induction of oxidative stress by ultraviolet B irradiation, the percentage of HEK cells undergoing apoptosis following UV-B irradiation was determined.

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COSUPPLEMENTATION WITH VITAMIN E AND COENZYME Q(10) REDUCES IRON-OVERLOADED INDUCED HEPATIC STEATOSIS IN TRANSGENIC MICE EXPRESSING THE HEPATITIS C VIRUS POLYPROTEIN
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Background: It was well known that vitamins E and/or C might be useful to prevent the formation of oxidation products by dietary oxidized fats. Coenzyme Q is a redox compound acting as an antioxidant. Reduced coenzyme Q can prevent lipid oxidation chain reaction by itself or by reducing other antioxidants such as vitamins E and/or C. We reported that iron-overloaded in the hepatitis C virus (HCV) transgenic mice (TgM) developed HCC through mitochondrial injury and chromosomal damage due to accumulations of hepatic oxidative stress (Gastroenterology: 130: 2087–2096). The aim of this study was to investigate whether or not Cosupplementation with Vitamin E (alpha-tocopherol) and Coenzyme Q10 (Coq10) is able to prevent oxidative stress and hepatic steatosis caused by dietary iron-overloaded in HCV TgM. Methods: 3 months old female were divided into four groups. These were fed iron excess diet (carbonyl iron 225 mg/kg diet) with 100 or 500 mg alpha-tocopherol/20 mg Coq10 control II: 500 mg alpha-tocopherol/20 mg, IV: 500 mg alpha-tocopherol/20 mg Cosupplementation after 6 months, hepatic steatosis was evaluated from mice liver. ROS production was observed using the dehydroethidium (DHE) dye, which could detect superoxide anion in the liver. ALT, Serum iron and transferin-iron saturation were measurement. Finally, mitochondrial complex I activity and GSH were examined as mitochondria function. Results: Serum ALT levels (I: 218±71 II: 120±26 III: 57±11 IV: 70±21) and the ratio of liver weight to body weight (I: 4.7±0.2 II: 4.1±0.4 III: 4.1±0.2 IV: 3.8±0.2) were significantly lower in HCV TgM treated with Cosupplementation (III and IV) than in those in control (I). ROS production was attenuated in II, III and IV than in those in I. Although inhibition of hepatic oxidative stress, hepatic steatosis was suppressed only in IV. These data suggested that high dose Vitamin need to Coenzyme Q for the antioxidant effects. Mitochondrial complex I activity in IV 20% increased than in those species) was determined by electrospray-ionization mass spectrometry. Hepatic α-Th was enriched in vivo in the adult rat by a single subcutaneous (SQ) injection of emulsified α-Th. α-Th transfer protein (α-TTP) was analyzed from liver homogenate by immunoblot. Results: Mitos from young rats showed >60% resistance to GCDC-induced MPT compared with adults. Young rats also had elevated α-Th in hepatic mitochondria (0.25±0.09 vs 0.12±0.01 nmol/mg pro, p<0.005) and reduced expression of α-TTP compared to adult rats. SQ α-Th treatment raised α-Th content in adult rat mito to that of young rats and significantly reduced susceptibility (i.e., increased resistance) to MPT induction (p=0.0004). Young rat mito contained similar total CL as adult mito, however, the major class of CL, tetra-linoleoyl CL, was significantly lower in young rats (1.92±0.14 vs. 3.01±0.13 nmol/mg pro, p<0.001). Conclusions: Resistance to bile acid-induced MPT in mito from young rat liver was associated with elevated mito α-Th (possibly due to reduced expression of α-TTP), and altered CL composition, which in combination would decrease the susceptibility of membrane lipids to oxidation. Enhancement of α-Th levels in adult mito reproduced the MPT resistance that was present in young rat mito. We conclude that mito membrane lipid changes in the developing rat may permit the young rat liver to cope with physiologically and pathologically elevated hepatic bile acid concentrations without inducing mito pathways of cell death. This process may be exploited as a target for reducing cholestatic injury.

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in I. Serum iron (I: 173±20, IV: 155±7) and transferin-iron saturation (I: 41±7, IV:38±2) were slightly reduced after cosupplement, Conclusion: CoQ(10) significantly enhances effect of vitamin E. Cosupplementation with Vitamin E and Coenzyme Q(10) could remove hepatic oxidative stress and might inhibit hepatic iron-overload.

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519 CORRELATION OF LIVER “COLLAGEN PROPORTIONATE AREA” BY COMPUTER ASSISTED IMAGE ANALYSIS AND HEPATIC VENOUS PRESSURE GRADIENT IN PATIENTS WITH RECURRENT HCV INFECTION AFTER LIVER TRANSPLANTATION
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Objectives: To assess whether the measure of liver collagen proportionate area (CPA) by computer assisted digital image analysis (DIA) is associated with hepatic venous pressure gradient (HVPG) in patients with recurrent HCV infection after liver transplantation.

Methods: 53 consecutive liver biopsies of 41 patients transplanted for HCV cirrhosis who had HVPG measured contemporaneously at the time of transjugular liver biopsy. Median HVPG was 5.5 mmHg (range 1-24). There was a significant positive association between digital image analysis of the CPA and HVPG by linear regression analysis. Results: The following people have nothing to disclose: Vincenza Calvaruso, Richard Standish, Pinelopi Manousou, Sergio Maimone, David Patch, James O’Beirne, Elias Xirouchakis, Alexandros Sigalas, Alice Corbani, Amar P. Dhillon, Andrew K. Burroughs

520 EVALUATION OF WEEK 12 RESPONSE ON SVR IN HCV POST TRANSPLANT PATIENTS UNDERGOING PEG/RBV THERAPY
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Background: Current guidelines for treatment of chronic HCV utilize EVR to indicate which patients are likely to achieve an SVR. Reports show a 72% SVR rate in patients with an EVR. The purpose of this study was to evaluate the week 12 viral response on SVR rate in chronic HCV post liver transplant patients. Methods: This is a secondary analysis of a multi-center randomized clinical trial of post OLT patients with recurrent HCV treated with 2 dosages of PEG IFN alfa.2b plus ribavirin 800 mg/day. PEG IFN dosage began at 0.5 mcg/kg/wk and increased in the high dose group to 1.5 mcg/kg/wk over 6 weeks. Results: The total sample contained 59 patients (27 low dose and 32 on high dose treatment). There were 39 males and 20 females ranging from 37 to 67 years of age (mean 51.4 ± 6.0). Race distribution was White 45 (76%), Black 3 (5%), Hispanic 10 (17%) and other 1 (2%). At treatment week 12, 32 (54%) patients achieved undetectable HCV RNA, 4 (7%) patients had ≥ 2 log decrease with positive virus, and 23 (39%) patients had <2 log decrease (Table 1). There was no difference in the use of pegylated IFN alfa.2b across race groups. There was no difference in the use of pegylated IFN alfa.2b across gender groups. There was no significant difference in the use of pegylated IFN alfa.2b across genotype groups. There was no significant difference in the use of pegylated IFN alfa.2b across treatment groups. Number of Patients with Undetectable HCV RNA

**5 patients discontinued prior to week 48 with side effects

Disclosures:
521 CHRONIC HEPATITIS E: A NEW ENTITY IN ORGAN TRANSPLANT PATIENTS

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Introduction: Hepatitis E virus (HEV) is a RNA virus that causes acute hepatitis in developing countries, but also sporadic cases in industrialized countries (non travel associated or autochthonous hepatitis E). Non travel associated hepatitis E have a predilection for middle aged and elderly males, are caused by genotype 3 and can carry significant morbidity especially when seen in the context of chronic liver disease. We describe for the first time a chronic evolution in organ transplant patients.

Patients: We identified 13 cases of hepatitis E infection that occurred in 13 organ-transplant patients who presented with unexplained elevation of liver enzymes levels. There were 3 liver-transplant patients, 8 kidney-transplant patients and 2 kidney-pancreas transplant patients. The median time since transplantation was 57 (6-168) months. Six were asymptomatic while the 7 remaining patients presented with fatigue. Two patients had been in contact with animals and none had travelled abroad recently. At diagnosis, there was a significant increase in liver enzymes levels, from 23 (12.95) to 115 (37-436) IU/L for AST (p=0.0015), from 26 (10-102) to 245 (66-874) IU/L for ALT (p=0.0015), from 32 (8-1164) to 132 (40-3482) IU/L for gGT (p=0.0015), and from 105 (26-226) to 249 (107-822) for alkaline phosphate (p=0.0015). Cytomegalovirus and hepatitis C virus Abs, as well as HBs anti-hepatitis A virus were negative and remained negative until last follow-up. HEV serology was negative in all patients but one. HEV RNA was positive in the sera of all patients, and in the stool (n=3) when looked for. Nine patients underwent a liver biopsy at diagnosis which revealed signs of non specific modest inflammation. HEV RNA became negative within three months in 7 patients (group I), while it remained positive in the 6 other patients (group II), respectively 9, 12, 13, 15, 16, and 27 months after diagnosis. The time between the transplantation and the diagnosis was significantly shorter in patients who developed chronic hepatitis, i.e. 82 ± 17 months in group I vs. 31.6 ± 10 in group II (p=0.02). Two patients who had chronic hepatitis E infection underwent a second liver biopsy, respectively 12 and 18 months after diagnosis. Both biopsies revealed signs of chronic active hepatitis associated to liver fibrosis. Conclusion: HEV infection can progress to chronicity in organ-transplant patients. HEV must be looked for in transplant patients with even moderately elevated transaminases.

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522 POST-LIVER TRANSPLANT SURVIVAL IN HEPATITIS C PATIENTS IS IMPROVING, NOT DECLINING

Jacqueline G. O’Leary, Lafaïne M. Grant, Henry Randall, Nicholas Onaca, Linda Jennings, Goran Klintmalm, Gary L. Davis.

Outcomes after orthotopic liver transplant (OLT) for chronic hepatitis C (HCV) have been reported to be worsening over the last 2 decades. We analyzed our center’s experience over 15 years to identify trends in post-OLT survival in patients with and without HCV. Methods: Patient and graft survival of adult primary OLT recipients from January 1991 to June 2006 at Baylor Regional Transplant Institute (n=2051) were evaluated by Kaplan-Meier analysis. Those with or without HCV were analyzed by era: 1:1991-1994 (n=509), 2:1995-1998 (n=459), 3:1999-2002 (n=532), and 4:2003-6/2006 (n=551). Differences in eras with disparate survivals were assessed by univariate and multivariate analysis. Results: Overall, patient and graft survival were significantly lower among HCV recipients than in others (p<0.0001). This difference was dependent on the era of transplantation with improvement in HCV patient (p=0.0015) and graft (p=0.0001) survival in sequential eras: 5-year patient survival of 61.5%, 62.6%, and 75.6% for eras 1, 2, and 3, respectively (era 4 not evaluable yet). The change is largely related to changes in listing criteria for hepatocellular carcinoma (HCC) beginning in era 3. Survival in those transplanted for HCC with HCV has improved dramatically over time (p<0.0001). In fact, there was no change in post-OLT patient survival over time (p=0.19) when those with HCC were excluded from the HCV cohort, while graft survival still improved in successive eras (p<0.007). There was no change in survival of non-HCV recipients between eras (p=0.14), although graft survival improved after 1994 and has remained stable since (p=0.02). The impact of potentially detrimental changes in recipient demographics over the eras including older patients, older donors, and a higher proportion with HCC have likely been ameliorated by positive trends including shorter cold ischemia time, fewer retransplants, greater use of tacrolimus and mycophenolate, and less steroid-resistant rejection. Conclusion: Post-transplant survival after OLT for chronic hepatitis C has improved significantly over the last 15 years despite demographic changes in patients and grafts that have been previously shown to impair survival. A major reason for this improvement is better selection of patients with concurrent hepatocellular carcinoma. No donor organs were obtained from executed prisoners or other institutionalized persons. The authors have no conflicts of interest.

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523 INTRAHEPATIC COVALENTLY CLOSED CIRCULAR DNA (CCC DNA) DETECTION IN PATIENTS TRANSPLANTED FOR HBV-RELATED CIRRHOSIS: A TOOL TO JUDGE FOR HBIG PROPHYLAXIS WITHDRAWAL IN LOW-RISK TRANSPLANT RECIPIENTS?

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INTRODUCTION: The outcome of orthotopic liver transplantation (OLT) for HBV-related cirrhosis remains influenced by the presence of HBV-DNA in the liver of the recipient. The hepatitis B surface antigen (HBsAg) positive recipients, who have undergone a recent HBIG prophylaxis have low risk of developing a positive post-OLT HBsAg test in the absence of high HBV-DNA levels. However, the level of HBV-DNA in the liver as a parameter of liver activity is not always available and the HBsAg status of the donor is not known in most OLT recipients. We assessed the intrahepatic viral DNA in a cohort of OLT recipients before liver transplantation and the correlation with the post-OLT HBsAg status. METHODS: Between December 1999 and May 2005, 532 patients underwent OLT at our institution. HBV-DNA was measured before transplantation using the polymerase chain reaction (PCR) according to the parameters of the Centers for Disease Control and Prevention (CDC) laboratory guidelines for a detection level of 5.0 IU/mL. RESULTS: Of the 532 OLT recipients, HBV-DNA was detected in 111 patients (21%). Of these, 45 patients (40.5%) were HBsAg positive before transplantation (31 patients with a recent HBIG prophylaxis withdrawal and 14 patients with a prior HBIG prophylaxis). Post-OLT HBsAg seroconversion occurred in 41 (91%) of the 45 patients, 30 patients (67%) with recently withdrawn HBIG prophylaxis, and 11 patients (78.6%) with a prior HBIG prophylaxis. Conclusion: The detection of HBV-DNA in OLT recipients before transplantation can be used as a tool to judge for HBIG prophylaxis withdrawal in low-risk recipients.
524 HBV GENOTYPE C IS ASSOCIATED WITH HIGHER RATES OF HCC AND POST-LIVER TRANSPLANT (OLT) MORTALITY COMPARED TO GENOTYPES A, B AND D

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Background/Aims: HBV genotype, precore and core promoter mutations have been reported to be associated with activity of liver disease and hepatocellular carcinoma (HCC). We hypothesized that these factors might impact indication for OLT and post-OLT outcomes. Methods: 130 patients in the NIH HBV OLT Study with available sera and detectable HBV DNA were tested for HBV genotype and precore (G1896A) and core promoter variant (A1762T/G1764A) using line probe assay. HBV DNA was quantified using Amplicor assay. Post-OLT survival was estimated using Kaplan-Meier analysis. Results: 82% of patients were men, mean age was 51 years; 48% were white, 41% Asian, 8% African Americans and 3% others. Indication for transplant was cirrhosis (62%), HCC (29%) and acute liver failure (7%). 51% of the patients were HBcAg positive. The distribution of HBV genotypes was A (36.2%), C (33.1%), D (14.6%), B (11.5%), and others (4.6%). Genotype C and B were predominantly found among Asians, A and D among White, and all but one African American had genotype A. Pre-core and core promoter variants were detectable in 42% and 89% of patients, respectively. HCC was the indication for listing among 51% of patients with genotype C but only 21% of patients with genotypes A and D and 13% of patients with genotype B. Univariate analysis showed that gender, Asian race, HBV genotype C, and presence of core promoter mutation were associated with HCC at listing. Multivariate analysis identified genotype C as the only factor associated with HCC (p<0.001). 70 patients underwent OLT, and 9 died during a median follow-up of 24 months (range 0-60). Patients with genotype C had significantly poorer post-OLT survival compared to those with genotype non-C. The probability of post-OLT survival at 1 and 4 years were 83% and 66% vs 94% and 91%, respectively for patients with genotype C vs. non-C (p = 0.024). Survival was similar among patients with genotypes A, B and D. Although genotype C was predominantly found in Asians, post-OLT survival of Asians was not worse than non-Asians. Similarly, despite a strong association between genotype C and HCC, post-OLT survival of patients with HCC was not different compared to those with cirrhosis. Cox regression identified HBV genotype C as the only predictor of post-OLT survival (p=0.04). Neither HBV genotype nor the presence of pre-core or core promoter variant was associated with HCC or HBV recurrence. Conclusions: In this multi-racial population of patients listed for OLT for HBV, genotype C was associated with significantly higher rates of HCC and post-OLT mortality but there was no association with HCC or HBV recurrence.

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525 EVALUATION OF THE INTRAHEPATIC EXPRESSION OF TWO INTERFERON-INDUCIBLE PROTEINS, MXA AND IFI16, DURING ACUTE REJECTION AND VIRAL REINFECTION OF LIVER ALLOGRAFTS

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Introduction. Character and strength of the adaptive immune response are influenced by signals from the innate immune response, including interferon alpha, that may be magnified by infection. In the transplantation setting, these processes may affect both infection control and allograft rejection. Our aim was to investigate the interplay between graft reinfection by hepatitis C virus (HCV), acute cellular rejection, and interferon-inducible protein expression (MxA and IFI16) among liver transplant recipients. Methods. Immunostaining for MxA and IFI16 expression was performed on formalin-fixed, paraffin embedded liver biopsy specimens, obtained from 28 patients (20 males, median age 52 years; 14 had been transplanted for HCV-related disease). The median interval between transplant operation and biopsy was 22 weeks. Eleven out of 28 biopsy specimens showed acute allograft rejection (Banff RA1 score 3-4: N=5; 5-6: N=5; ≥7: N=1). Leukocyte and macrophage infiltrates were characterized by CD45 and CD68 immunostaining, respectively. Results. The predominant MxA staining pattern was hepatocytic. IFI16 displayed a significant expression both in the hepatocellular and inflammatory compartments. MxA and IFI16 expression in hepatocytes were positively asso-
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Introduction/Aim: Successful treatment of chronic hepatitis C virus (HCV) infection is defined by undetectable serum HCV RNA six months following cessation of antiviral therapy (sustained virological response; SVR). This response seems highly durable in immunocompetent HCV patients, with rates >96% reported after several years of follow-up. However, it is unclear whether an immunosuppressed patient population will enjoy the same long term success. We therefore examined our liver transplant patient population with SVR to assess the durability of their response. Patients: Over 150 patients in the liver transplant program at our institution have been treated for recurrent HCV; to date, 75 have achieved an SVR. Qualitative HCVRNA determinations were made q3m for one year from cessation of treatment, and annually thereafter, as well as during any rise in liver enzymes. Results: 39/75 patients (52%) were Genotype 1 (G1), and 24 (32%) were G2 or G3, the remainder were other genotypes or unknown. Two patients were transplanted for HCV/hepatitis B coinfection, and had undetectable HBV DNA prior to treating HCV. All patients who had undetectable HCV RNA 3m following treatment achieved an SVR. Follow-up from SVR ranged from 3m to 84m. Two patients died during the follow-up period, one 8m and the other 22m from end of treatment; both were HCV RNA negative 2m prior to death. Only one patient relapsed during the observation period. This patient had received an HCV positive graft, and become HCV RNA negative on combination therapy, remaining so for 18m after end of treatment. Retransplantation with combined liver/kidney then took place and, 3m later, serum HCV RNA became detectable. Conclusions: An SVR achieved in liver transplant patients appears to be as durable as that seen in a non-transplant population, with the only relapse in a patient who underwent retransplantation and had marked augmentation of immunosuppression. Further, HCV RNA determination 3m after end of treatment may suffice to define SVR in this difficult-to-treat population.

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527 COMPARISON BETWEEN HEPATIC ELASTICITY MEASUREMENTS (FIBROSCAN®) AND LIVER BIOPSY IN PATIENTS TRANSPLANTED FOR HCV CIRRHOSIS

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Context and objectives: Liver biopsy is considered the gold standard to evaluate outcome post liver transplantation. Because of its non-invasiveness, hepatic elasticity measurement (Fibroscan®) has increasingly gained attention for the evaluation of fibrosis in transplanted patients. Our purpose was therefore to compare Fibroscan® values with those of liver biopsy in patients transplanted for HCV cirrhosis. Patients and methods: All consecutive patients transplanted for HCV cirrhosis who underwent a medical visit at Hôtel-Dieu Hospital between April and November 2006 and who had a recent liver biopsy or with indication for it were invited to participate in this study. 38 patients (27 men, mean age 58.3 years), between 44 and 75 years were included. We evaluated ALT values, viral load and genotype, treatment for chronic hepatitis, time between the biopsy and transplantation and between biopsy and Fibroscan® and possibility of any other hepatic comorbidity. The results of Fibroscan® were converted in Metavir Score values (using the values already validated for) and those were compared with the results of his biopsy. Results: 15 patients (39%) had fibrosis lower than F2 at liver biopsy and 3 (8%) were F4. For Fibroscan®, these results were 16 (42%) and 9 (24%), respectively. The mean duration between the transplantation and the biopsy was 76.8 months (3-172) and 3.2 months between the transplantation and the Fibroscan® (0.9). 17 patients (45%) had cleared HCV following therapy. At the time of transplantation, 8 patients (21%) presented already hepatocellular carcinoma on the explanted liver. The observed concordance between the methods was 0.71 (27 patients) and the expected concordance was 0.51. The sensibility of Fibroscan® was 0.76 (IC 0.58-0.94) and its specificity, 0.65 (IC 0.53-0.76) with predictive positive and negative values of 0.72 and 0.65, respectively. The prevalence and the corrected prevalence was 0.55 and 0.57. The positive likelihood ratio was calculated at 2.2 and the negative as 0.37. Intermethods reliability shows a kappa index at 0.41 (IC 0.09-0.73; statistical significance> 0.5). The comparison of all epidemiological and laboratory parameters between the two groups (concordants and non-concordants) did not show any statistically significant difference. Conclusions: Our data failed to show a close correlation between Fibroscan® and the liver biopsy in these patients. This discordance may be in part due to the high proportion of cases with mild fibrosis that all the more it emphasizes the need for specific validation of the Metavir Scores with Fibroscan® measurements in the context of transplantation which warrant further studies.

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528 HYPERIMMUNE ANTI-HBS PLasma FOR THE PREVEN-  
TIOn OF HBV RECURREnCE AFTER lIVER TRANSPLAN-  
tATION: EFFICACY, SAFETY, KINEtICS AND  
ECONOMICS IN 26 PATIENTS DURING 14-YEAR EX-  
PERIENCE OF TWO CENTERS  
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Background: Hepatitis B immune globulins (HBIG) in combina-  
tion with nucleos(t)ide analogues are used for the prevention of  
HBV graft re-infection after liver transplantation (LT). Several  
HBIG preparations with different biological activities are com-  
mercially available but associated costs for long-term treatment  
are very high. Thus, new strategies to lower post-LT costs are  
needed. Methods: In 1993 a blood center-based program for  
the production of hyperimmune anti-HBs plasma (HIP) was  
started in Geneva preparing fresh frozen plasma obtained by  
separation (separation-HIP) from HBV vaccinated donors with  
high titer anti-HBs (>20’000 IU/L). In 2000 the program was  
introduced at a regional Transfusion Center, far from the Trans-  
plant Center and the procedures were optimized by HIP pro-  
donation through plasma-apheresis (apheresis-HIP). Every  
apheresis procedure provided two preparations of 300cc  
leukocyte-deprived plasma (<106 WBC/ml). The plasma was  
frozen for four months and then tested again for the presence  
of viral markers. The total cost for production of one apheresis-HIP  
is 410$US. Both HIP formulations were produced according to  
the guidelines of the Swiss Red Cross Transfusion Service. The  
elimination kinetics of anti-HBs (antibody levels at time 0',  
directly after, 1h, 24h, 3d, 7d, 10d, 14d, 21d, 30d after trans-  
fusion) were assessed in 5 patients receiving apheresis-HIP and  
compared to commercial HBIG. Results: Since the introduction  
of HIP, 26 patients were treated with separation-HIP, and ten  
were switched to apheresis-HIP in 2000. Patients who were  
initially treated with commercial HBIG needed less frequent HBIG  
administrations after introduction of HIP: every 3-5 weeks with  
commercial HBIG vs 6-8 weeks with HIP. Mild transfusion reac-  
tions were observed occasionally with separation-HIP (n=5  
patients), and in this case patients were switched back to com-  
mercial HBIG. With apheresis HIP no side effects were  
observed ever since (10 patients over 7 years). The elimination  
kinetk of anti-HBs in apheresis-HIP were comparable to com-  
mercial formulations. The cost for commercial HBIG (7’500IU,  
4’050$US, median annual cost 48’600$US) was considerably  
higher compared to HIP (7’500IU, 410$US, median annual  
cost 4’100$US). Finally, with the introduction of HIP the median  
anti-HBs titer has risen with a less frequent need for HBIG  
administrations (from 100-200 to 200-300 IU/L). Conclusion:  
HIP is an effective, safe and economic treatment for the pre-  
vention of HBV recurrence after LT. It can be easily produced in  
any blood transfusion center and may be an attractive alterna-  
tive to commercial HBIG products, particularly in countries with  
limited resources.  
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Stefan Russman, Loriana Di Giammarino, Claudia Steineman, Andreas Cerny,  
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529 CAUCASIAN AMERICAN HEPATITIS B LIVER TRANSPLANT PATIENTS HAVE HIGHER RATES OF WAITLIST MORTALITY AND HBV RECURRENTnCE THAN ASIAN AMERICANS  
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Background/Aims: Previous studies suggested that Asian Amer-  
icans (AS) had worse outcomes after liver transplantation (OLT)  
than Caucasian Americans (CA), related in part to more  
advanced disease at presentation. We compared the indica-  
tions for OLT, severity of liver disease at listing, and pre- and  
post-OLT outcomes between AS and CA. Methods: Data from the  
NIH HBV OLT study that includes 15 US centers were  
reviewed. Patients self declared to be AS or CA were included;  
those who had HCV, HDV or HIV coinfection or were listed for  
repeat OLT were excluded. Outcomes were analyzed by Kaplan-Meier analysis and predictors of outcome were ana-

yzed by Cox regression. Results: A total of 251 patients, 135  
AS and 116 CA, were included; 76% AS and 82% CA were  
men; mean age at listing was comparable, 53 vs. 54 yr. At list- 
ing, a higher percent of AS had HCC (47% vs. 16%,  
p<0.0001), while a lower percent had acute liver failure (3%  
vs. 9%, p=0.03). HBcAg status and HBV DNA levels were simil- 
ar, and MELD scores among the cirrhotics were comparable.  
A similar percent of AS and CA was receiving antiviral therapy  
at listing: 57% vs. 60%. Among the patients who had cirrhosis  
at listing, 8 (12%) of 67 AS and 14 (16%) of 87 CA had new  
diagnosis of HCC (NS). Probability of OLT at 1, 3 and 5 yr  
were comparable, being 48%, 58%, and 66% for AS and  
49%, 59% and 64% for CA. AS had lower waitlist mortality,  
with the probability of death at 1, 3 and 5 yr being 1%, 3%  
and 12% vs. 9%, 17% and 22% for CA (p=0.009). This dif-  
ference remained when the analysis was restricted to patients  
with cirrhosis and no HCC. Cox regression identified race  
(p=0.014) but not OLT indication as a predictor of waitlist mortal-  
ty. Of the 85 (49%) AS and 69 (41%) CA transplanted, 11  
(13%) AS and 8 (12%) CA died during a median follow up of  
30 mos (0-67). Probability of post-OLT survival at 1, 3 and 5 yr  
were 90%, 85% and 85% for AS and 93%, 88% and 88% for  
CA (NS). Cox regression identified HCC recurrence (p=0.001)  
but not race, OLT indication (HCC vs. non-HCC) or HBV recur- 
rence as predictors of post-OLT mortality. One (1%) AS and 1  
(16%) CA had HBV recurrence, and Cox regression identified  
race (p=0.017) as a predictor of HBV recurrence. Five (9%)  
of 54 AS and 4 (13%) of 30 CA with HCC at transplantation had  
HCC recurrence (NS). Conclusions: AS were significantly more  
likely to have HCC at listing than CA, but the rates of new HCC  
diagnosis after listing and HCC recurrence post-OLT were com-  
parable. AS and CA had similar post-OLT mortality as CA, but  
CA had significantly higher waitlist mortality and HBV recur- 
rence rates than AS. Further analyses are ongoing to explain  
these discrepant outcomes.

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METABOLIC SYNDROME IS AN INDEPENDENT PREDICTOR OF FIBROSIS PROGRESSION IN PATIENTS WITH RECURRENT HEPATITIS C (HCV) AFTER LIVER TRANSPLANTATION (LT) USING SERIAL BIOPSY SPECIMENS

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While hyperinsulinemia and its associated metabolic syndrome (MS) have been implicated in the progression of hepatic fibrosis in HCV patients, little is known about consequences of MS after LT. 

Aims: Assess the interaction between MS and fibrosis progression in patients with recurrent HCV after LT using serial liver biopsies. 

Methods: Using electronic pathology database, we identified all LT/HCV patients with at least two post-transplant liver biopsies (1998–2006). MS was defined using ATP III criteria at 1 year post LT. Ludwig-Batts scoring system was used to stage all biopsies (408 biopsies from 95 patients). The first biopsy that showed progression post LT was used for the time-to-progression analysis. Univariable and multivariable logistic regression analysis was performed to identify factors associated with fibrosis progression. 

Results: MS was present in 50% of patients. Median follow-up was 22 (Q25, Q75: 12.0, 31.2) months during which 71% of subjects had fibrosis progression. Overall median rate of fibrosis progression was 0.08 units per month (Q25, Q75: 0.0, 0.17). By univariate analysis, high HCV RNA at 4 months post-LT (p<0.001), diabetes (p=0.046), CMV infection (p=0.006) and MS (p=0.049) were associated with progression of fibrosis. In multivariate analysis, MS was not associated with fibrosis progression during the first year post-LT (OR=1.06, 95% CI: 0.49-2.26) but was independently associated with progression of fibrosis beyond 1 year after LT (OR=6.3, p=0.017). High viral load at 4 months postLT (OR=1.1, p=0.004), steroid therapy for acute rejection (OR=1.9, p=0.05), and CMV infection (OR=1.9, p=0.01) were independently associated with fibrosis progression. A Kaplan-Meier plot below outlines the association between MS and fibrosis progression. 

Conclusion: MS, a potentially modifiable factor, is common and appears to be strongly associated with long-term fibrosis progression in the setting of recurrent HCV after LT.

The following people have nothing to disclose: Ibrahim A. Hanouneh, Charles M. Miller, Arthur J. McCullough, Rocio Lopez, Federico Aucejo, Ariel E. Feldstein, Nizar N. Zein

MULTICENTER RANDOMIZED TRIAL IN HCV-INFECTED PATIENTS TREATED WITH PEGINTERFERON ALFA-2A AND RIBAVIRIN FOLLOWED BY RIBAVIRIN ALONE AFTER LIVER TRANSPLANTATION: FINAL REPORT

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The aim of this randomized, double-blind study was to determine the effect of placebo or maintenance therapy with ribavirin (RBV) monotherapy, after a year of combination therapy with peginterferon-alfa 2a (PEG-IFNα-2a) and RBV, on continued eradication of HCV after liver transplantation (LT). 

Methods: The study enrolled 100 patients with recurrent HCV and a minimum of stage 1 fibrosis (METAVIR scoring) on a liver biopsy obtained 1–5 years after LT. At inclusion, the activity and fibrosis scores (METAVIR) were 1.67 ± 0.76 and 1.47 ± 0.70, respectively. PEG-IFNα-2a and RBV were initiated at 90 µg/wk and 600 mg/d, respectively, and increased to 180 µg/wk and 1000 mg/d or adjusted as a function of hematological tolerance. At week 52, combination therapy was discontinued and patients were randomized to RBV alone or placebo for a further 48 weeks (blinded). 75% of patients received tacrolimus and 25% cyclosporine. 

Growth factor use was permitted. Results: After one year of combined therapy, 63 patients were negative by PCR and randomized between RBV alone or placebo. At 78 weeks (6 months after combined therapy), a sustained virological response (SVR) was obtained in 40% of ITT patients (40/100). At 30 months (M30) (6 months after the end of the maintenance period), 47% of the patients randomized to RBV (16/34) and 55% of those randomized to placebo (16/29) had undetectable HCV-RNA (NS). At M30, the histological activity score was 1.52 ± 0.84 (vs 1.63 ± 0.74 at M12) in the RBV group and 1.72 ± 0.84 (vs 1.51 ± 0.88 at M12) in the placebo group (NS). At M30, the fibrosis score was 1.52 ± 0.84 (vs 1.63 ± 0.91 at M12) in the RBV group and 1.72 ± 0.84 (vs 1.51 ± 1.09 at M12) in the placebo group (NS). The fibrosis score marginally improved in the RBV group between M12 and M30 (-0.14 [CI : -0.5;-0.22]), whereas it worsened in the placebo group (+0.26 [CI : -0.02;+0.54]) (p=0.07). 

Conclusion: After 1 year’s combination therapy with peginterferon alfa-2a plus ribavirin, a SVR was achieved in 40% of patients following liver transplantation in ITT analysis. A main disadvantage of this study was the low SVR which can be attributed to low HCV-RNA at inclusion. The authors conclude that RBV plus placebo offers a good combination therapy for maintaining the cure of HCV after liver transplantation.

Disclosures:
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HEPATITIS C VIRUS (HCV) INFECTION IS A PROTECTIVE FACTOR FOR HEPATITIS B VIRUS REACTIVATION (HBVR) AFTER RECEIVING HEPATITIS B CORE POSITIVE (HBcAb+) DONORS FOR LIVER TRANSPLANTATION (LT)

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HBVr occurs with a variable frequency after receiving an HBCAb+ liver donor. However, there is little data on clinical, serological and virological factors associated with HBVr after LT. Aim: To determine the incidence of HBVr in LT recipients and to identify clinical, serological and virological factors associated with HBVr. Methods: Retrospective analysis of our LT population from June 1995 to May 2007. Non-HBV infected recipients with complete laboratory data post LT were studied. HBVr was defined as a positive HBsAg or HBV DNA >20,000 IU/ml post LT. Fisher’s exact test was used for group comparisons. Results: 60 of 550 LT recipients (10.9%) received HBCAb+ donors. 42 of 60 patients met inclusion criteria. 6 of 42 (14%) LT recipients had HBVr. Laminiviremia prophylaxis was used post LT in 6% of the recipients. 74% of the recipients had HCV infection. Median time for HBVr was 20.7 (5.8 to 110) months. 75% were male. Mean age was 54.3 years. We identified three different groups for HBV (table1). Of the 26 patients in the MG, one (4%) patient had HBVr. In the UG, 4 of 12 (33%) patients developed HBVr. The UG had 8.7 (95%CI, 1.1 to 69) times the risk of HBVr compared to the MG after LT (p=0.03). The remaining 4 patients were vaccinated and one (25%) patient had HBVr. There was no difference in HBVr in the vaccinated group compared to the MG and the UG. To investigate HBVr by LT recipient diagnosis, we compared HCV infected recipients (74%) to non-HCV recipients (26%). Of the 31 HCV infected recipients, 2 (6.4%, 95% CI, 0% to 21%) developed HBVr. In contrast, 4 (36%, 95% CI, 10% to 69%) of the non-HCV recipients had HBVr. The non-HCV recipients had 5.6 (95% CI, 1.2 to 27) times the risk for HBVr in comparison with HCV infected recipients (p=0.03). Since both HCV infected recipients and MG recipients appeared to be protected against HBVr, we tested if the combination was also protective. 3 (60%) of the 5 recipients in the MG with non-HCV diagnosis had HBVr compared to 0 of 22 recipients in the MG with HCV infection (p=0.003). Conclusions. Serological and virological characteristics have important implications to allocate HBCAb+ livers. Recipients in the matched group with HCV infection appear to be protected from HBV reactivation after LT. UG and non-HCV recipients have higher risk of HBV reactivation and therefore, HBV antiviral prophylaxis should be strongly considered. HBVr in the vaccinated group may have been explained for low HBsAb titers in the recipient.

Table 1

<table>
<thead>
<tr>
<th>Group</th>
<th>Matched group (MG)</th>
<th>Unmatched group (UG)</th>
<th>Vaccinated group</th>
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<tbody>
<tr>
<td>Donor</td>
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<td>or</td>
</tr>
<tr>
<td>Recipient</td>
<td>HBcAb+ / HBsAb+</td>
<td>HBcAb+ / HBsAb+</td>
<td>HBcAb+ / HBsAb+</td>
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</tbody>
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HIGH PREVALENCE OF GLOMERULONEPHRITIS IN PATIENTS WITH END-STAGE HCV-INDUCED CIRRHOSIS

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Patients undergoing liver transplantation for cirrhosis due to hepatitis C virus (HCV) infection have a greater frequency of renal insufficiency after liver transplantation compared to patients without HCV. It is speculated that there is a greater prevalence of glomerulonephritis (GN) at the time of liver transplantation, but there are no data to support this postulate. The aims of this study were to determine the prevalence and type of renal pathology with HCV-induced and nonviral-induced cirrhosis and to correlate renal pathology with serum and urinary markers of renal disease. METHODS: Forty adult patients undergoing liver transplantation for cirrhosis (including 30 patients with HCV-induced cirrhosis previously reported) were evaluated prior to liver transplantation for renal dysfunction (serum creatinine), hematuria (dipstick > trace blood), and proteinuria (urinary protein/creatinine ratio >0.3). At the end of transplantation, a needle biopsy of the right kidney was done. RESULTS: The clinical data for the six patients with nonviral-induced cirrhosis included: mean age 55 yr (44-68), 5 men, and cause of liver disease was 2 non-alcoholic steatohepatitis, 2 alcohol, 1 primary sclerosing cholangitis and 1 cryoprecipitable. Hepatoma was present in 1 patient, diabetes in 3 and hypertension in 4. The mean calculated MELD score was 23. Preoperatively, 5 patients had one or more abnormal noninvasive study [3 elevated serum creatinine, 2 hematuria, and 2 proteinuria]; 1 patient had normal studies. Kidney biopsy showed 3 patients with IgA nephropathy (IgAN) and 3 without GN. The clinical data for the 34 patients with HCV-induced cirrhosis included: mean age 52 yr (41-73), 23 men, 13 hypertensive, 8 diabetic, and 2 had hepatorenal syndrome at engraftment requiring hemodialysis. Mean calculated MELD score was 24. Preoperatively, 19 patients had at least one abnormal noninvasive study (13 elevated serum creatinine, 12 hematuria, and 7 proteinuria); 15 patients had normal studies. Kidney biopsy showed 29 patients with an immune-complex GN (14 membranoproliferative GN, 9 IgAN, and 6 mesangial GN). No patient in either group had cryoglobulins by electronmicroscopy or serum measurement. At liver engraftment, histologically recognizable glomerular disease affected 29/34 (85%) of patients with end-stage HCV cirrhosis versus 3/6 (50%) of patients with nonviral-induced cirrhosis (p = 0.05). CONCLUSIONS: Patients with HCV-induced cirrhosis have a higher prevalence of immune-complex GN compared to patients with nonviral-induced cirrhosis at the time of engraftment. These findings may explain the decline in renal function that commonly occurs after liver engraftment for HCV.

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Rapid Fibrosis in HCV-Patients After Liver Transplantation Is Associated with an Upregulation of Collagen Type I, MMP-9 and TIMP-1

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Introduction/Aim: Only a subset of hepatitis C virus (HCV)-infected patients develop progressive hepatic fibrosis after liver transplantation (LT). Whereas non-transplanted chronic HCV-patients develop cirrhosis after 15-20 years LT-HCV-patients may develop cholestatic fibrosis with very high mortality during the first year (rapid progression, RP) or chronic progression with high or low ALT resulting in cirrhosis after 2-3 years or later (slow progression, SP). There are no reliable markers to identify high-risk recipients who will develop cirrhosis shortly after LT. Activation of hepatic stellate cells is an early step in hepatic fibrogenesis leading to the expression of a plethora of extracellular matrix genes and enzymes. The aim of this pilot study was to define a subset of ECM molecules allowing early discrimination between RP and SP. Methods: Samples from routine liver biopsies obtained 6, 12, 36 and 48 months post LT (11 HCV-LT patients) and 3 non-transplanted controls were stored in RNA later before mRNA was isolated, transcribed to cDNA and subjected to TaqMan real time quantitative PCR using primers and probes for matrix metalloproteinases and inhibitors (MMP-2, MMP-9, TIMP-1), growth factors (CTGF, TGF-beta, collagens (type I, VI-alpha3) transglutaminase-2 and GAPDH. Grading and staging of fibrosis was performed by histology and liver function tests were determined. Results: By histology six and five patients, respectively, were identified to have SP or RP. Biopsies from non-transplanted livers and livers with SP showed no difference in the expression of all 8 markers whereas 3 of the 8 markers (MMP-9, TIMP-1 and CI) showed a higher expression in the RP group compared to controls and SP. These changes were seen more pronounced in liver biopsies one year after LT. Conclusions: Three markers of hepatic stellate cell activation and extracellular matrix turnover were identified which discriminate between slow and rapid fibrosis progression in HCV patients after LT. Further studies are ongoing to confirm these results in large patient cohorts. The identification of patients with a rapid progression at an early timepoint after LT shall allow an early intervention either by modification of immunosuppression and/or addition of antifibrotic treatment.

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Anti-HCV Immune Responses Modulate Recurrence of HCV Infection and Severity of Liver Histology After Liver Transplantation in HCV-Co-infected Patients

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Background: Liver transplantation (LT) in HIV/HCV co-infected patients is successful. Hepatitis C reinfection is the major cause of recipient mortality. The aim of this work was to evaluate the immune responses against HCV before and after LT in HIV/HCV co-infected patients. Methods: 14 patients with HIV viral load (VL)<50 copies/mL and CD4 counts>200/mm3 receiving a graft for HCV-cirrhosis were included. Patients were monitored for HCV and HIV VL, liver histology and immune responses in pre- and post-transplantation course (2 years). T cell responses were evaluated in blood by IFN-γ-ELSpot assays using recombinant (r) proteins: core, NS3, and NS4 (HCV), HIV-1-p24 and CMV, PPD and candida, and 16 pools of HCV-peptides (core, NS3, and NS5) and 1 pool of HIV-1-p24-peptides. Results: Before LT and 2 years after, median CD4 counts and HIV-1VL were 276 and 238/mm3 and 5.73 and 6.40 log IU/mL. During the follow-up, T-cell responses against opportunistic antigens were detected in 13/14 patients with median frequency of 245 SFC/106 PBMC (range: 50 to 1677). 11/16 patients had responses against rHIV-1-p24 protein with a median frequency of 110 SFC/106 PBMC (range: 50 to 930). HCV-specific CD4 T cell responses were detected in 4 patients and directed against core in 3 patients (from 67 to 187 SFC/106 PBMC) and NS3 and NS4 in the fourth patient (87 and 50 SFC/106 PBMC). Ex vivo CD8 responses against HIV-1-p24 were observed in 7/11 patients with a median frequency of 195 SFC/106 PBMC (range: 60 to 875) whereas responses against HCV-epitopes were detected in 1 patient and directed against core and NS5 (153 and 220 SFC/106 PBMC respectively). In 3 patients without ex vivo CD8 response, in vitro HCV-peptide stimulation lead to generate responses directed against core in 1 patient (2340 SFC/106 PBMC) and NS3 in 3 patients (from 203, 280 and 1460 SFC/106 PBMC). Interestingly, 6/7 patients with anti-HCV responses had also anti-candida responses vs 1/7 patients without anti-HCV responses. The seven patients with anti-HCV responses had F0(n=3) or F1(n=2) or F2(n=2) (METAVIR) score at 2 years. 3/7 had undetectable HCV-VL. In patients with no anti-HCV response, the scores observed were: F1(n=1), F2(n=1), F3(n=2) and F4(n=3). Conclusions: Overall, higher levels of IFN-γ secretion to HCV and candida are associated with less severe fibrosis. Our results demonstrate that immune responses against HCV modulate recurrence of HCV infection and severity of liver histology after LT in HIV/HCV co-infected patients.

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Background: Severe hepatitis C (HCV) recurrence is the most important influencing factor of survival after liver transplantation (LT).Portal hypertension (PH) and fibrosis stage (FS) at 12th month after LT have been associated to severe recurrence and poor survival. Several serum biological markers have been proposed as predictors of fibrosis progression in non-transplanted HCV infected patients. Aims: To analyze the relationship between the early expression (3 mo) of a serum panel of biological markers related to progression of liver damage (TNF-alpha, IP-10, MCP-1, sCAM-1, sVCAM-1, VEGF, HGF, Ang2, MMP9, TIMP-1 and hyaluronic acid) and severity of HCV recurrence after LT. Patients and Methods: Thirty-seven consecutive LT recipients due to HCV (n=19) or alcoholic (n=18) cirrhosis were included. All patients received tacrolimus or cyclosporin based immunosuppression with steroids. In all cases rejection and technical complications were excluded at the time of evaluation. Serum samples were collected at 3 mo after LT. Severe HCV recurrence was defined as fibrosis stage ≥F1 (Metavir score) in the one-year protocol biopsy and/or the existence of a HVPG value ≥6mmHg. Values are expressed as median (IQR). Results: At one year, 12 out 19 patients had severe HCV recurrence. No liver fibrosis was found in the biopsies performed in non-HCV patients. Patients with severe HCV recurrence compared to non-severe HCV recurrence and to non-HCV alcoholic patients presented at 3 mo significantly higher level of IP10 [(820 (2409), 348 (280), 180 (240) pg/ml], sCAM-1 [2466 (839), 1266 (916), 1354 (1330) ng/ml] and hyaluronic acid [624 (246), 434 (351), 326 (207) pg/ml]. ROC curves were able to identify significant value to predict severe recurrence in HCV positive patients: IP10>=590: 0.74 (0.5-0.9); sCAM-1>1481: 0.89 (0.7-1); hyaluronic acid>461: 0.70 (0.5-0.9). Conclusions: Serum levels of IP10, sCAM-1 and hyaluronic acid measured early after LT are associated to severe HCV recurrence. Evaluation of these biomarkers may be useful for early identification of patients with bad prognosis in which a more aggressive therapeutic approach could be necessary.

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537 HLA-DRW LOCUS DISPARITY IS ASSOCIATED WITH WORSE OUTCOME IN HCV TRANSPLANT RECIPIENTS

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Background & Aim: Host immune response may account for varying severity of Hepatitis C (HCV) recurrence after liver transplantation (LT). Data on HLA matching is conflicting, and could also influence survival, but this has not been evaluated. Our aim was to assess donor-recipient HLA disparity and severity of HCV recurrence, rejection, and survival at 3 months and long term after LT. Methods: Consecutive HCV cirrhotics, 229 transplants -17 retransplants; 180 males (78.6%). Mean age recipients 51.8(21-66); mean age donor 40.9(11-73). Median follow up was 52 months [range 1-216]. Hepatocellular carcinoma in 76 (31%); 74 had acute Hepatitis C (increased ALT>2x normal and consistent histology without features of cellular rejection, duct loss or other cause of liver injury). Overall 79(34%) died: 13 recurrent HCV cirrhosis, 5 chronic rejection, 37 other transplant related causes, 22 transplant unrelated cause, 2 sepsis. Serological typing for HLA class A and B, and microcytotoxicity assay for class II (DR and DQ) HLA disparity at each locus was performed and evaluated by multivariate analysis for recurrence and survival with respect to clinical data: donor and recipient age, donor and recipient gender, tacrolimus/cyclosporin monotherapy or any combination of immunosuppressive therapy, diabetes mellitus, HCV genotype, histological acute hepatitis C and occurrence and/or therapy of rejection episodes Results: HCV recurrence was evaluated histologically (Ishak scoring) from 1 year after LT (165 of 197 alive at 1st year); mean follow up 63.4 months -median 3 biopsies/patient (1-8). At last follow up 93 (37.7%) had fibrosis stage ≥3 and 72 (29.1%) had stage ≥4. Deaths occurred in 17% of fibrosis stage ≥3 and 26% stage ≥4. HLA locus disparity did not correlate with early deaths within 3 months, acute or chronic rejection, recurrent HCV or retransplantation. Severe fibrosis (≥4) stage was not associated with HLA disparity by Cox regression. However DRw locus disparity was significantly associated with patient survival (p=0.01). By Kaplan Meier analysis 75% survival if no mismatches, 62.5% with 1 mismatch, and 44.4% with 2 mismatches (x2=8.087, p=0.018). Conclusions: DRw locus mismatch does not associate with severity of HCV recurrence, but does correlate with survival after liver transplantation in HCV cirrhotic patients, independently of factors known to affect HCV recurrence.

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PATIENTS WITH RECURRENT HEPATITIS C

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Background and aims: We previously demonstrated that TE reliably predicts severity of graft damage in LT patients. However, to what extent TE helps monitoring patients with recurrent hepatitis C under protocol liver biopsies (LB) needs to be assessed.

Methods: 34 patients (28 males, median age 57 years) with recurrent hepatitis C, who underwent sequential protocol LB and TE examinations twice, during a follow-up period of 18 months (first examinations at 6-96 months, median 6, from LT). Grading and staging were assessed according to Ishak score (grading 0-18; staging 0-6). TE examination was considered adequate if at least ten valid measurements for each patient were obtained with a >65% success rate. Changes in liver fibrosis and TE values were assessed as follows: worsening fibrosis (wF) or improvement fibrosis (iF) was defined as ≥1 point staging increase or decrease, respectively; worsening TE (wTE) or improvement (iTE) was defined as ≥30% increase or decrease of TE baseline value. Four groups were identified: wF/wTE=group 1; iF/iTE or stable F/stable TE=group 2; wF/stable TE=group 3; iF/wTE=group 4. Results: Median length of LB was 30 mm (range 15-60). At first examination median grading was 7 (range 1-11), staging 1 (0-5), TE 6.9 kPa (4.0-20.2). At second examination median grading 7 (range 1-13), staging 2 (1-6), TE 8.5 kPa (4.1-27.1). Changes in histological grading and staging positively correlated with changes in TE values (r=0.47, p=0.005 and r=0.68, p<0.0001, respectively). 11/34 (32%) patients were in group 1, 17/34 (50%) were in group 2, 3/34 (9%) were in group 3, and 3/34 (9%) were in group 4. In particular, in the group 3 staging worsened in one patient from 0 to 1, in the other two from 1 to 2; only one had grading decreased from 9 to 5. In the group 4: one with stable staging had grading increased from 8 to 13; the second developed de novo autoimmune hepatitis during follow-up, and the third had severe intrahepatic siderosis. Overall, sensitivity and specificity of TE in predicting wF were 79% and 85%, respectively. Conclusions: In patients with recurrent hepatitis C TE accurately predicted wF. TE could spare the need for protocol LB in patients with iT or stable TE.

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Background and Aim The recurrence of hepatitis C following liver transplantation (LT) is universal and it frequently leads to progressive fibrosis and cirrhosis with poor outcome. The aim was to evaluate the progression of fibrosis on protocol liver biopsies in patients transplanted for HCV cirrhosis. Materials and Methods A cohort of patients transplanted (1999-2005) for HCV cirrhosis who underwent protocol liver biopsies consecutively performed at our Gastroenterology Unit at 6, 12 and 24 months after LT were included in the study. Histological stage of fibrosis was evaluated according to Scheuer (score 0-4). The fibrosis progression rate (FPR) was expressed as fibrosis unit per month (FU/mo) and compared over 2 periods (6-12 mo; 12-24 mo after LT). When indicated, patients underwent PEG-IFN/Ribavirin antiviral therapy. Statistical analysis: Wilcoxon rank, Friedman and Kruskal Wallis tests. Results 54 patients underwent 3 serial and consecutive liver biopsies, M/F 20/34, age 55±7yrs, follow-up 24-95mo. Overall, at 6, 12 and 24 months after LT the stage of fibrosis 3 or 4 was reported in 3.7%, 7.4% and 13% of patients respectively, stage of fibrosis (mean value) was 1.17, 1.20, 1.70 respectively, FPR was 0.005 FU/mo (6-12 mo) and 0.0416 FU/mo (12-24 mo). 9/54 (16.7%) underwent antiviral therapy, SVR was seen in 4/9 (44.4%) patients. At 6, 12 and 24 months after LT the stage of fibrosis 3 or 4 was reported in 0%, 11%, and 22% of treated and in 4.4%, 6.7% and 11.1% of non-treated patients, the stage of fibrosis was 1.44, 1.61, 2.11 in treated (p<ns) and 1.12, 1.12, 1.61 (p=.003) in non treated patients, FPR was 0.0283 and 0.0416 in treated and 0 and 0.0416 in non treated patients at 6-12 and 12-24 mo respectively. According to SVR, at 6, 12 and 24 mo after LT, stage of fibrosis 3 or 4 was 0%, 0%, 0% in SVR+ and 0%, 20% and 40% in SVR- patients, stage of fibrosis was 1.25, 1.25 and 1.5 in SVR+ and 1.6, 1.9 and 2.6 in SVR- patients, FPR was 0.00 and 0.02 for SVR+ and 0.05 and 0.06 for no SVR at 6-12 and 12-24 mo after LT respectively (p<ns). Conclusion The fibrosis progression due to HCV recurrence is accelerated between the 1st and 2nd year compared to the early period after LT, with a lower stage and slower FPR seen in patients who were not selected for antiviral therapy. In patients without SVR, pre-cirrhosis or cirrhosis is seen in 40% of cases at 2 years after LT.

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A PROSPECTIVE STUDY ON THE SAFETY AND EFFICACY OF LAMIVUDINE AND ADEFOVIR DIPIVOXIL PROPHYLAXIS IN HBSAG POSITIVE LIVER TRANSPLANTATION CANDIDATES

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Combination lamivudine (LAM) plus high-dose intravenous heaptatitis B (HBV) immunoglobulin (HBIG) provides safe and effective prophylaxis against recurrent HBV following liver transplantation (OLTx) in HBsAg positive candidates, with recurrence rates less than 10%. However, this regimen is associated with life-long monthly clinic visits, parenteral administration of HBIG, and high cost (US$120K first year, 60-90K per annum thereafter). The aims of this study were to assess the safety and efficacy of oral antiviral prophylaxis with combination adefovir dipivoxil (ADV) and LAM without HBIG in the prevention of recurrent HBV. This prospective, open label, multi-centre study enrolled consecutive adult candidates for OLTx with HBsAg positive end-stage cirrhosis. Patients with prior LAM exposure were excluded if they had clinical or virological LAM resistance. Patients received LAM 100mg daily and ADV 10mg daily when listed for OLTx. Following OLTx, patients also received IM HBIG 8000IU daily for one week only. Primary endpoint was recurrence of HBsAg. Secondary endpoints were detection of HBV DNA, graft or patient loss, and significant renal dysfunction (increase in serum creatinine >50 µmol/L) Patients with a baseline creatinine of >180 µmol/L, HIV or HCV co-infection were excluded. No grafts were obtained from executed prisoners or other institutionalized persons. 26 patients were enrolled in the study, of whom 16 (62%) had hepatocellular carcinoma. 6 were delisted (2 for improvement, 4 for disease progression), 19 have been transplanted, and 1 remains listed at the time of writing. At baseline, 41% of patients were HBsAg positive and 81% had detectable HBV DNA, with a median level of 3.3 log10 IU/mL (range 0-8). Median duration of pre-OLTx antiviral therapy was 3.6 months (range 0-17). At OLTx 50% of patients had detectable HBV DNA and in these patients the median load was 2.6 log10 IU/mL (range 1.5-4.7), but no patient had genotypic LAM or ADV resistance. All 19 post-OLTx patients survived without recurrent hepatitis B with a median follow-up of 11.7 months (range 1-40). The median increase in serum creatinine from baseline for post-OLTx patients was 38 µmol/L. There have been no other treatment related adverse events. Combination ADV/LAM antiviral therapy is safe and effective in HBsAg positive candidates awaiting OLTx. Pre-OLTx therapy is well tolerated, achieves viral suppression, prevents LAM resistance and may rescue some patients from OLTx. Post-OLTx combination ADV/LAM therapy prevents HBV graft infection without the significant cost and inconvenience of long-term regular HBIG administration.

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WHAT IS THE RISK OF HBV TRANSMISSION IN RECIPIENTS OF LIVER GRAFT FROM HBC POSITIVE ANTIBODY, HBS NEGATIVE ANTIGEN DONORS? A SINGLE CENTER EXPERIENCE OVER 10 YEARS

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Background: Liver graft from HBc positive antibody (HBcAb), HBs antigen negative (HBsAg) donors are used to expand the pool of graft despite a known risk of HBV transmission. Various prophylaxis strategies were used in small series to prevent the risk of HBV transmission. The aim of the present study was to report our experience over 10 years using a prophylaxis with HBs antibody immunoglobulins (HBIG). Methods: From 1997 to 2006, 80 HBcAb + liver grafts out of 947 grafts (8.4%) were used. These grafts were used for 21 HBsAg positive recipients and 59 HBsAg negative recipients (HCV cirrhosis n=22, alcoholic cirrhosis n=14, amyloid neuropathy n=11, miscellaneous n=12). Among the 59 HBsAg negative recipients, 30 were HBsAb and HBcAb negative, 13 were HBsAb positive and HBcAb negative and 16 HBcAb positive +/- HBsAg positive. HBsAg positive recipients received a prophylaxis regimen using HBIG +/- antivirals according to pre-transplant HBV viral load. Among HBsAg negative recipients, 44 received HBIG prophylaxis using 10000 IU IV during anhepatic phase and reinjection of 5000 IU IV when HBsAb was below 100 IU/L. Results: Mean follow-up was 47.8 months (1-115.8). HBV infection occurred in 13 recipients (16.2%) during follow-up at a mean delay of 15 months (0-45) after transplantation. HBV DNA became positive in 13 patients and HBsAg in 12. No HBV infection occurred in HBsAg positive recipients. HBV infection occurred in 12 out of 30 HBsAb and HBcAb negative recipients (40%) and 1 out of 13 HBsAb positive recipients (7.7%). Among the 30 HBsAb and HBcAb negative recipients, HBV infection occurred in 7 out of 20 who received HBIG prophylaxis (35%) and 5 out of 9 who received no prophylaxis (55%). HBV infection of the graft was treated with lamivudine in 13 recipients and adefovir was added on in 6 lamivudine-resistant patients. A HBsAg / HBsAb seroconversion was observed in 3 recipients during follow-up. Conclusions: The use of liver grafts from HBcAb positive, HBsAg negative donors is relevant to expand the liver graft pool. These grafts should be used in priority for HBsAg positive or HBV immune recipients (past infection or HBV vaccination). This study confirms a high risk of HBV transmission for HBsAb and HBcAb negative recipients despite HBIG prophylaxis. Future studies should evaluate a combined prophylaxis with HBIG and antivirals in this group of recipients.

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RESPONSE OF FIBROSING CHOLESTATIC RECURRENT HEPATITIS C FOLLOWING LIVER TRANSPLANTATION TO COMBINATION INTERFERON/RIBAVIRIN THERAPY

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Introduction/Aim: Fibrosing cholestatic hepatitis C (FCHC) is an uncommon (<5%) presentation of recurrent HCV following liver
transplantation. It is characterized by very high bilirubin and alkaline phosphatase and modestly elevated transaminases, with a biopsy demonstrating marked cholestasis, minimal necroinflammation, bridging fibrosis, and numerous apoptotic bodies. Outcome is uniformly poor, and retransplantation generally unsuccessful. We examined the response of FCHC to interferon/ribavirin therapy, using data collected from three Canadian liver transplant programs. Patients: 15 patients from the University of Toronto, University of Western Ontario and McGill University Liver Transplant Programs who developed FCHC following liver transplantation from 2000-2005 and received combination therapy with interferon or PEG-interferon and ribavirin were included. All cases were biopsy-proven, and antiviral treatment was commenced at 30-75% of standard doses; growth factors were administered as required. Results: 10/15 patients were male; mean age at transplant was 54y [range 39-69y]. Nine received deceased donor, and six living donor, grafts. Mean time from transplant to FCHC diagnosis was 3m [range 1.5 to 6m]. Ten patients had Genotype 1, 2 G2, 3 G4. Mean pretreatment viral load was >1E6 IU/ml. 4 pts received standard IFN and 11 PEG IFN, in addition to ribavirin. 5/15 patients (33%) achieved an SVR, and 10/15 had no response. 9 of these patients succumbed to liver failure 2-22m following FCHC diagnosis; one patient is alive 6yrs after transplant with decompensated graft cirrhosis. Two of the five SVR patients have died, one from systemic sepsis 22m post transplant, and the other 17m post transplant from biliary tract complications. Conclusions: Combination therapy with interferon and ribavirin offers some hope in the management of FCHC, although the overall prognosis remains poor.

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545 PREDICTING CARDIOVASCULAR EVENTS AFTER LIVER TRANSPLANTATION

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Cardiovascular disease (CVD) is a leading cause of late death post-liver transplantation (LT). CVD risk factors are common in LT patients. The optimal method and timing of risk stratification for CVD post-LT are not established. The Framingham risk score (FRS) estimates the risk of death or developing symptomatic coronary heart disease (CHD) in non-LT patients. In European cohorts, FRS increased post-LT, but had limited discriminatory capability. In our LT patients at a U.S. center, we evaluated changes in CV risk factors over time and subsequent CV events (CVE), defined as the need for intervention (surgical/medical) in CHD or peripheral vascular disease, transient ischemic attack, or cerebrovascular accident. Methods: This retrospective review of 120 consecutive first LT recipients (65% male, mean age of 55 ± 10 yrs) followed subjects for a mean of 1601 days [range 233-1907] post-LT. Fasting total, HDL and LDL-cholesterol (TC, HDL-C, LDL-C), glucose, weight and blood pressure (BP) were measured and FRS calculated at pre-LT and regular intervals post-LT (at 4 and 12 months, then annually). Diabetes mellitus (DM) was defined as plasma glucose >126 mg/dL and/or the continuing requirement for DM therapy. Hypertension (HTN) was defined as systolic BP >140, diastolic BP >90 or the use of anti-hypertensive agents. The change over time (slope) for risk factors was calculated and the association between risk factor trajectory and CV events were examined with logistic regression models. Results: 9 non-fatal CVE's occurred in our cohort over the follow-up period. CV risk factor data are shown (see Table) for selected visits. Increasing body mass index (BMI) increased the odds of developing DM (OR 1.07 [95% CI 1.07 - 1.13], p<0.01). The FRS slope correlated with CVE, (OR 1.4 [95% CI 1.19], p=0.04). Conclusions: CV risk factors are prevalent in U.S. LT patients. Changes in FRS over time identify patients at risk for CVE post-LT, with the odds of CVE increasing by 40% for each 1-unit increase in slope. Risk factor modification studies in this population appear warranted.

CV Risk Factors Pre and Selected Visits Post-LT

<table>
<thead>
<tr>
<th></th>
<th>Pre-LT</th>
<th>4-month visit post-LT</th>
<th>Year 4 visit Post-LT</th>
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<tr>
<td></td>
<td>n=120</td>
<td>n=20</td>
<td>n=59</td>
</tr>
<tr>
<td>Mean time post-LT (days) ± SD</td>
<td>-</td>
<td>152 ± 6</td>
<td>160 ± 64</td>
</tr>
<tr>
<td>BMI &gt;30 (%)</td>
<td>35%</td>
<td>36%</td>
<td>43%</td>
</tr>
<tr>
<td>BMI &gt;35 (%)</td>
<td>14%</td>
<td>11%</td>
<td>15%</td>
</tr>
<tr>
<td>Mean TC (mg/dL) ± SD</td>
<td>151.5 ± 49.5</td>
<td>174.1 ± 50</td>
<td>181.8 ± 46.9</td>
</tr>
<tr>
<td>Mean HDL-C (mg/dL) ± SD</td>
<td>40.2 ± 19.7</td>
<td>40.8 ± 14.3</td>
<td>48.8 ± 14.2</td>
</tr>
<tr>
<td>Mean LDL-C (mg/dL) ± SD</td>
<td>92.1 ± 37</td>
<td>97.8 ± 42.4</td>
<td>96.8 ± 34.8</td>
</tr>
<tr>
<td>Prevalence of HTN (%)</td>
<td>-</td>
<td>59%</td>
<td>72%</td>
</tr>
<tr>
<td>Prevalence of DM (%)</td>
<td>24%</td>
<td>38%</td>
<td>36%</td>
</tr>
<tr>
<td>FRS &gt; SD</td>
<td>5 ± 5.2</td>
<td>7.5 ± 7.2</td>
<td>7.7 ± 6.7</td>
</tr>
</tbody>
</table>

Disclosures: The following people have nothing to disclose: Michael Herman, Surakit Pungpapong, Jaime Aranda-Michel, Barry Rosser, Denise M. Harnois, Rolland C. Dickson, Raj Satyanarayana, Daniel Yip, Andrew Keaveny

546 TRANSPLANTATION TRENDS IN RECIPIENTS OVER THE AGE OF 65

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Liver transplantation (LT) provides long-term survival for adults with end-stage liver disease. As a result of improved survival and an aging United States population the demand for LT in older patients is expected to increase. The aim of this study is to describe the transplantation trends in the older recipient (65 yrs and older). Method: Using the UNOS database, we identified all LT recipients between 1990 and 2006. The data collected included demographics, diagnosis, LT year, survival information and UNOS region. We used Kaplan Meier method to calculate overall survival (1, 3, 5 and 10 yr) and Cox regression modeling techniques for predictors of survival. Result: During the study period 5630 (7.6%) LT recipients were >65 years. Of these, there were 3030 (56.5%) males with a median age of 67 (range 65 – 87). There were 4256 (79.4%) Caucasians, Hispanic (10.3%), African Americans (3.6%) and rest (6.7%). Hepatitis C (21%) was the most common indication followed by cryptogenic (15.1%), alcoholic liver (13%), Hepato-cellular Carcinoma (10.4%), Primary biliary cirrhosis 9.2%, Primary sclerosing cholangitis 5.1% and others 26.2%. There was an increase in LT for older patients from 4.1% in 1990 to 10.2% in 2006 (p=0.002), as well as a regional variation (p<0.001). The 10-yr patient and graft survival was 60% and 57% for <65 yrs vs. 42% and 40% for >65 yrs (p <0.0001). With age stratification (65 – 75 yrs vs. >75yrs), there was no difference in overall survival but when adjusted for race there was a significant difference in graft survival with a 10 yr (Caucasian 40%, Hisp 44% and African Americans 19%) (p=0.04). No other predictors of survival were identified. Conclusion: The demand for LT in recipients older than 65...
years is increasing; there is regional variability and a change in LT indication (cryptogenic 2nd most common). Although their survival is reduced when compared to recipients <65 yrs, there appears to be no difference in unadjusted survival with age stratification above 65 years. Among ethnic minorities, African Americans have a disproportionately lower % LT and a decreased survival.

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THE EFFECT OF DISEASE RECURRENCE ON GRAFT SURVIVAL FOLLOWING ORTHOTOPIC LIVER TRANSPLANTATION
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Disease recurrence following transplantation is well recognised but since the impact of recurrence on graft survival is less clear we undertook a retrospective analysis to determine both the frequency and impact of disease recurrence on graft survival. All 1840 adult patients who underwent liver transplantation between 1982 and 2004 were included. Disease recurrence was diagnosed on clinical, serological, histological or radiological grounds as appropriate. Where graft loss was due to factors associated with the indication (such as a return to alcohol consumption), the graft loss was attributed to disease recurrence. The most common disease indication was primary biliary cirrhosis (PBC) so these patients were used as the comparison group. To eliminate those grafts lost where recurrence was unlikely only those 1499 patients surviving more than 90 days after transplantation were included in the analysis. The risk of graft loss from disease recurrence for first grafts was calculated using the Cox regression model. The risk of graft loss from recurrent disease was greatest, when compared to PBC, in those transplanted for hepatitis C virus (HCV) (hazard ratio (HR) 11.6; 95% confidence interval (CI) 5.1-26.6), primary sclerosing cholangitis (PSC) (HR 6.0; 95% CI 2.5-14.2) and autoimmune hepatitis (AIH) (HR 4.1; 95% CI 1.3-12.6). The overall risk of graft loss was also significantly greater in HCV (HR 2.1 vs. PBC; 95% CI 1.5-3.0), PSC (HR 1.6 vs. PBC; 95% CI 1.2-2.3) and AIH (HR 1.6; 95% CI 1.0-2.4) although when recurrent disease was excluded as a cause of graft loss there was no difference in the overall risk of graft loss (HCV: HR 1.4; 95% CI 0.9-2.1, PSC: HR 1.4; 95% CI 0.9-2.0, AIH: 1.4; 0.9-2.2 all vs. PBC). When the rate of disease recurrence and the rate of graft loss from disease recurrence were compared, proportionally more grafts were lost in HCV (14.3% of grafts lost to recurrent disease vs. 77% of grafts affected), PSC (8.4% vs. 37%), and AIH (6.2% vs. 28%) than in PBC (1.3% vs. 24%). There was no statistically significant difference in the risk of graft loss due to recurrent disease, when compared with PBC, for patients transplanted for alcohol related liver disease (ALD) (HR 1.0;95% CI 0.2-4.9), non-alcoholic steatohepatitis (HR 2.2;95% CI 0.6-8.4) and fulminant hepatic failure (HR 1.7;95% CI 0.4-6.6). Disease recurrence is a significant cause of graft loss particularly in HCV, PSC and AIH. Graft loss from disease recurrence in part explains the increased overall rate of graft loss in these groups.

Disclosures:
The following people have nothing to disclose: Ian A. Rowe, Kerry Webb, Bridget K. Gunson, Naimish Mehta, Sayeed Haque, James M. Neuberger

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NATURAL HISTORY AND PROGNOSTIC INDICATORS IN CIRROTIC PATIENTS WITH PULMONARY HYPERTENSION
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Porto-pulmonary hypertension (pPH) is a circulatory complication defined by elevated pulmonary artery pressure (mPAP) and pulmonary vascular resistance (PVR) but normal pulmonary artery occlusion pressure (mPAOP). Recently, the transpulmonary gradient (TPG) (mPAP-mPAOP) has been proposed as another diagnostic tool. pPH may be reversible after liver transplantation and a debate has started on the evaluation of such patients on the waiting list, but data on the natural history of pulmonary hypertension (PH) in cirrhosis are scarce, in particular outside of pre-transplant cohorts. Aim: To assess natural history and prognostic indicators in cirrhotic patients with PH in a large single-centre cohort. Methods: 729 consecutive patients with suspected cirrhosis referred to our unit between 12/1995 and 12/2004 received full clinical and haemodynamic assessment which included Child-Pugh/MELD score, the hepatic venous pressure gradient (HVPG) and cardiopulmonary pressures. Survival time/time-to-transplant (ST/TtT) was calculated as a combined end point (Kaplan-Meier method; cut-off 31/11/2006), and MELD/Child-Pugh, HVPG and cardiopulmonary morbidity were introduced as variables in a multivariate cox-regression model. Results: 650 patients met study criteria (Child-Pugh A/B/C 28%/45%/27%; no malignancy) including 575 without PH (mPAP<25mmHg), their survival predicted by Child-Pugh/MELD and HVPG (4% transplanted). mPAP exceeded 25mmHg in 75 patients (12%), in 25 (4%) secondary to cardiopulmonary disease. Survival was similar without out PH and with secondary PH (ST/TtT 1905±71 vs. 1590±274 days / MELD 15±7 vs. 17±8, ns.), although liver-related scores lost their predictive value in the latter. In the remaining 50 patients TPG, but not mPAP or PVR, was an independent prognostic indicator (p<0.05), and 3-month-survival was best predicted by a TPG cut-off of 12mmHg using ROC curves. Thus, an mPAP>25mmHg and a TPG≥12mmHg (mPAP 35±13mmHg, mPAOP normal) were associated with the poorest outcome (15 patients [2%] with ST/TtT 734±196 vs. 1905±71 days in patients without PH, p<0.05; MELD 18±8 vs. 15±7, ns.). While liver-related scores failed to predict survival in this group (only one patient transplanted), the patients carried the same 3-month-prognosis as patients with MELD<26 but no PH, even with a MELD<14. Conclusion: In our cohort, an mPAP>25mmHg and TPG≥12mmHg in the absence of secondary PH selects cirrhotic patients with the worst prognosis. These
parameters may be sufficient to diagnose POPH in the presence of the hyperdynamic circulation following cirrhosis. The poor prognosis argues for prioritizing such patients on the waiting-list irrespective of low MELD scores.

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The following people have nothing to disclose: Matthias M. Dollinger, Alexander Zipprich, Stefan Rogowski, Susanne Behl, Wolfgang E. Fleig

549 EXCELLENT OUTCOME OF PATIENTS WITH ACUTE ALCOHOLIC HEPATITIS AFTER LIVER TRANSPLANTATION

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Liver transplantation (LT) is the only curative therapeutic option for patients with acute and chronic liver failure. Besides chronic hepatitis C liver cirrhosis due to alcoholic liver disease is the most common indication for LT. Patients with acute alcoholic hepatitis (AH) have an extremely poor prognosis, as this disease is associated with a 30-50% mortality within one month. No curative therapeutic strategies are available for patients with severe AH, except LT. However, the role of LT in the management of AH has been discussed controversially and so far, no data regarding long-term outcome of these patients following LT have been reported. The aim of this retrospective study was to analyse the long-term survival of patients who underwent LT due to severe AH in our center. Between 1995 and 2006 11 out of 651 patients (1.7%) with severe AH were transplanted. Three patients were female, 8 male with a median age of 43 (29-54) years. All patients had a Maddrey discriminant factor >32; the median MELD score was 27.5 (16-35). A thorough psychiatric evaluation, including family members and local physicians, was performed in all patients prior to LT. Patients with stable psychosocial environment, as an indirect parameter for a patient's compliance, were listed for LT. The period of abstinence ranged from few days to 3 months. A specific psychiatric therapy was started as soon as possible after LT. The patient survival rates of the AH group were compared to those of patients transplanted for alcoholic liver cirrhosis and of the overall LT cohort. The mean follow-up of AH patients was 3.9 ± 3.1 (0.6-9.4) years. Immunosuppression consisted of cyclosporine or tacrolimus in combination with prednisolone (taper within 3-6 ms) and mycophenolate mofetil. The actuarial patient survival of all AH patients at 1-, 2-, and 5 years were 100%, 100% and 98%, respectively; n=487). Including alcohol liver cirrhosis (90%, 88% and 78%, n=153), however, the differences were not statistically significant. Three patients with AH died 1.2, 3.9 and 9.4 years post LT due to cholangiopitis secondary to intrahepatic biliary strictures, suicide and oropharyngeal carcinoma. Two of these patients developed recurrent alcohol abuse, whereas nine patients had no evidence of recurrent disease and remained completely abstinent after LT. This study showed an excellent long-term survival of patients with acute alcoholic hepatitis. An extensive interdisciplinary management of these patients, especially in the postoperative care, is mandatory in order to achieve this excellent outcome.

Disclosures:
The following people have nothing to disclose: Ivo Graziaiadei, Karin Nachbaur, Barbara Sperner-Unterweger, Walter Mark, Raimund Margreiter, Wolfgang Vogel

550 EVALUATION OF THE BENEFIT ON RENAL FUNCTION OF A DELAYED INTRODUCTION OF TACROLIMUS IN LIVER TRANSPLANT RECIPIENTS AT 13 FRENCH CENTERS

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Although calcineurin inhibitors have demonstrated efficacy in liver transplantation, they have a potential to impair renal function. Delayed tacrolimus (TAC) administration may reduce the risk of renal dysfunction. Methods: A prospective study included primary LTx recipients randomised either to delayed introduction of TAC (Day 5) + Daclizumab (DAC) (Group A) or to immediate TAC administration (Group B). In both groups, TAC T0 was 15 ng/ml until Week 4 and 5−15 ng/ml thereafter. MMF was given at 2 g/d for 2 months, and corticosteroids (CS) at standard doses. Pts with a serum creatinine (Scr) > 180 umol/l at 12 hours (H12) were not included. The primary endpoint was the rate of pts with a mean Scr > 130 umol/l at Month 6. Results: Analyses were performed on 207 pts, 98 (Group A) and 101 pts (Group B) (ITT population). Baseline characteristics were similar. At Month 6, mean TAC T0 was 9.6 (Group A) and 11.2 ng/ml (Group B), and patient and graft survival were 100% and 98.9%, respectively. Two pts required dialysis sessions (DS) in group A (1 pt had 10 DS during the first month and 1 pt 1 DS thereafter). All six pts required DS within the first month in group B (1 of them required 37 DS). Incidence and time to first BPAR were 20.3% (A) and 24.2% (B) pts. At least one serious adverse event related to treatment was reported in 26.5% pts in Group A and 37.6% pts in Group B. Insulin therapy > 30 days was reported in 9.2% and 11.8% pts respectively. Conclusions: Delayed TAC administration with DAC and MMF is a safe immunosuppressive therapy for LTx pts with no increased BPAR. Benefit on renal function (Scr) at 6 months was not statistically different to that with immediate TAC administration. A trend towards better GFR was observed for pts with good renal function at H12 and with delayed TAC administration. The impact on longer-term renal function will be assessed at 1- and 2-year follow-up.
Renal function assessment

<table>
<thead>
<tr>
<th>Post surgery (H12)</th>
<th>TAC delayed (n=58)</th>
<th>TAC immediate (n=101)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>SCR unisol. (mean ± SD)</td>
<td>91 ± 27</td>
<td>87.5 ± 25</td>
<td>0.328</td>
</tr>
<tr>
<td>For pts with SCR &gt; 100 at H12</td>
<td>119 ± 18 (n=26)</td>
<td>118 ± 14 (n=29)</td>
<td>0.898</td>
</tr>
<tr>
<td>For pts with SCR &gt; 100 at H12</td>
<td>75 ± 15 (n=62)</td>
<td>75 ± 15 (n=72)</td>
<td>0.957</td>
</tr>
<tr>
<td>At Month 6</td>
<td>22.4%</td>
<td>29.7%</td>
<td>0.244</td>
</tr>
<tr>
<td>SCR &gt; 130 with at least 1 BPAR</td>
<td>5.1%</td>
<td>7.9%</td>
<td>0.423</td>
</tr>
<tr>
<td>SCR ≤ 130 with no BPAR</td>
<td>62.9%</td>
<td>52.5%</td>
<td>0.165</td>
</tr>
<tr>
<td>GFR*</td>
<td>72 ± 29</td>
<td>66 ± 23</td>
<td>0.09</td>
</tr>
<tr>
<td>For pts with SCR &gt; 100 at H12</td>
<td>64.5 ± 22</td>
<td>62 ± 25</td>
<td>0.667</td>
</tr>
<tr>
<td>For pts with SCR &gt; 100 at H12</td>
<td>76.5 ± 32</td>
<td>67.5 ± 23</td>
<td>0.066</td>
</tr>
<tr>
<td>ENP/ N pts</td>
<td>10 / 1</td>
<td>57 / 6</td>
<td>0.07</td>
</tr>
</tbody>
</table>

GFR : Glomerular filtration rate (Cockroft formula - ml/min)  
Number of DS during the first month

551 THE ISCHEMIC PRECONDITIONING (IPC) PARADOX IN LIVER TRANSPLANTATION (LT) – EVIDENCE FROM A PROSPECTIVE RANDOMIZED TRIAL

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Introduction: Animal studies show that IPC of liver is uniformly beneficial in LT with potential to expand available deceased donor (DD) organ pool. However, evidence from the few clinical trials in LT is conflicting. Herein we report the results from a recently completed, largest prospective randomized study to date of IPC in DD-LT. Methods: Between Oct 2003 and June 2006, 101 DD were randomized based on donor age (< or ≥ 50) to either 10 min of IPC (N=50) or No IPC (N=51). IPC was induced by hepatic hilar clamping soon after laparotomy. All other care in DD and recipients was standard. The primary objective was to test the hypothesis that 10 min of IPC would improve post LT graft reperfusion (RP) injury and function. Secondary objectives were to determine whether IPC would decrease 1) systemic inflammatory cytokine response 2) biopsyc-confirmed acute rejection (AR) in the first month and 3) hospital stay. Intent-to-treat based analyses were applied. Results: Both groups of donors were comparable with an identical donor risk index of 1.5 ± 0.3. The recipient groups were comparable except for significantly fewer males (66 vs. 86%, p=0.01) and lower MELD score (median 18 vs.23, p=0.04) in the IPC group. Contrary to expectation IPC led to a significant increase in biochemical RP injury (Table). Donor and recipient TNF-α, IL-6 and donor IL-10 plasma levels at various time points were similar in both groups. IPC recipients had significantly higher median IL-10 levels on day 1. (237 vs. 112 pg/ml, p=0.03) and had fewer biopsy-confirmed moderate/severe AR (2/50 vs. 8/51, p=0.09). Median length of stay [11 days] was the same in both groups. One and two-year patient survival in IPC vs. No IPC were 88 and 81% vs. 79 and 64%, respectively (p=0.10). Graft survivals were 84 and 76% vs. 77 and 66%, respectively (p=0.25). Conclusions: Despite the initial worsening of biochemical RP injury, IPC increases circulating levels of the anti-inflammatory cytokine, IL-10, in liver recipients in association with a decreased risk of severe rejection – an IPC paradox. IPC alone provides limited benefit in DD-LT, and may need to be combined with other preconditioning measures to derive maximal benefit.

552 MELD-NA IS SUPERIOR TO OTHER ORGAN ALLOCATION SYSTEMS

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Introduction There is ongoing analysis of the optimal method of organ allocation. The MELD system is inaccurate for up to 20% of the population on the liver transplant waiting list. This is most likely due to significant clinical variables such as ascites not being accounted for. We analyzed several scoring systems for end-stage liver disease to assess which predicted survival on the waiting list. Methods All patients listed with UK Transplant (UKT) at our centre, between January 2000 and December 2003, were included in the study. Patients were excluded if they were super-urgently listed, listed for multiple organ transplants, non-NHS entitled for liver transplant or their indication for transplant was amyloidosis. The scoring systems examined were the Child-Pugh score, MELD score, MELD-Na score and three variants of the Child Pugh score incorporating creatinine at the time of listing with UKT. A positive outcome was surviving to transplantation or being delisted due to improvement obviating the need for transplant. A negative outcome was death on the transplant list or being delisted due to the developments of contraindications for transplant. Receiver-operating characteristic (ROC) curves were generated for each scoring system based on these outcome measures and the areas under the curve (AUC) were compared using the Hanley-McNeil method. Results Seven hundred and eighty seven patients were listed. After exclusions, 490 patients were analysed in the study. The median age of the patients was 55 years and the predominant aetiologies were alcoholic liver disease and hepatitis C. The median Child-Pugh score was 9 and the median MELD score was 15. There were 416 patients with a positive outcome including 402 transplants. The remaining 74 patients with a negative outcome had significantly higher CPS (11 vs 9), MELD (18 vs 14), MELD-Na (38 vs 16) and modified CP scores (All p values <0.0001). There was no difference in time on the waiting list (64 vs 68 days; p=0.18). The AUC for all scoring systems was >0.705 (p<0.001) indicating that they performed well and were clinically applicable. However, MELD-Na was significantly better than the other scoring systems with an AUC of 0.828 (p<0.001). Conclusion All scoring systems analysed performed adequately in predicting a negative outcome on the transplant waiting list with no difference between CP score and MELD score. However, MELD-Na was significantly better than all the other scoring systems at predicting waiting list mortality and thus any changes in organ allocation warrant comparison with this scoring system.
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LONG TERM RESULTS OF A RANDOMIZED TRIAL OF TACROLIMUS MONOTHERAPY VERSUS TRIPLE THERAPY IN HCV CIRRHOSIS LIVER TRANSPLANT RECIPIENTS

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Introduction
Less potent immunosuppression has been considered to be beneficial in reducing severity of HCV recurrence. However some reports suggest benefit with low dose steroid maintenance. Optimal immunosuppression regimen is unknown. Aim of the study To evaluate benefit of tacrolimus monotherapy versus triple therapy in a randomized trial. Patients and methods 88 consecutive patients with first transplant for HCV cirrhosis with/without HCC were randomized to tacrolimus (MT) 0.1 mg/kg/day in 2 divided doses, or the same tacrolimus dose with 1mg/kg azathioprine and 20mg prednisolone daily (TT) the latter was tapered off to zero at 3-6 months. Patients had scheduled biopsies with Hepatic Venous Pressure Gradient (HVPG) measurement yearly. Fibrosis was staged using Ishak score and stage 4(F4) was the predetermined end-point. Cox regression was used to evaluate factors associated with F4: age and gender of recipient and donor, histological acute hepatitis, rejection episodes, HCC before LT, addition of MMF, and allocated treatment. Results Randomization resulted in no significant differences in pre, peri or post operative variables (median follow up 48 months). Mortality was not significantly different: 8 of 43 in MT at 1m, 1m, 1m, 2m, 3m, 4m, 6m and 5 of 45 in TT at 5m, 7m, 11m, 37m, and 66m. Retransplantation in 3 MT and 3 TT; frequency of scheduled biopsies was similar. During follow up (re-transplants censored), 13 MT and 6 TT reached F4. Cox regression revealed that randomization to therapy and episodes of acute hepatitis were two factors independently associated with F4 (p=0.036). Kaplan Meier analysis showed TT patients had significantly slower progression (p=0.048) than MT patients. HVPG gradient was available in 33 MT and 30 TT patients. Kaplan Meier analysis showed a significantly shorter time to reach HVPG>10mmHg in MT group compared to TT group (p=0.038). Conclusion The use of low dose azathioprine long term and short term low dose prednisolone in addition to tacrolimus in HCV cirrhosis recipients resulted in a slower onset of histological severe fibrosis and portal hypertension compared to tacrolimus alone, independent of other factors known to affect fibrosis. This randomized trial supports observational reports of the benefit of low dose steroids, as well as azathioprine, used as maintenance immunosuppression after liver transplantation for HCV positive recipients.

Disclosures:
The following people have nothing to disclose: Pinelopi Manousou, Dimitris Samonakis, Alice Corbani, E. Cholongitas, Alexandros Sigalas, Elias Xirochakis, Vincenza Calvaruso, Federica Grillo, David Patch, James O’Beirne, Amar P. Dhillon, Keith Rolles, Brian Davidson, Andrew K. Burroughs

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GRAFT SURVIVAL AFTER LIVING DONOR OR CADAVERIC LIVER TRANSPLANTATION: AN ANALYSIS STRATIFIED BY DONOR RISK

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Introduction: Living donor liver transplantation (LDLT) has become an accepted alternative to transplantation with a cadaveric organ. Recently, cadaveric donor characteristics as codified by the Donor Risk Index (DRI) have illuminated the requirement to balance donor qualities and recipient risk of death on the wait list as a means to optimize the survival benefit of transplantation. Within this framework, the role of the living donor graft remains unclear. Methods: Utilizing United Network for Organ Sharing data from 2002 to 2006, we analyzed graft survival comparing 4 different graft types stratified by donor risk characteristics: low-risk living donor (LRD – Relative Risk [RR] of graft loss <1.7), high-risk living donor (HRRD, RR >1.7), low-risk deceased donor (LRDD, <1.7) and high-risk deceased donor (HRDD, RR >1.7). Graft survival was modeled using Cox regression while controlling for recipient characteristics, which included age, race, diagnosis, dialysis, medical condition, life support and Model for End-Stage Liver Disease (MELD) score. Results: 98% of LDLT transplants were performed in recipients with a MELD score of 25 or less. Overall 3 year graft survival for LRD, HRRD, LRDD, and HRDD was 77%, 65%, 80%, and 67%, respectively (p-value < 0.001) (Fig. 1). The relative risk of graft loss, in reference to the LRDD group, was 1.7 for HRRD (95% CI 1.05-2.75), 0.9 for LRDD (95% CI 0.72-1.12), and 1.4 for HRDD (95% CI 1.18-1.86). Conclusions: Survival with high risk living donor grafts is similar to high DRI cadaveric organs in patients with low to moderate MELD scores. Consideration of donor characteristics relative to recipient risk of wait list death should be considered in LDLT.

Disclosures:
The following people have nothing to disclose: Siddharth Patel, Ian Lent, Adel Bozorgzadeh, Mark Orloff, Georgios Tsoufas, Randeep Kashyap, Ashok Jain, Peter Abt
555 SERUM SODIUM LEVELS ARE NOT INDEPENDENT OF FLUID STATUS AND DISEASE SEVERITY IN LIVER TRANSPLANT WAITING LIST CANDIDATES

Patrick G. Northup1, Curtis K. Argo1, Abdullah M. Al-Osaimi1, Timothy M. Schmitt2; 1Gastroenterology and Hepatology, University of Virginia Health System, Charlottesville, VA; 2Surgery, University of Virginia Health System, Charlottesville, VA

Introduction: Serum sodium levels in liver transplant waiting list candidates have been shown to predict waiting list mortality. Controversy surrounds this because it is clinically evident that sodium levels are highly dependent on fluid status, renal function, diuretic use, TIPS, and general liver disease severity. We hypothesize that most fluctuations in sodium levels are dependent on other clinical measures and are not true independent factors in waiting list death risk. Methods: Organ Procurement and Transplantation Network (OPTN) liver transplant waiting list data between February 2002 and April 2004 were analyzed for all adult, non-status 1 candidates. All consecutive waiting list laboratory data, including serum sodium for each transplant candidate were analyzed. Waiting list death rates were calculated for patients with varying levels of hyponatremia. Multivariate survival models were constructed to analyze the independent predictors of sodium variation. Multivariate survival models were then constructed to compare 90-day waiting list death rates adjusting for those factors that influence sodium levels. Results: 100,133 serum sodium levels from 26,502 candidates on the waiting list were analyzed. 17.6% of all candidates had at least one sodium below 135 mg/dL and 6.9% had one sodium reported below 130 mg/dL. Statistically significant independent predictors of sodium levels less than 130 mg/dL included receiving a TIPS while on the waiting list (OR 1.70, 95%CI 1.14-2.54, p<0.01), large volume ascites (OR 4.00, 95%CI 3.25-4.93, p<0.0001), serum albumin (OR 0.43, 95%CI 0.40-0.46, p<0.0001), serum total bilirubin (OR 1.02, 95%CI 1.01-1.03, p<0.0001), and serum creatinine (OR 1.07, 95%CI 1.03-1.11, p<0.0001). These predictive variables were responsible for more than 75% of the variation in serum sodium. In a 90-day multivariate waiting list survival model including the above variables, serum sodium was not an independent predictor of mortality (p=0.17). In a similar proportional hazards model after adjusting for the above factors, serum sodium remained statistically significant (p<0.0001) but only improved the fit of the overall survival model by less than 0.08% Conclusions: Serum sodium measurements are highly dependent on other clinical factors and can be manipulated by addressing these clinical factors. When factors that influence sodium are adjusted, serum sodium has a minimal influence on liver transplant waiting list mortality. Including sodium in the allocation policy might add additional subjectivity to the MELD system.

Disclosures: The following people have nothing to disclose: Patrick G. Northup, Curtis K. Argo, Abdullah M. Al-Osaimi, Timothy M. Schmitt

556 SAFETY AND EFFICACY OF THYMoglobulin FOR STEROID RESISTANT ACUTE CELLULAR REJECTION FOLLOWING LIVER TRANSPLANTATION


Safety and Efficacy of Thymoglobulin for Steroid Resistant Acute Cellular Rejection Following Liver Transplantation A.K. Sahajpal, V. Madhok, K. Shivakumar, J.E. Hay, M.R. Charlton, C.B. Rosen, R.H. Wiesner, K.V.N. Menon Introduction: Steroid resistant acute cellular rejection (ACR) following liver transplantation (LT) is commonly treated with OKT3 which is often associated with serious adverse events. The most common adverse events associated with its use are fever, chills, leucopenia and thrombocytopenia. There is limited data on its role in the treatment or steroid resistant ACR in LT. Aim: The aim of our study was to assess the safety and efficacy of thymoglobulin for steroid resistant ACR compared to OKT3 in LT. Methods: Twenty-two consecutive patients (11 females) who received Thymoglobulin (ATG) for steroid resistant ACR following LT at the Mayo Clinic, Rochester from September 2003 to July 2006 were included in the analysis. They were compared with 19 patients who received OKT3 for the same indication during the study period. Patients were selected for antibody treatment if they continued to exhibit histological signs of ACR following bolus corticosteroid therapy for ACR (three doses of 1000 mg of methyl prednisolone or 200 mg of dexamethasone intravenously on alternate days). OKT3 was dosed at 5 mg/kg daily for 7 to 10 days while ATG was dosed at 1.5 mg/kg/dose for 7 doses over 10 days along with standard premedication. Results: The primary immunosuppression was tacrolimus in all but 2 patients in the OKT3 group who received Neoral. Following bolus corticosteroid therapy moderate cellular rejection was seen in 45% of patients in the ATG group and 19% of patients in the OKT3 group. ATG therapy was associated with a lower incidence of adverse effects (fever, hypotension, chills and infection) compared to OKT3 therapy (29% versus 71%, p = 0.002). The number of patients requiring intensive care for hypotension and infectious complications was higher in the OKT3 group compared to the ATG group (21% versus 0%, p = 0.045). No patient treated with ATG required dose adjustments for cytopenias, weight change or fever. Conclusion: ATG was as efficacious as and better tolerated than OKT3 for the treatment of steroid resistant ACR with a significantly greater number of patients on OKT3 requiring intensive care. ATG is therefore a safe and effective agent for the treatment of steroid resistant ACR following LT.

Disclosures: The following people have nothing to disclose: Ajay K. Sahajpal, Vishnu Madhok, K. Shivakumar, J. E. Hay, Michael R. Charlton, C. B. Rosen, R. Wiesner, K. V. Menon

557 MYCOPHENolate MOFetil PLUS LOW DOSE CALCineurin INHIBITOR FOR RENAL DYSFUNCTION IN LIVER TRANSPLANT: A 24-MONTH CONTROLLED CLINICAL TRIAL

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Renal dysfunction is a major complication of long-term immunosuppressive therapy with calcineurin inhibitors (CNI) in liver transplantation (LT). In this setting, we tried to assess the long-term efficacy and outcome of tapering CNI to a low through level and introducing Mycophenolate Mofetil (MMF). Thirty patients were matched for sex, age, months after LT, immunosuppressive treatment, creatinine level, presence of diabetes and calculated glomerular filtration rate (GFR) via Cockroft-Gault method, with a parallel control group who had received
CNI only. MMF was introduced and CNI dose was progressively reduced. The following variables were analyzed: side effects and rate of rejection, serum creatinine reduction by at least 10% with respect to baseline value (10%-creatinine) and/or a GFR increase of at least 10% with respect to baseline value (10%-GFR), change from baseline of creatinine and GFR (Δserum creatinine = serum creatinine level at baseline – serum creatinine level at 24 month; ΔGFR = GFR at 24 month – GFR at baseline). Thirty patients (25 M; mean age 61±7 yrs; creatinine 1.77±0.51 mg/dL; GFR 48.4±14 mL/min) were converted to the combined therapy after a median of 63 months from LT and were compared with 30 patients (25 M; mean age 60±7 yrs; median time from LT 63 months; creatinine 1.62±0.25 mg/dL; GFR 48.9±11.2 mL/min) used as contemporaneous control group. After 2 years, serum creatinine decreased in the MMF to 1.57±0.71 mg/dL and GFR increased to 56.8±17.2 mL/min (p<0.001). The controls experienced a worsening of both serum creatinine (1.7±0.35 mg/dL, p=0.013) and GFR (47.3±11.1 mL/min, p=0.006). The logistic regression models employing 10%-creatinine and 10%-GFR as dependent variables, showed the use of MMF as the only statistically significant parameter associated with improvement of renal function (Odds Ratio (OR) 5.7 / 95% Confidence Interval (CI) 1.7-18.9, p=0.004; OR 3.8 / 95% CI 1.7-11.4, p=0.019; respectively). Multiple linear regression analysis identified only MMF as independent predictor of Δcreatinine (Regression coefficient B: -0.26 / 95% CI -0.1-0.41; p=0.002) and ΔGFR (Regression coefficient B: -9.05 / 95% CI -4.9-13.2; p<0.001). The mean decrease of Δcreatinine was 0.18±0.36 mg/dL in MMF group, whereas we observed a mean increase of 0.08±0.23 mg/dL in controls. In respect to ΔGFR, in MMF group we observed a mean increase of 7.4±8.3 mL/min versus a mean decrease of 1.7±7.7 mL/min in controls. No rejection episode was observed (3 in the controls). This study demonstrates the long-term efficacy and safety of MMF plus low dose CNI in reducing nephrotoxicity in LT recipients.

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558
SMOKING RELATED MORBIDITY AND MORTALITY POST LIVER TRANSPLANTATION
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Introduction: The negative health implications of cigarette smoking in the non-transplant setting are well recognised. However, the morbidity and mortality associated with tobacco use amongst liver transplant recipients specifically remains unclear. The aim of this study was therefore to assess the influence of smoking on both short and long term outcomes post liver transplantation. Methods: A single-centre retrospective case-note study of 135 consecutive patients transplanted between 01/01/96 and 31/12/00. Smoking status was based on documentation during liver transplant assessment. Patients who did not have smoking status recorded or who were described as ex-smokers were excluded from further analysis. Mean follow-up time was 8.8+/−1.4 years. Results: Of the remaining 108 patients, 29% were smokers and 71% were non-smokers. No difference was observed between the two groups with regards age, gender, prevalence of HCC, Child Pugh score, listing renal function, and the presence of pre-transplant diabetes, hypertension and cardiovascular disease. 35% of smokers had alcoholic liver disease compared with 13% of non-smokers (p<0.01). Post transplant a similar proportion of patients in each group received cyclosporin, and were treated for diabetes, hypertension and hypercholesterolaemia. During the immediate post-operative period smokers were more likely to develop pneumonia (p<0.01). However, there was no difference in the rate of systemic bacterial/fungal infection, CMV viraemia, acute cellular rejection, need for renal replacement therapy and survival to discharge. Long term the incidence of pneumonia, bacterial/fungal infection, CMV viraemia, late acute rejection and chronic rejection did not differ between the two groups although smokers had increased sepsis-related mortality (16% vs 4%, p=0.03). Smokers did not demonstrate increased incidence of cardiovascular disease but did have increased cardiovascular-related mortality (13% vs 1%, p<0.01). Non-smokers were more likely to develop de novo malignancy (26% vs 3%, p<0.01), although malignancy and de novo malignancy-related mortality between the two groups was similar. Smoking at the time of liver transplantation was associated with reduced survival during the followup period (58% vs 78%, p=0.04). On multivariate analysis smoking (OR 2.80; 95% CI 1.11-7.03, p=0.03) and post operative renal replacement therapy (OR 3.15; 95% CI 1.04-9.49, p=0.04) were the only independent predictors of mortality. Conclusions: Smoking is associated with significant morbidity and mortality post liver transplantation. Prospective studies are required to assess the impact of smoking cessation on long term outcome.

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The following people have nothing to disclose: Joanna A. Leithead, James W. Ferguson, Peter C. Hayes

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MULTICENTER, RANDOMIZED TRIAL OF CONVERSION TO EVEROLIMUS WITH CALCINEURIN INHIBITOR MINIMIZATION OR DISCONTINUATION IN LIVER TRANSPLANT PATIENTS WITH RENAL IMPAIRMENT
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Liver transplant recipients frequently experience renal impairment due to calcineurin inhibitor (CNI)-related nephrotoxicity, adversely affecting quality of life and life expectancy. An investigational study was undertaken in which treatment with the proliferation signal inhibitor everolimus was initiated in liver transplant patients with impaired renal function, with concomitant reduction or discontinuation of CNI therapy. The objective was to determine the effect of this immunosuppressive protocol on renal function. Methods. This was a prospective, randomized, six-month, multicenter, open-label trial. A total of 144 adult liver transplant recipients with CNI-related renal impairment were randomized to one of two groups. Patients in Group A remained on the baseline dose of CNI and their baseline
adjunctive therapy (mycophenolic acid [MPA] or azathioprine, with or without corticosteroids). In Group B, MPA or azathioprine was discontinued on day 1 and everolimus initiated at 3mg/day. The everolimus dose was then adjusted to target post-defined trough (CO) ranges. The CNI dose was reduced by 50% on day 1, and by a further 50% if everolimus C0 was at least 3ng/mL and calculated glomerular filtration rate (cGFR) was <10mL/min above baseline. If cGFR did not reach 10mL/min or less above baseline, CNI was withdrawn. To be eligible for inclusion, patients had to (a) have received a primary liver transplant 12-60 months previously (b) have CNI minimum 20mL/min and maximum 60mL/min at baseline and (b) be receiving tacrolimus (Prograf®) or cyclosporine (CsA, Neoral®) immunosuppression. Key exclusion criteria were an identifiable reason for renal dysfunction other than CNI toxicity; proteinuria 1.0g/24h or greater; or acute rejection <6 months previously. The primary endpoint was the change in cGFR (Cockcroft-Gault formula) from baseline to month 6 after introduction of everolimus. Power calculations determined that the study has 80% power to detect a difference of 8mL/min in the change in cGFR from baseline to month 6. Results: Complete six-month data on the total study population (n=144) will be presented. Results will include the primary endpoint (change in cGFR), efficacy variables including biopsy-proven acute rejection, graft loss and death, and safety variables. Conclusion: Findings from this randomized trial will determine the effect of conversion to concentration-controlled everolimus with a minimized/eliminated CNI regimen on renal function and efficacy in liver transplant patients with renal impairment. Results at six months may suggest a novel therapeutic approach for patients experiencing renal insufficiency following liver transplantation.

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The following people have nothing to disclose: Frederic Nevens, Martina Sterneck, Gerald Mettelzaar, Jerome Dumortier, Emiliana Giostro, Karim Boudjema, Evaristo Vara, Paolo de Simone

560 LIVER TRANSPLANTATION FOR BUDD-CHIARI SYNDROME: A 20-YEAR NATIONAL REGISTRY ANALYSIS

Dorry L. Segev1, Geoffrey C. Nguyen2, Jayme E. Locke1, Christopher E. Simpkins1, Robert A. Montgomery1, Warren R. Maley1, Paul J. Thuluvath2; 1Transplant Surgery, Johns Hopkins Medical Institutions, Baltimore, MD; 2Hepatology, Johns Hopkins Medical Institutions, Baltimore, MD

Several treatment options exist for the management of Budd-Chiari syndrome (BCS), yet the relative role and timing of liver transplantation remain poorly defined. Small case series published to date have not been able to delineate the impact of comorbidities and thrombo-embolic complications of BCS on graft and patient survival after liver transplantation. To better understand the outcomes after liver transplantation for BCS, we analyzed 510 liver transplants performed for this disease in the United States between 1987 and 2006. Risk factors predicting graft loss or patient death included increased recipient age, hyperbilirubinemia, elevated creatinine, life support or hospitalization at the time of transplantation, prior transplantation, prior abdominal surgery, increased donor age, and prolonged cold ischemic time. Prior transjugular intrahepatic portosystemic shunt (TIPS) was not associated with worse outcomes. Transplantation in the MELD era was associated with significantly lower risk of graft loss (HR 0.50, 95% CI 0.30-0.86, p=0.012) and death (HR 0.52, 95% CI 0.29-0.93, p=0.027). Similarly, MELD era was associated with significantly lower risk of early graft loss (OR 0.35, 95% CI 0.16-0.79, p=0.012) and early death (OR 0.37, 95% CI 0.14-0.95, p=0.040). However, patients with BCS transplanted in the MELD era were less likely to have life support, hospitalization, prior transplants, and prolonged cold ischemia times. Conclusion: Outcomes of liver transplantation for BCS are excellent, with further improvements since 2002 associated with a selection shift imposed by MELD-based organ allocation.
graft rejection in five (10.9%) patients. Four (8.7%) patients developed malignancy involving the skin, the larynx, and unknown sites (n=2) at year 2 to 8 of follow-up. Only one case of CMV infection was reported. CONCLUSION: Combined liver-heart and liver-heart-kidney transplantation is a viable option for candidates who need the combined organ transplantation, with outcomes comparable to those of single organ recipients.

**Patient and Graft Survival**

<table>
<thead>
<tr>
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<th>1 year</th>
<th>3 years</th>
<th>5 years</th>
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<tr>
<td>Patient Survival</td>
<td>84.8%</td>
<td>79.5%</td>
<td>75.6%</td>
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<tr>
<td>Liver Graft Survival</td>
<td>82.4%</td>
<td>77.3%</td>
<td>55.5%</td>
</tr>
<tr>
<td>Heart Graft Survival</td>
<td>84.3%</td>
<td>79.5%</td>
<td>75.6%</td>
</tr>
<tr>
<td>Kidney Graft Survival</td>
<td>66.5%</td>
<td>66.7%</td>
<td>66.7%</td>
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</tbody>
</table>

Comparative OPTN/SRTR Unadjusted Patient Survival Data as of May 1, 2006

HHS/HRSA/HSB/DOT

Disclosures:
The following people have nothing to disclose: Helen S. Te, Smruti R. Mohanty, Nancy Reau, Kapuluru G. Reddy, Rohit Satoskar, Donald M. Jensen

562 AMYLOID CARDIOMYOPATHY FOLLOWING LIVER TRANSPLANTATION FOR FAMILIAL AMYLOID POLYNEUROPATHY ATTR MET30 (PORTUGUESE TYPE); RESULTS FROM THE COLLABORATIVE BRITISH-SWEDISH STUDY

Arie J. Stangou1, Mark Monaghan2, John G. O’Grady1, Chris Bachman3, Thodore Kyriakides4, Mohamed Rela1, Bo-Goran Ericzon5, Nigel Heaton1, Ole Suhr2; 1Institute of Liver Studies, King's College Hospital, Amyloid Treatment Programme, London, United Kingdom; 2Amyloid Treatment Programme, Cardiology Dept, King’s College Hospital, London, United Kingdom; 3Amyloid Centre, Department of Medicine, Umeer, Sweden; 4Cyprus institute of Neurology and Genetics, Department of Neurology, Cyprus, Cyprus; 5Department of Surgery and Transplantation, Karolinska Institute, Stockholm, Sweden

Progressive amyloid cardiomyopathy following liver transplantation for familial amyloid polyneuropathy (FAP) is a paradoxic phenomenon due to continuing wild-type transthyretin deposition, observed in the rare, non-Met30 variants. Conflicting reports however have emerged, between US/ Northern European and South American/ Mediterranean series, regarding its potential occurrence in the commonest world-wide portuguese type Met30 mutation. We investigated the long term cardiac outcomes after LT for Met30, in a collaborative British-Swedish study comprising all Met30 patients transplanted in our centres in the past 13 years, amongst whom all major FAP European foci are represented. Eighty-five Met30 patients underwent LT between 1992-2005 at King’s College Hospital in London, UK, and Karolinska Institute in Sweden. 51 patients (60%) were of Scandinavian origin, and 34 (40%) Mediterranean (23 Cypriot, 7 Greek, 4 Portuguese). Median age at LT was 42 years (22-70) and symptoms duration 3 yrs (1-10). Median age at disease onset was 43 years in the Scandinavian and 34.5 in Mediterranean patients (p<0.01). Comprehensive echocardiography and intraventricular septal (IVS) readings were obtained before and annually after LT. IVS thickness changes of ≥2 mm were considered significant. Age of 40 years was used as the cut off between early- vs late-onset ATTRMet30. Forty five patients (52.9%) had early-onset and 40 (47.1%) late-onset Met30. Pre LT mean IVS thickness of 10.25±1.8 in early-onset was significantly different to 11.6±3.2mm in late-onset Met30 cases (p<0.015). At median follow up of 73 months after LT (12-174), 57.6% of patients (n=49) exhibited stable, and 15.3% (n=13) improved echo parameters; 3.5% (n=3) had IVS thickening with normal echoes, but 23.5% (n=20) showed IVS thickening and progression to amyloidotic echocardiograms; 2 cases developed heart failure. Autopsy confirmed cardiac amyloid with increased wild-type TTR content. Multiple linear regression and binary logistic regression analysis identified age at onset of FAP (<0.04), ethnic origin (p<0.01), and length of post-LT fol-up (<0.03) as factors with significant impact on amyloid cardiomyopathy post LT. There was no significant correlation with pre LT IVS thickness or disease duration, and no correlation with the post LT course of FAP features or rate of extracardiac amyloid turnover on amyloid scintigraphy. Late-onset presentation and North European origin were independent risk factors. Progressive amyloid cardiomyopathy can occur after LT in patients with the common ATTRMet30 variant. Late-onset disease and non-Mediterranean origin are independent risk factors.

Disclosures:
The following people have nothing to disclose: Arie J. Stangou, Mark Monaghan, John G. O’Grady, Chris Bachman, Thodore Kyriakides, Mohamed Rela, Bo-Goran Ericzon, Nigel Heaton, Ole Suhr

563 A NATIONWIDE SURVEY OF ATTITUDES TOWARD LIVER TRANSPLANTATION IN ALCOHOLIC LIVER DISEASE

Rohit Satoskar, Andrew Aronsohn, Helen S. Te, Smruti R. Mohanty, Kapuluru G. Reddy, Nancy Reau, Donald M. Jensen, Medicine, Center for Liver Diseases, University of Chicago Hospitals, Chicago, IL

AIMS: To better understand the relative importance of various psychosocial factors involved in decision making surrounding liver transplantation (LT) for alcoholic liver disease (ALD). METHODS: A 36 item survey was sent to 89 transplant centers in the U.S. Recipients were asked to provide demographics and respond to questions aimed at determining the perceived importance of various psychosocial factors surrounding LT in ALD. Chi square analysis was used for comparison of categorical variables. RESULTS: 117 responses were received. 60% of responses were from physicians, and the remainder from nurses, transplant coordinators, social workers and PAs. The mean degree of favorability of each individual psychosocial parameter when initially considering an alcoholic for LT was determined. “In counseling regularly” and “good family support” were most favorable, while “lives alone” and “failure of 2 prior rehab attempts” were most unfavorable. Perceived compliance had a significant effect on transplant status (Table 1). When assigning relative importance to 8 different factors, ranking from highest to lowest was: 1) excellent family support,2) abstinent for >6 months,3) in counseling with good attendance,4) good insight into alcohol abuse,5) good compliance with office visits, 6) random alcohol screen negative x3,7) working full-time,8) has health insurance. 57% of respondents would consider LT in a patient with severe alcoholic hepatitis (AH) if medical therapy was unlikely to prevent death. In this scenario, the most common reason for denying LT was likelihood of recidivism. Non-physicians were more likely to consider LT in AH compared to physicians (73% vs. 46%, p<0.01). Transplant volume and provider experience had no effect on responses. CONCLUSIONS: There is considerable variability in opinions regarding the effect of psychosocial factors on select-
ing LT candidates in the setting of ALD, but good family support and 6 months abstinence were consistently important. About half of the respondents would consider LT for a patient with alcoholic hepatitis if death was imminent. A strong divergence in opinion among transplant team members highlights a need for a methodical, non-biased, evidence-based approach to evaluating psychosocial factors in considering LT eligibility of patients with ALD.

Table 1. Effect of psychosocial parameters on transplant status

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Delay listing (% of respondents)</th>
<th>Place on list (% of respondents)</th>
<th>Remove from list (% of respondents)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No longer attending AA</td>
<td>63</td>
<td>52</td>
<td>2</td>
</tr>
<tr>
<td>Missed app., with counselor</td>
<td>85</td>
<td>81</td>
<td>10</td>
</tr>
<tr>
<td>Slipped without epilepsy while in counseling</td>
<td>82</td>
<td>75</td>
<td>13</td>
</tr>
<tr>
<td>Poor compliance with office visits</td>
<td>74</td>
<td>71</td>
<td>24</td>
</tr>
</tbody>
</table>

Data shown from separate questions, sums do not total 100%.

Disclosures: The following people have nothing to disclose: Rohit Satoskar, Andrew Aronsohn, Helen S. Te, Smriti R. Mahanty, Kapuluru G. Reddy, Nancy Reau, Donald M. Jensen

564 IMPLEMENTATION OF A LIVER ALLOCATION SYSTEM BASED ON MELD SCORE IN THREE TRANSPLANT CENTERS OF THE SAME CITY. CAN THE SCORE DIFFERENCES BE SOLVED?

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MELD score is considered an objective and reproducible measure of liver disease severity. However, MELD score can be influenced by specific laboratory methodologies. In order to implement an organ allocation system based on MELD score in the three Hospitals of Barcelona with liver transplant program, all patients in the waiting list at the three centers were studied simultaneously: blood samples were obtained from each patient and divided in three aliquots, which were processed in the three laboratories. Two laboratories (A, C) expressed creatinine and bilirubin in mg/dl and one (B) in µmol/l. Therefore, a conversion factor was used in laboratory B [creatinine x 0.0113 and bilirubin x 0.0588]. MELD was calculated according to the reported mathematical formula. Results: 74 patients, 51 from hospital A, 15 from B and 8 from C, were studied; 4 cases had a missing value and were excluded. Overall, there were significant differences in the three laboratory parameters, the greatest difference being in INR. Mean values and 95% CI for each variable are shown in the table. MELD score was identical in 564 patients with ALD.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Laboratory A</th>
<th>Laboratory B</th>
<th>Laboratory C</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Creatinine mg/dl</td>
<td>1.23 (1.05-1.42)</td>
<td>1.20 (1.13-1.25)</td>
<td>1.21 (1.03-1.41)</td>
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<tr>
<td>Bilirubin mg/dl</td>
<td>2.96 (2.29-3.64)</td>
<td>3.28 (2.47-4.90)</td>
<td>3.11 (2.38-3.84)</td>
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<tr>
<td>INR</td>
<td>1.40 (1.32-1.48)</td>
<td>1.52 (1.42-1.61)</td>
<td>1.67 (1.51-1.83)</td>
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</tr>
<tr>
<td>MELD</td>
<td>14.3 (13.8-16.3)</td>
<td>15.1 (13.1-15.4)</td>
<td>15.9 (14.6-17.3)</td>
<td>0.001</td>
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<tr>
<td>MELD range</td>
<td>6-29</td>
<td>6-31</td>
<td>6-40</td>
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</tbody>
</table>

Disclosures: The following people have nothing to disclose: Xavier Xiol, Pere Gines, Lluís Castells, Alba Ribalta, Jorge Twose, Clara Ventura, Joan Carles Reverter, Xavier Fuentes, Roser Deulofeu

565 DECEPTION DURING PSYCHOLOGICAL EVALUATION FOR LIVER TRANSPLANT IN ALCOHOLIC CIRRHOSIS: A DUI CORROBORATION STUDY

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Allocation of livers to alcoholic patients is a controversial issue. Proper selection of alcoholic liver recipients is essential to avoid potential recidivism & ethical/societal conflict regarding liver donation. Psychological evaluation (PE), which includes interview/alcohol history, is one aspect of the process used to define transplant suitability. Deception is evidence of active disease in alcoholism. Driving under the influence (DUI) of alcohol is objective evidence of alcohol abuse. However, verifying DUI status during PE through official records is not the standard approach. The aim was to determine whether there is deception on the part of patients regarding their alcohol abuse as corroborated by official department of transportation (DOT) DUI rate during PE. Method: 69 charts of alcoholic cirrhotics who underwent PE were reviewed for demographic data, detailed alcohol abuse/abstinence history & DUIs admitted on PE. The Wisconsin DOT was queried for DUIs before PE. Discrepancies between PE DUI and DOT DUIs were compared. The case was then presented to the psychologist who had conducted the original PE without identifiers. Psychosocial recommendation for transplant was then re-evaluated in light of discrepancies between PE & DOT DUI numbers. Results: 6 non-drivers were excluded; the remaining had a mean age of 53±1 yrs, 17F, 15 also had HCV & had 29±2 yrs of alcohol abuse. They had been alcohol abstinent 2.8±0.8 yrs before PE and 30% had received formal abstinence therapy. DUIs that were not originally admitted were found in 16 cases; 63% were in the period patients claimed to be sober. Two of 16 had been rejected for transplant due to other causes. Representing the case to the psychologist with the new knowledge of DUIs would have changed the recommendation for transplant for the remaining 14 (22%) cases because of the deception it uncovered as a sign of active alcoholism, which is significantly different from the pre-DOT record recommendation (Table). Importantly, there were no other significant differences in other variables during PE between groups. Conclusions: Evaluation of official driving records to exclude DUI in alcoholic cirrhotic patients may increase identification of active alcoholism during PE. Prospective follow-up of these patients post-transplant will be helpful in identifying recidivism.
OUTCOMES OF TRANSPLANTATION WITH PREVIOUSLY REFUSED DONOR LIVERS IN REGION 8
Sheila L. Eswaran, Elizabeth R. Lyden, Timothy M. McCashland; Gastroenterology and Hepatology, University of Nebraska Medical Center, Omaha, NE

Intro: There is a 10-20% mortality for patients awaiting liver transplantation (LT). There are various refusal reasons (RR) for donor livers (DLs). After refusal, DLs may be offered to another patient or center. Aim: Evaluate the frequency of RR and outcomes of LT with previously refused DLs. Methods: We requested data from UNOS-OPTN between July 2004-June 2006 in Region 8 regarding donors and recipients who received living and deceased livers on the first offer (Group A) and who received previously refused livers (Group B). We determined graft survival (GS) and patient survival (PS). Split livers were excluded. Results: Eleven centers were offered 764 DLs (Group A=315; 41% and Group B=437; 59%). The mean number of DL refusals was 12.64 (range 1-221). The most common RRs were donor age/quality (37%), donor size/weight (35%) and transplant center personnel limitations (10%), including heavy work load, operational, or surgeon unavailable. Group B had more male recipients (59% vs 72%; p=0.0003), older donor age (35 vs 38 yrs; p=0.013), longer cold ischemic times (CIT) (6.23 vs 7.42 hrs; p=0.0001), shorter warm ischemic times (WIT) (47.18 vs 42.8 hrs; p=0.003) and shorter length of hospitalization post LT (19 vs 14 days; p=0.003). There was no difference in 1 month (92% vs 94%) or 1 year (83% vs 85%) GS (p=0.20, 95% CI). There was no difference in 1 month (92% vs 94%) and 1 year (83% vs 85%) GS (p=0.20, 95% CI). (Figure) Group B had a longer PS (86% vs 91%) at one year (p=0.0069, 95% CI); however, when adjusted for gender, donor age, CIT, WIT and length of hospitalization post LT, a previously refused status was no longer associated with a longer PS. Conclusion: There were differences between recipients who received first offer DLs and recipients who received refused DLs with respect to recipient gender, donor age, CIT, WIT and length of hospitalization post LT; however, there was no difference in PS of the two groups after adjusting for these LT variables. GS is equivalent in LT with previously refused DLs compared with first offer DLs.

Disclosures:
The following people have nothing to disclose: Jasmohan S. Bajaj, Muhammad Hafeezullah, Andrea Thompson, Kia Saenian, Jose Franco, Rebecca C. Anderson

567 DOWNSTAGING/BRIDGING OF HCC PATIENTS TO TRANSPLANTATION USING Y90 RADIOTHERAPY: PATHOLOGIC RESULTS FROM 21 EXPLANTS
Laura M. Kulik, Bassel Atassi, Robert Lewandowski, Mary Mulcahy, Michael Abecassis, Riad Salem; Northwestern University, Chicago, IL

Purpose: To present pathologic explant and survival outcomes in 21 patients with unresectable HCC that were bridged or downstaged to transplantation using Y90 radiotherapy. Material and Methods: 251 patients with unresectable HCC were treated over a 7 years period with Y90 intra-arterial radiotherapy (TheraSphere). Of these, 21 patients were transplanted. Pathologic analysis was performed in all explants. Findings were stratified by complete or partial necrosis of the target lesion. Survival analysis was performed from first treatment (intention-to-treat) and from transplantation. Results: There were 18 males and 3 females. Mean age was 60. Etiology of disease was: Hep C (n=6), Hep B (n=4), PBC (n=1), Hep C + alcohol (n=4), alcohol (n=3) and cryptogenic (n=3). There were 10, 10 and 1 patients with Child A, B and C disease respectively. There were 9 patients with Okuda 1 and 12 with Okuda 2 disease. Tumor distribution was: 16 unilobar and 5 bilobar. Target tumor dose was 120 Gy. Toxicities included fatigue (42%), anorexia (7%), pain (5%) and nausea (5%). Mean AFP at time of treatment for the 21 patients was 1647.4 ng/ml. Mean AFP prior to transplantation was 1105.2 ng/ml (n=17). Median time from first treatment to transplant was 114 days (mean 142 days, range 10-311). Complete necrosis by pathologic analysis was noted in 14 of 21 patients (66%). Pathologic findings included coagulative necrosis and peripheral ring fibrosis containing benign tissue. Recurrence has been documented in 4 patients; 2 with complete necrosis and 2 with partial necrosis. Mean time to recurrence was 250 days (median: 167 days). 19 of 21 patients are still alive with a median follow-up of 142 days (range 10-311). Complete necrosis by pathologic analysis was noted in 14 of 21 patients (66%). Pathologic findings included coagulative necrosis and peripheral ring fibrosis containing benign tissue. Recurrence has been documented in 4 patients; 2 with complete necrosis and 2 with partial necrosis. Mean time to recurrence was 250 days (median: 167 days). 19 of 21 patients are still alive with a median follow-up of time of treatment of 428 days (range: 131-1461) and 219 days (range: 63-1390) from time of transplantation. The 2 deaths were attributed to disease recurrence. Conclusion: Treatment with Y90 achieves complete pathologic necrosis in the majority of targeted lesions in patients bridged or downstaged to transplantation. Longer follow-up will be required to assess treatment benefit with regards to recurrence-free and overall survival.

Disclosures:
The following people have nothing to disclose: Sheila Eswaran, Elizabeth R. Lyden, Timothy M. McCashland
Background: While malnutrition reduces survival after OLT, it remains unclear whether obesity, which is increasingly present in American recipients, has similar deleterious effects. Aim: To compare the impact of obesity on patient and graft survival after OLT in a large, multi-institutional cohort of adult American patients. Methods: The United Network for Organ Sharing (UNOS) database identified 47,796 adult patients who underwent 53,719 OLT’s between 1987 and 2005. BMI groups (A: <20, B: 20–24, C: 25-34, D: 35-39, E: 40+) were compared using log-rank and chi-squared statistics. Patient and graft survivals were assessed using Kaplan-Meier survival curves. Demographic and medical variables were studied using univariate and multivariate analyses. Results: At a median follow-up interval of 1,018 d (range: 0-6,261 d), 5-yr patient survivals were: Group A=69%, B=73%, C=74%, D=73% and E=69% (p=0.001 for A and E). Five-yr graft survivals were: Group A=58%, B=63%, C=64%, D=64% and E=60% (p=0.001 for A and E). The 30-d post-transplant mortality rate was 10% for E, compared to only 6% for A, B, C, and D (p<0.001). Patients with BMI >or=35 (D+E), had a higher proportion of cardiovascular deaths (15% vs. 11% for A, B, and C; p<0.001) and higher incidence of myocardial infarction (5% vs. 3% for A, B, and C; p=0.04). In contrast, group A had a higher proportion of deaths due to infection (23% vs. 21% for B, C, D, and E, p=0.02) or complications of rejection (3% vs. 2% for B, C, D, and E, p=0.009). Patients with BMI >or=35 (D+E) had a higher proportion of infection-related graft failure (20% vs. 17% for A, B, and C, p=0.04). Group A had the highest proportion of graft failure due to chronic rejection (14% vs. 9% for B, C, D, and E, p=0.005) Univariate analysis showed race, era of transplantation, preoperative ICU care, and the need for mechanical ventilation to be significant prognostic variables (p=0.001 to 0.03). Multivariate analysis identified era (p=0.006) and the need for mechanical ventilation (p=0.030) as independent prognostic variables. Interestingly, the presence of obesity did not significantly affect 5-yr patient and graft survivals in HCV+ patients. Conclusions: Malnutrition (BMI <20) and morbid obesity (BMI >40) are associated with significant decreases in patient and allograft survivals after OLT. BMI >40 was associated with significantly higher 30 d mortality than any other group. BMI >or=35 was associated with significantly higher cardiovascular mortality and infection-related allograft failure. The adverse impact of obesity on post-OLT survival indicates that OLT should be delayed, whenever possible, until a BMI<35 is achieved.

Disclosures: The following people have nothing to disclose: Joel A. Rodriguez, John M. Vierling, Thomas A. Aloia, Natasha S. Becker, Christine A. O’Mahony, John A. Goss; 2Liver Transplant Unit, Hospital Universitario Virgen del Rocio, Sevilla, Spain; 3Pathology, Hospital Universitario Virgen del Rocio, Sevilla, Spain; 4Liver Transplant Unit, Hospital Universitario Virgen del Rocio, Sevilla, Spain

CONTROL ID: 368722 TITLE: C4d IN DE NOVO IMMUNE HEPATITIS AFTER LIVER TRANSPLANTATION

Isabel Aguilera1, Jose Manuel Sousa2, Ingeborg Wichmann3, Francisco Gavilan2, Angel Bernardos4, Antonio Núñez-Roldán1; 1Immunology, Hospital Universitario Virgen del Rocio, Sevilla, Spain; 2Hepatology, Hospital Universitario Virgen del Rocio, Sevilla, Spain; 3Pathology, Hospital Universitario Virgen del Rocio, Sevilla, Spain; 4Liver Transplant Unit, Hospital Universitario Virgen del Rocio, Sevilla, Spain

C4D IN DE NOVO IMMUNE HEPATITIS AFTER LIVER TRANSPLANTATION

Body: In contrast to other solid organs, the liver allograft appears to be relatively resistant to antibody-mediated rejection. C4d staining plus documentation of circulating donor specific antibodies in the presence of graft dysfunction is accepted by most clinicians as evidence of active humoral rejection in renal and heart transplantation. In liver, there are a few reports in which the use of C4d immunostaining is analyzed as a possible marker of humoral rejection in ABO-incompatible liver transplantation (1) or to differentiate acute rejection from recurrent hepatitis C (2). Objective: To study immunohistochemical C4d staining in patients diagnosed of de novo immune hepatitis (IH). Results: We retrospectively analyzed a total of 7 biopsies obtained from patients with the null GSTT1 genotype that have received a graft from a positive donor. We have described that in about 80% of the cases the recipients with this mismatch produce anti-GSTT1 antibodies and in most of the cases develop de novo immune hepatitis. Biopsies were always performed for clinical indications and specimens ranged from 12 to 85 months post-transplant. We have followed a scoring system of capillaries, arteries and veins is evaluated in a scale of 0, none; 1+, weak; 2+, moderate and 3+, strong (Table 1). Six patients present C4d deposits in portal areas with different degrees of extension. Only one of them is C4d negative in a biopsy performed after treatment coinciding with disappear-

Heating after Liver Transplantation

Body: Abstract

C4d staining in patients diagnosed of de novo immune hepatitis (IH). Results: We retrospectively analyzed a total of 7 biopsies obtained from patients with the null GSTT1 genotype that have received a graft from a positive donor. We have described that in about 80% of the cases the recipients with this mismatch produce anti-GSTT1 antibodies and in most of the cases develop de novo immune hepatitis. Biopsies were always performed for clinical indications and specimens ranged from 12 to 85 months post-transplant. We have followed a scoring method described by Troxell ML (3) in which C4d positive staining is achieved.

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570 NO U.S. ORTHOTOPIC LIVER TRANSPLANT (OLT) OUTCOMES FOR ACUTE LIVER FAILURE (ALF) IMPROVED IN THE LAST DECADE?

Christine A. O’Mahony, Sanjeet Patel, Jessica Suarez, John M. Vierling, Neal R. Barashes, Natasha S. Becker, Norma Flores, Wanda Samuels, Rise Stribling, Norman L. Sussman, John A. Goss; Baylor College of Medicine, Houston, TX

BACKGROUND: OLT for ALF has been associated with decreased patient and allograft survival rates. The risk factors causing these decreased survival rates remain largely undefined. AIM: To determine if patient and allograft survival rates have improved in the current era of transplantation and to identify risk factors leading to mortality and allograft failure in patients undergoing OLT for ALF. METHODS: Data from all patients who underwent OLT for ALF between 1990-1993 (Era-1) and 2000-2003 (Era-2) were obtained from the United Network for Organ Sharing. Multigorgan transplant recipients and patients <18 years old were excluded. Group comparisons were performed using Chi squared and student’s T-test. Unadjusted survival rates were estimated with the Kaplan-Meier product limit estimate. A multivariate Cox proportional hazards model was then used to compare patient mortality or graft failure rates after adjusting for important donor, graft, and recipient characteristics. RESULTS: A total of 1037 patients who underwent OLT for ALF were identified from the UNOS database. The average age (29 years), gender (61% female), SGPT (1335 U/L), creatinine (1.8 mg/dl), bilirubin (21 mg/dl), albumin (2.85 g/dl) and time on the waitlist (3.3 days) were similar for both groups. However, the patients transplanted in Era-2 experienced a significantly shorter cold ischemia time (10.8 hrs vs. 7.8 hrs, p<0.05) as well as a shorter warm ischemia time (65 minutes vs. 43 minutes, p=0.05). One year patient as well as allograft survival rates were significantly higher in the patients who underwent OLT in Era-2 with a one year patient survival of 73% in Era 1 compared to 82% in Era-2 (p<0.05) and a graft survival of 63% in Era-1 and 75% in Era-2 (p=0.05). In patients that underwent OLT in Era-1, factors that independently influenced survival were a cold ischemia time greater than 12 hours (HR 2.26, p<0.05), and mechanical ventilation at the time of transplant (HR 1.63, p<0.05). In patients that underwent OLT in Era-2, recipient age > 60 yrs (HR 2.13, p<0.05), donor age > 60 yrs (HR 1.94, p<0.05), and mechanical ventilation at the time of transplant (HR 1.84, p=0.05) were independent risk factors for mortality. CONCLUSION: Over the last decade there has been little change in demographics in the patient population undergoing OLT for ALF. However, patient mortality and allograft failure rates have decreased significantly.

Disclosures: The following people have nothing to disclose: Christine A. O’Mahony, Sanjeet Patel, Jessica Suarez, John M. Vierling, Neal R. Barashes, Natasha S. Becker, Norma Flores, Wanda Samuels, Rise Stribling, Norman L. Sussman, John A. Goss

571 PRETRANSPLANT RISK FACTORS FOR ADVANCED CHRONIC KIDNEY DISEASE IN LIVER TRANSPLANTATION

Santiago Tome1,2, Julio Pascual2, Milagros Samaniego2, Adnan Said2, Luis Fernandez2, Tony D’Alessandro2, Aji Djamali2, Michael R. Lucey2, John Pirsch2, Hans Sollinger2, Stuart J. Knechtle2; 1Hospital Universitario de Santiago, Santiago de Compostela, Spain; 2Division of Transplantation, Departments of Surgery and Medicine, University of Wisconsin, Madison, WI

Body: Severe renal failure is increasingly frequent after liver transplantation (LT). The US Registry showed a 18% cumulative incidence of severe chronic kidney disease (sCKD) [estimated glomerular filtration rate eGFR<30mL/min] 5 years after LT. To select the population in whom a special effort is needed for nephroprotection, we have evaluated the impact of patient characteristics on the development of sCKD as the primary endpoint. All adult primary LT performed at our institution between 1994 and 2005 were analyzed. Patients with pre-LT dialysis need, pre-LT Scr>2.5 mg/dL, recipients of retransplants or combined organs, and patients surviving <30 days after LT were excluded. Univariate analysis and Cox proportional hazards model were used for determining pre-LT factors related with the end-point. Finally, 651 patients fulfilled these criteria. Kaplan-Meier estimates of freedom of sCKD were 92% at 3 months, 84% at 1 year, 81.2% at 3 yr, 77% at 5 yr and only 69% at 10 yr. Risk factors for sCKD in the univariate analysis were: age at LT (HR 1.05/yr, p=0.0001), body mass index (protective: HR 0.92/unit, p<0.0001), calcium status (protective: HR 0.55, p=0.03), male sex (protective: HR 0.30, p<0.0001), pre-LT Scr (HR 1.73 each mg/dl, p=0.0065), pre-LT eGFR (protective: HR 0.98 each ml/min, p<0.0001), pre-LT ALT (protective: HR 0.99/unit, p=0.04). Donor factors, other analytical parameters (pre-LT albumin, INR, bilirubin, MELD), liver disease type, HCV+ status, need of TIPS, clinical situation (ascites, encephalopathy) and perioperative factors (v v bypass need and time, blood products) were all non-significant factors for sCKD during the follow-up. The Cox multivariate analysis confirmed that age at LT (HR 1.032/yr, p=0.002), body mass index (protective: HR 0.94/unit, p=0.0006), calcium status (protective: HR 0.50, p=0.02), male sex (protective: HR 0.31, p=0.0001), and pre-LT eGFR (protective: HR 0.98 each ml/min) were the only significant risk factors. Pre-LT Scr lost significance when eGFR was included. The 10-year incidence estimate for developing severe-CKD almost doubles the previously reported rate at 5 years. Preventive measures based on the described risk factors are urgently needed. Nephroprotection would be particularly relevant in older, non-caucasian, females with pre-LT renal mild dysfunction.

Disclosures: The following people have nothing to disclose: Santiago Tome, Julio Pascual, Milagros Samaniego, Adnan Said, Luis Fernandez, Tony D’Alessandro, Aji Djamali, Michael R. Lucey, John Pirsch, Hans Sollinger, Stuart J. Knechtle

572 THE EFFECT OF HEPATIC ENCEPHALOPATHY ON POST LIVER TRANSPLANTATION (LT) MORBIDITY AND MORTALITY: D. BRANDMAN, S.W. BIGGINS, J.P. ROBERTS, N.A. TERRAULT

Danielle Brandman1, Scott W. Biggins1, John P. Roberts2, Norah Terraault1; 1Medicine, University of California, San Francisco, San Francisco, CA; 2Surgery, University of California, San Francisco, San Francisco, CA

BACKGROUND: Hepatic encephalopathy (HE) does not enhance the prediction of wait-list mortality of MELD. However, the effect of HE of post-LT morbidity and mortality is largely
unknown. AIM: To determine the effect of severe HE on post-LT outcomes and to evaluate the relationship serum sodium and severe HE. METHODS: In this retrospective cohort of adult patients undergoing first LT for end-stage liver disease from 02/2002-12/2005, severity of HE was determined by the transplant surgeon immediately prior to LT (grade 0-2 non-severe HE, grade 3-4 severe HE), with re-review of those with grade 2 HE to include additional severe HE cases. The primary outcomes were survival at 90 days and 1-year post-LT, and length of stay (LOS). RESULTS: Of the 450 LT recipients during the study period, 368 met eligibility criteria (MELD 28 ±8.5; age 55 ± 8.9; 36% HCC), and 44 (12%) had severe HE. Gender, race, and underlying liver disease were similar between groups. MELD score at LT was higher in the severe HE group (36 ±5.8 vs 27 ±8.2 non-severe HE, p<0.0001), and severe HE subjects were less likely to have HCC at LT (11.4% vs. 38.9%, p<0.0001). Compared to the non-severe HE group, patients with severe HE more frequently had serum sodium (Na+) changes of ≥15 points in the peri-LT period (1 wk prior to LT to 1 wk post-LT), 25% vs 6% (p<0.001), as well as lower pre-LT nadir Na+ (133 ±0.95 vs 135 ±0.31, p=0.04). No difference was detected between the groups in the proportion with pre-LT <126 (18% vs. 14%, 95% CI 0.59:3.18, p=0.46). While 90-day mortality was not significantly different between groups, there was a trend toward higher 1-year mortality in the severe HE group (86.4%, CI 72.1-93.6 vs. 93.2%, CI 89.9-95.5 in non-severe HE group, p=0.11). In Cox models, severe HE was associated with 1-yr mortality (HR 2.06, 95% CI 0.84:5.09, p=0.12) compared to non-severe HE, but as expected, the association was weakened when MELD was included (HR 1.42, 95% CI 0.53:3.83 in bivariate model). LOS (days) was significantly longer for severe HE than non-severe HE patients (28 ±34 vs. 12 ±22, p=0.0001), and the association between severe HE and LOS was independent of MELD, recipient age, presence of HCC, pre-LT Na+ <126, peri-LT change in Na+, or rejection episodes. CONCLUSIONS: Severe HE was associated with a higher 1-year mortality, but this effect was, in large part, accounted for by MELD. Patients with severe HE have significantly longer LOS, a marker of increased morbidity, and this association was independent of MELD. Whether the large changes in peri-LT serum Na+, more frequently seen in the severe HE group, contribute to post-LT morbidity requires further study.

Disclosures: The following people have nothing to disclose: George V. Mazariegos, Zurab Machaidze, Kyle Sohys, Geoffrey Bond, Robert Squires, Rakesh Sindhi

574 USE OF MULTIVISCERAL TRANSPLANTATION IN THE MANAGEMENT OF CIRRHTIC PATIENTS WITH DIFFUSE PORTOMESENTERIC THROMBOSIS
Richard S. Mangus, A Joseph Tector, Jonathan A. Fridell, Marwan Kazimi, Rodrigo Vianna; Transplant Division, Dept of Surgery, Indiana University, School of Medicine, Indianapolis, IN

Introduction. The presence of portal vein thrombus in a cirrhotic patient is a poor prognostic indicator and can be exclusionary criteria for orthotopic liver transplantation (OLT). The majority of these patients can be successfully managed intraoperatively at the time of transplant with either low dissection and thrombectomy or portal vein bypass. The absence of a large tributary can preclude the anastomosis of the donor portal vein. In this situation, alternative vascular reconstructions have been proposed, including cavo-portal hemitransposition, renalportal vein graft interposition and utilization of the hepatic artery for portal flow. Although these techniques are able to restore blood flow to the donor portal vein, portal hypertension and its complications continue to exist after OLT. We describe our experience with 15 patients with diffuse portomesenteric thrombosis who were listed for OLT with multivisceral transplant (MVT) back-up. Patients in whom portal flow could not be established intraoperatively underwent MVT to completely replace the portomesenteric system. Methods. All cirrhotic patients at our center referred for OLT evaluation undergo abdominal imaging with dual-phase computed tomography (CT) scanning. Those with portal or mesenteric thrombus undergo additional imaging with magnetic resonance (MR) scanning. We identified 15 patients with diffuse mesenteric thrombosis who were listed for OLT with MVT back-up. At the time of transplantation, organs were procured on-block as a donor graft. In patients in whom portal flow could not be established, MVT was performed, which included transplantation of the liver, pancreaticoduodenal complex, small intestine and stomach. Results. Fifteen survival is 97.3% (109/112) and 94% (109/116) respectively, at a mean follow-up time of 34.4 m +/-.16.3 (range 6.1-67.9 m). 3 children died from recurrent hepatic malignancy (n=2), and cardiac arrest (n=1). Re-transplantation was performed successfully in 3 patients; one patient required two re-transplants for thrombotic complications. Steroid responsive rejection was seen in 45 (40.1%) patients within the first 90 days post-transplant. IS maintenance at current follow-up is: TAC (TID n=1, BID n=48; QD n=39, QOD or less n=12), SRL (QD n=4, BID n=2, single dose or less n=2). 85 children (78%) are free of maintenance steroids and the others are being weaned off steroids instituted during treatment of ACR. Viral drug associated complications (hypertension, renal insufficiency) and infection is favorable as compared to historical controls.

Disclosures: The following people have nothing to disclose: George V. Mazariegos, Zurab Machaidze, Kyle Sohys, Geoffrey Bond, Robert Squires, Rakesh Sindhi

573 LONG-TERM OUTCOME WITH RATG INDUCTION AND STEROID-FREE IMMUNOSUPPRESSION IN PEDIATRIC LIVER TRANSPLANTATION(PLTX)
George V. Mazariegos, Zurab Machaidze, Kyle Sohys, Geoffrey Bond, Robert Squires, Rakesh Sindhi; Transplant Surgery, Children’s Hospital of Pittsburgh, Pittsburgh, PA

Background: Steroid-free immunosuppression (IS) has significant potential benefits in pediatric liver transplantation. Methods: Outcomes with a steroid-free IS protocol with a rATG pre-conditioning regimen and tacrolimus (TAC) monotherapy were reviewed. Results: Between 8/2001-9/2006, 112 consecutive patients with a median age of 5.4 y (range 1.4 m-21.7 y) received 116 liver allografts (90 whole organs, 6 cadaveric split, 20 living donor, and 5 simultaneous cadaveric kidney allografts). Pre-treatment and induction therapy was with 4.5 mg/kg rATG. Oral tacrolimus (TAC) was started within 24 hours post-transplant to achieve a target trough of 10-15 ng/ml. 8 patients were switched to sirolimus (SRL) to minimize potential long-term calcineurin toxicity. Overall patient and graft
patients were listed for OLT with MVT back-up. Thirteen patients had no identifiable portal flow upon exploration and underwent MVT. Two patients had adequate portal vein flow and underwent standard OLT. There was one perioperative death, in an MVT patient related to heart failure. All patients had good initial graft function. Ninety-day survival was 92% (12/13) for patients receiving MVT and 100% (2/2) for patients undergoing OLT. Conclusions: For cirrhotic patients with inadequate portal vein inflow, multivisceral transplantation offers a life-saving alternative. This management option has been successfully utilized at our center in 15 patients.

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LONG-TERM OUTCOMES FOR PATIENTS TRANSPLANTED FOR ACETAMINOPHEN INDUCED ACUTE LIVER FAILURE; NO WORSE THAN FOR OTHER ETILOGIES
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Introduction: Acetaminophen hepatotoxicity is the most common cause of acute liver failure (ALF) in the UK. Concern has been raised about compliance and long-term outcomes of liver transplantation (LT) in this patient group, given the frequent history of suicide, substance abuse and difficulty in psychological assessment of encephalopathic patients requiring emergency LT. We compared medical, psychosocial and long-term outcome of patients who had undergone emergency LT for acetaminophen induced ALF (AALF) with age and sex matched patients undergoing emergency LT for non-acetaminophen etiologies of ALF (NAALF) and elective LT for chronic liver disease (CLD). Design: A review was undertaken of all patients transplanted for AALF (n=36) between 1999-2004 at King’s College Hospital; comparing to those transplanted for NAALF (n=35) and CLD (n=34) during the same period. Patients transplanted for AALF and NAALF met King’s College Criteria for super-urgent listing. The CLD cohort had been listed as part of standard multidisciplinary process. Statistical analysis utilized non-parametric testing and survival analysis used log-rank testing. Results: AALF patients had a higher median INR (P < 0.004) and APACHE II score on the day of listing (P < 0.02) compared to the NAALF and CLD groups. More AALF patients required hemofiltration pre-LT (AALF 94%, NAALF 43%, CLD 3%, P < 0.001). More than 95% of AALF patients were mechanically ventilated prior to LT (vs. 3% CLD, P < 0.001). Median follow-up for all groups was 1068 (585-1467) days with no differences in 1 or 3-year survival (AALF 1yr 84% 3yr 78%, NAALF 88%/82%, CLD 83/73%, P >0.8 log rank). There were no significant differences in frequency of acute cellular rejection, chronic ductopenic rejection or graft failure between groups. Twenty (56%) AALF patients had a formal psychiatric diagnosis prior to LT (NAALF 0%, CLD 6%; P < 0.001). Nine (25%) AALF patients had previously attempted suicide. All AALF patients underwent psychiatric review following recovery. Post-LT, one AALF patient attempted suicide requiring psychiatric admission. Twenty AALF patients required long term psychiatric follow-up post-LT (P < 0.001 vs. both) and 11 psychiatric medications (P < 0.01 vs. both). During follow-up, AALF patients attended 79% of their appointments, fewer than controls (CLD 91%, P = 0.104, NAALF 93%, P = 0.009). Conclusions: Despite a high prevalence of psychiatric disturbance, short and long term outcomes in patients undergoing super-urgent LT for AALF were comparable to those with NAALF and those transplanted electively for CLD. Long term psychiatric follow-up may contribute to low post-LT rates of suicide and graft loss.

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SEXUAL DYSFUNCTION BEFORE AND AFTER LIVER TRANSPLANTATION (LT)
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Sexual disorders are frequently described in patients with cirrhosis, often related to the endocrine consequences of advanced liver disease. It is assumed that LT will help to recover from these symptoms. Aim: To report on the prevalence of sexual dysfunction in liver transplant patients before and after LT, emphasizing the potential recovery of pre-LT disorders and the occurrence of de novo post-LT disorders. Methods: During a direct interview, a sexual history was taken by a sexology medicine specialist in all liver transplant recipients seen at the outpatient clinic between May and December 2006. Exclusion criteria were: age < 18 or > 70, LT < 6 months, evolutive cancer, interferon treatment for HCV reinfection, severe renal or cardiac dysfunction. The following domains were assessed: hypoactive sexual desire in men and women, erectile dysfunction in men and inadequate vaginal lubrication in women, premature or delayed or absent ejaculation in men, orgasmic disorder in women, pain with intercourse in men, dyspareunia or vaginismus in women. Results: 93 pts fulfilled the inclusion criteria and 89 accepted to complete the study. There were 64 males, mean age 54 ± 9 yrs and 25 females, mean age 51 ± 10 yrs. Indication for LT were: alcoholic liver disease in 52, virus in 15, alcohol plus virus in 9, others in 13. The mean delay form LT was 55 months (6-225). Evolution of sexual disorders before and after LT: hypoactive sexual desire was present in 29% of male and 40% of female, and improved in 73.6% of male and 0% of female. Erectile dysfunction was present in 39% of male and improved in 68% of them. Inadequate lubrication was present in 24% of female and improved in none. Premature ejaculation was present in 21% of male and improved in 7% of them. Orgasmic disorder was present in 14% of male and 36% of female and improved in 66% of male and 11% of female. Pain was absent in male and present in 1 female before and after LT. De novo sexual disorders after LT: hypoactive sexual desire was present in 6.2% of male and 12% of female. Erectile dysfunction was present in 14% of male. Premature ejaculation was present in 3% of male. Orgasmic disorder was present in 3% of male. Conclusion: Sexual disorders were frequent in liver transplant patients before LT. They frequently improved after LT in male, but almost never in female. This point emphasizes the unclear impact of advanced liver disease in women’s sexual dysfunction. De novo sexual disorders after LT were unusual. Overall, 34.8% of liver transplant recipients experienced sexual disorder. This will need further attention.

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577 IMPLICATIONS OF PREOPERATIVE PULMONARY FUNCTION TESTING FOR POST LIVER-TRANSPLANT OUTCOMES

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Aim: To assess whether prolonged ICU stay, post-operative pulmonary complications (PPCs) and death post-liver transplant are predicted by pre-transplant pulmonary function tests (PFTs). Background: Pulmonary complications are common post-transplant with an estimated incidence of 41 to 98%. Up to 25% of deaths within the first week of any surgical procedure are attributable to pulmonary complications and the highest risk procedures are abdominal. Although PFTs are routinely obtained to assess surgical risk, their usefulness in liver transplantation has not been evaluated. Methods: The study cohort encompasses all liver transplants at our center between 1990 and 2005. Outcomes including death, length of ICU stay, and PPCs such as pneumonia, pneumothorax, and ARDS were verified using both medical records and an existing transplant database. PFTs are routinely measured before transplant at our center and are expressed as percentage of predicted values, based on the European Community for Coal and Steel reference set. Other data collected included age, gender, race, smoking history, etiology of liver disease, BMI, MELD score, cold and warm ischemia time. Individuals with hepatopulmonary syndrome or portopulmonary hypertension were excluded. Multiple linear regression was performed to assess the impact of these independent variables on length of ICU stay, while logistic regression and Cox proportional hazards models assessed their impact on PPCs and mortality. Results: Of 537 transplants, preoperative PFT data were available for 325. 5 year mortality was 34%. Length of ICU stay varied from 0 to 101 days. The cumulative incidence of pneumonia, pneumothorax, and ARDS was 12%, 6%, and 5% respectively; for PPCs overall it was 15%. Total lung capacity (TLC) was a predictor of length of ICU stay (p=0.018; CI: -0.04 to -0.004). A measured TLC 50% below predicted translates to an increase in ICU length of stay of 1 day. There were no significant predictors of PPCs. Independent predictors of mortality included TLC with hazard ratio 0.95 (p-value 0.011; CI 0.91-0.99) and residual volume (RV): hazard ratio 1.02 (p=0.04; CI: 1.0-1.04). For each 1% decrease in TLC the mortality risk at any time is increased by 5%. Smoking history and MELD score were not significant independent predictors. Discussion: These data suggest that post-transplant pulmonary complications are common and that while impaired PFTs do not predict PPCs they do predict length of ICU stay and mortality. PFT abnormalities may reflect severity of underlying liver disease as well as intrinsic lung disease.

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578 SIROLIMUS IN LIVER TRANSPLANT RECIPIENTS WITH RENAL DYSFUNCTION OFFERS NO ADVANTAGE OVER LOW DOSE CALCINEURIN INHIBITORS

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Aim: The purpose of this study is to compare the clinical experience with sirolimus in liver transplant recipients with chronic renal insufficiency with a matched control group maintained on reduced dose calcineurin inhibitors. Methods: This is a retrospective study of patients transplanted between 2001 and 2006. 57 liver patients with renal insufficiency and on sirolimus were matched for gender, year of transplant, and baseline creatinine clearance to a control group of 57 liver patients on low dose calcineurin inhibitors. Results: Baseline characteristics were similar between the 2 groups. a) Sirolimus tolerability and rejection: Drug-related side effects were more common in the sirolimus group compared with the control group (36 vs 24 patients, p=0.025). Rejection, retransplantation and death rates were similar. b) Renal function: Renal replacement rate was similar between the 2 groups (sirolimus=21% v control=12%, p=NS). The creatinine clearance of both the sirolimus (37 +/- 1.6 vs. 35 +/- 14 ml/min, p=ns) and the control groups (38 +/- 1.4 vs 37 +/- 1.6 ml/min, p=ns) was unchanged at 12 months after intervention. However, the change in creatinine clearance relative to the time of intervention was significantly worse in the sirolimus group compared with the control group at 6 (p=0.045) and 12 months after intervention (p=0.001). Stratification according to creatinine clearance at baseline, showed no difference between the groups with creatinine clearances of 30-45 ml/min or 45-60 ml/min. However, in the lower creatinine clearance group(< 30 ml/min), the sirolimus group (n=17) demonstrated significantly worse creatinine clearance at the 3 (20 +/- 1.4 vs 25 +/- 1.1 ml/min, p<0.012) and 6 months (19 +/- 2 vs 25 +/- 1.5 ml/min, p<0.035) post intervention. Conclusion: This study demonstrates that conversion from a calcineurin inhibitor protocol to sirolimus in liver transplant patients with chronic renal insufficiency is associated with stabilization of renal function, but confers no advantage over low dose calcineurin inhibitors. In addition, at creatinine clearance levels lower than 30 ml/min, the sirolimus group deteriorated faster than the control group. This finding as well as the excess number of side effects seen in the sirolimus group make the conservative approach of reduced calcineurin inhibition the preferable option.

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579 THE IMPACT OF ISCHAEMIC PRECONDITIONING ON GENE EXPRESSION IN HUMAN LIVER TRANSPLANTATION

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Introduction In liver transplantation ischaemia / reperfusion (IR) is associated with an inflammatory process that leads to cell loss and affects allograft outcome. Ischaemic preconditioning (IP) has been demonstrated to be effective in protecting organs from IR. The molecular basis of this protective effect is poorly understood. This study assesses the gene expression profile in liver allografts during transplantation and evaluates the impact
of IP. Methods IP in cadaver donor livers was performed by clamping the porta-hepatis for 10 minutes followed by 30 minutes of reperfusion prior to organ removal. Pre- and post-transplant liver biopsies were obtained from livers subjected to IP (n=19) or no preconditioning (IR) (n=16). Total RNA was extracted and hybridised to GeneChip oligonucleotide microarrays (HG-U133A; Affymetrix) (3 allografts from each group). Real-time RT-PCR was subsequently performed in all pre- and post-transplant biopsies obtained from both groups to revalidate the findings. Results IP livers showed better liver function in terms of serum transaminases, INR and lactate following transplantation. Microarray analysis of IR group showed increased expression of 55 genes involved in cell death, immune response, cell stress, proliferation and metabolism. IP group showed attenuation of expression of these genes. Expression of most significant 10 genes was revalidated in all patients using real time RT-PCR and correlated with clinical outcome. In the IR group, elevated expression of ADAMTS1 was associated with higher levels of day 2 serum lactate (r=0.502; p=0.04), day 7 (r=0.639; p=0.008) and 14 (r=0.515; p=0.04) bilirubin post-transplant. High expression of CCL20 was associated with higher day 7 (r=0.648; p=0.01) and day 14 (r=0.621; p=0.01) serum bilirubin. High expression of DUSP6 was associated with higher day 1 (r=0.601; p=0.01) and day 2 (r=0.601; p=0.01) INR. Increased IL8 was also associated with high levels of serum bilirubin in day 7 (r=0.517; p=0.04) and day 14 (r=0.629; p=0.009). In the IP group increased expression of WEE1 was associated with a lower incidence of acute rejection (r=0.478; p=0.03). Conclusions IP protection to liver allografts is mediated by reduced expression of genes that have an impact on graft function following transplantation.

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A MULTI-INSTITUTIONAL ANALYSIS OF 235 PATIENTS UNDERGOING OLT FOR HEPATOPULMONARY SYNDROME

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Introduction: Orthotopic liver transplantation (OLT) is the only curative treatment for hepatopulmonary syndrome (HPS). Given the irreversibility of lung disease after the disease process becomes advanced, patients with HPS should have expedited evaluation and prioritization for OLT. The UNOS liver allograft allocation policy has been modified to reflect this, with MELD exception points given to candidates with HPS. Aim: To review the United States experience with OLT for hepatopulmonary syndrome and to determine the current effect of wait-list time on outcomes after OLT in this patient population. Methods: The UNOS database was used to identify 235 patients who underwent primary OLT with an exception for hepatopulmonary syndrome. Demographic, clinical, and disease severity variables were collected and analyzed. Survival was calculated using Kaplan-Meier analyses and compared with log-rank tests. Univariate analysis was performed to determine prognostic factors for patient survival after OLT. Wait-list time was analyzed in detail to determine its effect on patient survival after OLT. Results: The 235 study patients with HPS were 59% male and 76% Caucasian. The median age was 50 years (range: 0-70 years). Compared to patients undergoing OLT for other indications (n=16,828), HPS patients waited longer (median wait time: 152 days vs. 83 days; p=0.001), were less likely to be Status 1 (1% vs. 7%; p=0.001), and had a lower PELD/MELD score (13 vs. 17; p=0.001). Additionally, HPS patients had a lower bilirubin (median: 2.3 mg/dL vs. 3.3 mg/dL, respectively; p=0.001) and a lower creatinine (median: 0.8 mg/dL vs. 1.0 mg/dL, respectively; p<0.005) at transplant. One and 3-year patient survival rates were similar: for HPS patients 88% and 85% and for all other patients 87% and 79% (p=0.5425). In univariate analysis, only creatinine greater than 1.6 mg/dL predicted poor survival (p<0.05). Wait-time analyses showed no effect on patient survival after OLT (all p-values >0.05; Table 1).

Conclusions: Patients undergoing OLT with HPS have less severe liver disease when compared to patients undergoing OLT for other indications. They experience similar survivals to the overall population of patients undergoing OLT. Shortening wait-time further for HPS patients will not currently improve patient survivals.

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ABUNDANCE OF PD-1 AND PD-L1 EXPRESSING CD8 T-CELLS IN THE LIVER MAY BE KEY TO HEPATIC TOLEROGENICITY

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Background: The reason for the high tolerogenicity of the liver is unknown. CD4+CD25+ regulatory T-cells (T-regs) and expression of programmed death-1 (PD-1) and its ligands - members of the CD28/B7 super-family - on activated T, B, and dendritic cells (DCs), are central to the maintenance of tolerance in the experimental animal. Aims: To compare the frequency of regulatory cells and molecules within peripheral blood (PBMC) and hepatic (HMC) mononuclear cells in health. Methods: HMC were isolated from 12 liver perfusates collected from donor livers before allograft implantation, while PBMC were obtained from 9 healthy subjects (median age 31 years, range 21-48; all females). T-regs, PD-1 and PD-L1 expressing cells were evaluated by FACS using anti-CD4, -CD8, -CD25 and -PD-L1 monoclonal antibodies. Up-regulation of PD-1 and PD-L1 was induced by 500 ng/ml lipopolysaccharide (LPS) 4-hour stimulation. Results: CD4/CD8 T-cell ratio was 0.3 for HMC and 1.1 for PBMC. The frequencies of PD-1 expressing cells (10.47±0.19 vs 6.31±0.78, P=0.04) and PD-L1 expressing CD8 T-cells (4.57±1.58 vs 1.38±0.27, P=0.03) and PD-L1 expressing CD8 T-cells (0.36±0.01 vs 0.12±0.03, P=0.05) were higher in HMC than PBMC. Amongst CD8 T-cells, the percentage of PD-1 (14.4±3.29 vs 5.6±1.9, P=0.04) and PD-L1 (1.34±0.05 vs 0.5±0.001, P=0.01) was higher in HMC than PBMC. Frequencies of CD4/PD-1 (2.86±0.74 vs 2.67±0.28), PD-L1 (0.85±0.25 vs 1.35±0.43), BDCA-1/PD-L1 (0.54±0.19 vs 0.48±0.09) and CD4/PD-L1 (0.3±0.09 vs 0.26±0.06) expressing cells were similar in HMC and PBMC. After LPS stimulation, the CD4/CD8 T-cell ratio remained the same.
same in HMC and PBMC; PD-1 expressing cells (11.1±2.62 vs 7.37±1.32, P=0.12); CD8-PD-1 (5.47±1.91 vs 1.0±0.13, P=0.05) and CD8-PD-L1 T-cells (0.72±0.39 vs 0.26±0.03, P=0.09) remained higher in HMC than PBMC; amongst CD8 T-cells, the percentage of PD-1 remained also higher in HMC than PBMC (12.3±3.3 vs 4.95±0.78, P=0.07). The frequencies of all other cells were similar in HMC and PBMC. In contrast, the frequency of Tregs was lower in HMC than in PBMC before (1.43±0.38 vs 4.15±1.02, P=0.03) and after LPS stimulation (2.74±0.54 vs 4.66±0.95, P=0.06). Summary and conclusion: PD-1 and PD-L1 expressing CD8 T-cells are significantly more frequent in the liver than in the peripheral blood. These cells may be more important than conventional Tregs, which are fewer in the liver than in the blood, in imparting tolerogenicity.

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582 STEROID AVOIDANCE IN LIVER TRANSPLANTATION: META-ANALYSIS AND META-REGRESSION OF RANDOMIZED TRIALS

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Steroid use after liver transplantation (LT) has been associated with post-transplant diabetes, hypertension, hyperlipidemia, obesity, and hepatitis C (HCV) recurrence. With the dramatic rise in HCV as an indication for LT, efforts to decrease the recurrence of this disease after LT are critical. We performed meta-analysis and meta-regression of 30 publications representing 19 unique randomized trials that compared steroid-free with steroid-based immunosuppression (IS). There were no differences in terms of death, graft loss, and infection. Steroid-free recipients demonstrated a trend towards reduced hypertension (RR 0.84, p=0.08), as well as statistically significant decreases in cholesterol (SMD -0.41, p=0.001) and CMV infection (RR 0.52, p=0.001). In studies where steroids were replaced by another IS agent, the risk of diabetes (RR 0.29, p<0.001), rejection (RR 0.68, p=0.03), and severe rejection (RR 0.37, p=0.001) were markedly lower in the steroid-free arms. In studies where steroids were not replaced, the rates of rejection were somewhat higher in the steroid-free arms (RR 1.31, p=0.02) and the advantage of reduced diabetes was attenuated (RR 0.74, p=0.2). HCV recurrence was lower with steroid avoidance and, although no individual trial reached statistical significance, meta-analysis was able to demonstrate this important effect (RR 0.90, p=0.03). We conclude that there are no short-term detrimental effects with steroid avoidance but, in light of the heterogeneity and relatively small size of the trials performed to date, we believe that only a large, multi-center trial will define the role of steroid free regimens in LT.

583 LIVER TRANSPLANTATION FOR HEPATIC COMPLICATIONS OF SICKLE CELL DISEASE - FEASIBLE BUT WATCH OUT FOR SPECIFIC MORBIDITY

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Introduction: Patients with sickle cell disease (SCD) often develop severe hepatic complications. The experience with liver transplantation (LT) in patients with SCD and subsequent liver failure is limited. We describe a series of five adult patients transplanted in our center for liver complications associated with SCD between 1992 and 2006. Patients and methods: We reviewed charts of 5 patients (3 M, 2 F; mean age 37.6 yrs [35-47]). Type of SCD was S/S in 2 patients, S/C in 1, S/B in 2. Conclusions: LT is a feasible option for patients with SCD hepatopathy if liver failure is the
major threat to survival. However, LT is associated with unusual morbidity. In order to improve the outcome we therefore suggest a careful pretransplant screening for co morbidities, in particular neurological. Postoperatively, attention should be paid to neurological toxicity of calcineurine inhibitors and induction treatment might be necessary in these patients at high risk of acute rejection. Measures have to be taken to prevent sickle cell crisis post transplant by maintaining hemoglobin S fraction below 30% and total hemoglobin count above 8 g/dl at least during the first six months.

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584 HIGH CIRCULATING REGULATORY T CELL LEVELS IN LONG-TERM STABLE LIVER TRANSPLANTS TREATED WITH CYCLOSPORINE COMPARED TO TACROLIMUS

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Background/Aims: Regulatory T cell (Tregs) has been identified to play a pivotal role in the control of solid organ transplantation. In the setting of adult orthotopic liver transplantation (OLT), little is known about the extent of these cells in long-term stable transplantation recipients with maintenance immunosuppression. Methods: This prospective study enrolled total 85 (58 living donor, 27 deceased donor) consecutive recipients with sustained stable liver function over 2 years (range, 2-13 years) after OLT. We determined the frequency of CD4+CD25+ T cell in peripheral blood mononuclear cell (PBMC) of 85 liver transplantation recipients annually between January 2005 and December 2006. In 10 liver biopsy tissue during study period, Foxp3+ cells were analyzed using immunohistochemistry. We investigated the possible link between immunosuppressive drug and the frequency of CD4+CD25+ T cell. Results: In overall patients, the mean frequency of Tregs was 4.7% (range, 1-17.3). When the cut-off level of high frequency of CD4+CD25+ Treg cell was arbitrarily defined over 6% among CD4+ T cells, 29.4% (25/85) of long-term stable recipients showed high frequency of Tregs cell over 2 years after OLT. The proportion of patients with high Tregs over 6% was 41.1% (12/29) in CsA group, 13.8% (4/29) in FK506 group, especially in living donor liver transplantation (LDLT) (P=0.03). Eight of 10 patients had Foxp3 positive cells in liver biopsy tissue. Conclusions: If 6% Tregs among PBMC was arbitrarily the cut off level of Tregs cell frequency predicting clinical tolerance, approximately one fourth of 85 long-term stable liver transplantation recipients meet this criteria. In addition, this study suggests that cyclosporin A may induce the tolerance than FK506 in long-term stable LDLT patients.

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585 TRENDS IN LIVER TRANSPLANTATION FOR HEPATOCELULAR CARCINOMA IN THE UNITED STATES

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Background: Liver transplantation (LT) is an effective treatment for select patients with hepatocellular carcinoma (HCC). HCC patients who meet criteria are prioritized for liver allocation in 11 US geographic regions. It is unclear if the priority allocated for HCC accurately reflects risk of death or withdrawal from the waiting list. Furthermore, details regarding regional differences in transplantation rates, deaths on the waiting list or waiting list removal for HCC are unknown. Aims: 1) To evaluate transplantation rates and waiting list mortality for HCC patients and non-HCC patients listed for LT, and 2) To compare regional rates of LT, waiting list mortality and waiting list removal in patients with HCC. Methods: Data regarding 71,428 adult deceased donor LT candidates who were on the list between February 2001 and January 2007 were obtained from the United Network of Organ Sharing. Patients with a primary or secondary diagnosis of HCC or those with an HCC exception were identified. Rates of transplantation, waiting list removal and death on the waiting list were expressed as the number of events/patient year according to the pre-MELD period and four periods of MELD priority assigned to HCC patients. Rates of transplantation, regional rates of transplantation for HCC, waiting list removal and waiting list mortality were compared using unadjusted crude rate analysis. Results: LT rates for HCC increased from 0.2/patient year prior to the MELD era to 1.5/patient year in MELD era 4 (P<0.05). HCC LT rates increased during the 4 MELD eras (1.1/patient year-1.5/patient year, P<0.05). LT rates for HCC varied according to UNOS region (range 0.77/patient year in region 5 to 3.68/patient year in region 3, P<0.05). LT rates were higher for HCC patients compared to non-HCC patients (1.25/patient year vs. 0.28/patient year respectively, P<0.05), and death rates on the waiting list were lower in HCC patients (0.08/patient year vs. 0.11/patient year, P<0.05). Waiting list removals were similar for HCC patients and non-HCC patients (0.27/patient year vs. 0.26/patient year respectively, P=NS). Conclusions: 1) Rates of LT for HCC have increased, however, rates vary substantially according to geographic region; 2) Unadjusted waiting list mortality is lower in HCC patients than non-HCC patients despite equivalent drop out rates. Appraisal of the long term impact of allocation policies for HCC is therefore necessary to provide equitable access to LT.

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586 HOMA-IR AND MICROALBUMINURIA ARE ASSOCIATED WITH MICROVASCULAR COMPLICATIONS IN PRE-LIVER TRANSPLANT PATIENTS - A CASE-CONTROL STUDY

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BACKGROUND: Death from cardiovascular disease (CVD) is the most common cause of late mortality in liver transplant (LT) recipients. Many patients with macrovascular complications are not listed for a LT, but the presence and significance of microvascular complications has not been well established in the LT population. Microalbuminuria is an established risk factor for CVD, but its importance in cirrhotics also is not well characterized. METHODS: In this case-control study, we examined the prevalence of microalbuminuria and microvascular complications in 45 cirrhotics with diabetes (DM) and 45 cirrhotics without DM awaiting LT. Patients were matched by age, sex, race and etiology of liver disease. We collected demographic and laboratory data including fasting serum insulin and glucose levels, urine creatinine and microalbumin, macro-
and micro-vascular complications, and other established risk factors for CVD. The homeostatic model assessment for determining insulin resistance (HOMA-IR) was also calculated.

RESULTS: The DM and non-DM groups did not differ in body mass index, tobacco use, presence of hypertension (HTN), or family history (FH) of CVD; the presence of peripheral vascular disease (p=0.041), microvascular complications (p=0.0001), hyperlipidemia (p=0.029), hypertriglyceridemia (p=0.0001), and HOMA-IR (p=0.233) differed between the two groups.

34 (38%) patients had microvascular complications; the patient’s sex, race, FH of DM and CVD, tobacco use and microvascular complications were not significant between the groups. Elevated triglycerides, microalbuminuria, serum creatinine, duration of DM, duration of HTN and insulin requirement were significantly associated with the presence of microvascular complications. 14 (15%) patients had significant microalbuminuria, defined as an albumin/creatinine ratio >0.03. There was a significant association between microalbuminuria and the presence of HTN (p=0.014), hypertriglyceridemia (p=0.008) and a trend toward a higher MELD score (p=0.058). The HOMA-IR was not associated with microalbuminuria, but was associated significantly with the presence of microvascular complications (p=0.015). CONCLUSIONS: At our transplant center, 38% of patients awaiting LT have microvascular complications and 15% have significant microalbuminuria. The HOMA-IR and microalbuminuria are associated with microvascular complications. Microalbuminuria and HOMA-IR could be used to risk-stratify patients for CVD prior to LT and aid in selecting patients who need more rigorous testing for the presence of CVD prior to LT.

GUARDIAN MARKETING SERVICES

UNIVARIATE ANALYSIS

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The following people have nothing to disclose: Michael D. Voigt, Obiora E. Onwuameze, Douglas R. LaBrecque, Warren N. Schmidt, Kyle Brown, Daniel A. Katz, Frank A. Mitros

588 RECURRENT AUTOIMMUNE HEPATITIS AFTER LIVER TRANSPLANTATION AT A CENTER PRACTICING CORTICOSTEROID MINIMIZATION

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Introduction: Since 1997 our center has attempted to minimize corticosteroid (CS) use in all of our liver transplantation (OLT) recipients. In the mid-1990’s we initiated a prednisone-withdrawal protocol for chronic patients. In 1997, we began a series of trials to minimize CS use in all of our de novo liver recipients starting from the time of transplantation. In 1997, CS were administered for only 14 days after transplantation and then in 2000, CS use was reduced to only 3 days after transplantation. In 1997, patients with autoimmune hepatitis (AIH) typically require CS after transplantation, we reviewed our experience in this cohort of patients to determine 1) patient outcome including recurrent disease and 2) long-term requirement for CS use in AIH patients. Methods: From 1988 to 2006, 1,102 OLTs were performed in 1,032 adults at the University of Colorado. Of these, 66 patients (6 %) with AIH received 68 allografts. Recurrence was defined by a clinically worsening exam and histological evidence from biopsy. Bivariate and multivariate analyses were used to evaluate predictors of CS withdrawal. Twelve potential predictors of CS discontinuation were considered: age, gender, presence of inflammatory bowel disease (IBD), type of graft (cadaver or living donor), recurrence of AIH, warm ischemia time, follow up time (time since transplant), and immunosuppressant (IS) regimen (cyclosporine, tacrolimus, sirolimus, azathioprine, and mycophenolate mofetil). Results: Overall survival at 5 years was 91%. The 1, 5 year recurrence-free survival was 86, 59 %, respectively. Risk of recurrent AIH at 1, 3, and 5 years was 12, 36, and 41 %.
respectively. The incidence of disease recurrence was 23 of 66 patients or 34.8%. Of the 23 patients that developed recurrent disease none received a second transplant because of recurrent disease. At time of review 36% of patients were on IS monotherapy verses 56% on two drug and 8% on three IS drug therapy. CS was withdrawn in 50% of patients at time of review. Only two factors on the multivariate analyzes were associated negatively with PD withdrawal: 1) an increasing dose of IS and 2) the presence of IBD. Recurrent disease did not influence CS withdrawal. Conclusions: 1) Outcomes in AIH patients were quite favorable and none of the patients required re-transplantation for recurrent AIH. 2) Using a CS minimization approach, one-half of the patients were able to remain CS-free.

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PRE-TRANSPLANT VARIABLES PREDICT QUALIFY OF LIFE IN LIVER TRANSPLANT RECIPIENTS
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Introduction: With an increasing number of liver transplant recipients living, understanding quality of life issues is essential. Our goal is to specifically identify the pre-transplant predictors of post-transplant quality of life in liver transplant recipients. Understanding how these variables will affect quality of life can help determine if specific interventions will further improve quality of life post-transplant. Methods: Three hundred and eight adult liver transplant recipients seen at UCLA were administered the Short Form 36 and a questionnaire regarding work history and insurance coverage. Variables associated with post-transplant quality of life were studied in a multivariate analysis. Interaction terms were used to examine effect modification.

Results: The mean age of participants (± standard deviation [SD]) was 51 (± 13.99) years. Most patients (50%) were transplanted for viral hepatitis. Post-transplant quality of life domains were associated with a number of pre-transplant factors: Physical Functioning (work hours, gender, ethnicity), Role-Physical (work hours, body mass index, renal failure, ascites, and pay change post transplant), Bodily Pain (ascites, pay change), General Health (encephalopathy, ascites), Vitality (ascites), Social Functioning (disease etiology, ascites), Role-Emotional (ascites), and Mental Health Factors (work hours). Conclusions: Our results demonstrate that pre-transplant factors such as work hours, body mass index, renal failure, and disease etiology predicted impaired quality of life in liver transplant recipients. In addition, pre-transplant manifestations of portal hypertension such as ascites and encephalopathy continue to affect patients even after transplantation. Interventions to modify these pre-transplant variables may further improve quality of life in liver transplant recipients.

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<td>Ethnicity</td>
<td>Physical Functioning</td>
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Disclosures:
The following people have nothing to disclose: Sammy Saab, Bijal Surti, Ayman Ibrahim, Francisco A. Durazo, Steven-Huy B. Han, Hasan Yersiz, Douglas Farmer, R M. Ghobrial, Leonard Goldstein, Myron J. Tong, Ronald Busuttil

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MICROVASCULAR INVASION DOES NOT IMPACT SURVIVAL IN PATIENTS UNDERGOING ORTHOTOPIC LIVER TRANSPLANTATION (OLT) FOR HEPATOCELLULAR CARCINOMA (HCC) WHO HAD PRE-OLT LOCAL THERAPY
Christine A. O’Mahony, Sanjeev Patel, John M. Vierling, Natasha S. Becker, Norman L. Sussman, Norma Flores, Kimberly Talley, Rise Shribling, John A. Goss; Baylor College of Medicine, Houston, TX

BACKGROUND: The increasing incidence of HCC in the U.S. has resulted in the transplantation of a larger proportion of patients with HCC. Despite advances in preoperative staging, the risk factors for recurrent HCC remain poorly defined, especially in patients undergoing pre-OLT transarterial chemoembolization (TACE) and/or radiofrequency ablation (RFA). AIM: To identify prognostic variables for recurrence of HCC post-OLT in patients receiving pre-OLT local therapy. METHODS: We conducted a retrospective review of all patients transplanted with HCC at our institution from February 1997-May 2006. Patient survival rates were determined using the Kaplan-Meier product-limit estimate. Demographic, tumor, and pathologic characteristics were tested for their prognostic significance using univariate and multivariate analyses. RESULTS: There were 131 patients with HCC identified among 715 adult OLT recipients in this time period with a mean follow-up of 35 months. 88% of the patients underwent pre-OLT TACE, while 48% of the patients underwent pre-OLT TACE combined with RFA. 12% (15/131) of the patients did not receive pre-OLT local therapy. Of these 15 patients, 6/15 tumors were discovered incidentally, 3/15 were transplanted prior to pre-OLT local therapy, 2/15 were too ill for TACE, and 4/15 were unknown. The overall patient survival was 96%, 89%, and 85% at 1, 3, and 5 years, respectively. 9/131 patients experienced recurrence of the tumor. The average time for recurrence was 1.5 years with all recurrences occurring by 3 years post-OLT. As shown in table 1, a total of 23 tumors had microvascular invasion. Only 2 of these tumors recurred. 3 tumors exhibited macrovascular invasion, with no recurrences. 5/20 patients with tumors >5cm experienced a recurrence (p<0.05) while none of the 13 patients with <3 tumors had a recurrence. Only 2/11 of the poorly differentiated tumors recurred. Tumor size > 5 cm was associated with a significant increase in the risk for recurrence (HR 2.44, p<.05) Microvascular invasion, macrovascular invasion, >3 tumors, pre-OLT TACE and/or RFA,
and tumor grade did not predict tumor free survival (Table 1).

**CONCLUSION:** Patients with HCC can undergo OLT with the expectation of excellent outcomes. Tumor size > 5cm significantly increased the risk of recurrence. In contrast, neither microvascular invasion, nor macrovascular invasion predicted recurrence in these patients undergoing pre-OLT local therapy.

**Table 1**

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<th>Micronodular Invasion No.23</th>
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<th>Total # # Tumors≤5 No.13</th>
<th>Poorly Differentiated No.11</th>
<th>TACE N=115</th>
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<td>N=2 (Recurrence N=15)</td>
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Disclosures:
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**OCCURRENCE OF ADRENAL INSUFFICIENCY AFTER LIVER TRANSPLANTATION: RISK FACTORS FOR AN UNEXPECTEDLY FREQUENT COMPLICATION**

**Elisa Fumolo¹, Elisabetta Rossi¹, Silvia Fumagalli¹, Davide Bitetto¹, Ezio Forisari¹, Carlo Fabris¹, Edmondo Falletti¹, Rosalba Minisini², Mario Pirisi², Pierluigi Toniutto¹.¹Medical Liver Transplant Unit, Azienda Ospedaliero-Universitaria, Udine, Italy; ²Dpt of Clinical & Experimental Medicine, University of Eastern Piedmont “A. Avogadro, Novara, Italy**

Introduction. Immune-suppressive therapy after liver transplantation (OLT) is based on the use of calcineurin inhibitors in association with corticosteroids. This can lead to the development of adrenal insufficiency after the cessation of prolonged corticosteroid administration. This study aimed to investigate the prevalence and associated factors to the development of adrenal insufficiency after OLT. Methods. Eighty seven patients who underwent OLT were studied. Adrenal function testing was performed when the patients were taking 5 mg of prednisone daily for at least one week. The adrenal function was evaluated by means of the 250 µg standard corticotrophin test. Serum cortisol levels were assessed basally, 30’ and 60’ after corticotrophin administration; the test was defined pathological when serum cortisol did not double the basal value and did not reach at least 20 µg/dL. Results. The corticotrophin test was found to be pathological in 23/87 patients, more frequently in patients who had taken a total dose of prednisone >1350 mg (19/52 Vs 4/35, p<0.01) and in those who were treated with prednisone for >105 days (17/49 Vs 6/38, p<0.05). Furthermore, a pathological test was found to be associated at a level of p<0.10 with recipient male gender, recipient body mass index (BMI) ≤25 Kg/m2 and the alcoholic aetiology of liver disease. No significant association was found between corticotrophin test and duration of ischaemia and of transplant operation, number of blood transfusions, days of stay and presence of septic episodes in intensive care unit. Total dose of prednisone (p<0.01), BMI ≤25 Kg/m2 (p<0.05) and alcoholic aetiology of liver disease (p<0.05) were the variables independently associated with the presence of a pathological corticotrophin test. No significant difference was detected between patients with normal and pathological test regarding the parameters classically considered to be associated with adrenal insufficiency. Conclusions. Adrenal insufficiency is frequent in liver transplanted patients and cannot be suspected on the basis of the common clinical and laboratory pertinent parameters. Thus it may be suggested to perform the corticotrophin test in all liver transplanted patients especially in those malnourished patients with alcoholic liver disease who were treated with higher doses of prednisone.

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**RENAL DYSFUNCTION FOLLOWING LIVER TRANSPLANTATION: A COMPARISON OF NOVEL RENAL BIOMARKERS IN THE POST TRANSPLANT PERIOD**

**Andrew J. Portal¹, Matthew Bruce¹, Mark Austin¹, Constantine J. Karvellas¹, Mark McPhail¹, Funmi Awopevi¹, Roy Sherwood¹, John G. O’Grady¹, Nigel Heaton¹, Paolo Muiesan¹, Julia Wendon¹, Michael Heneghan¹, Institute of Liver Studies, Kings College Hospital, London, United Kingdom; ²Department of Biochemistry, Kings College Hospital, London, United Kingdom**

Introduction Renal dysfunction is a common occurrence both before and after liver transplantation (LT). Because standard markers of renal function are poor reflections of true glomerular filtration (GFR) in liver disease patients, we assessed the ability of two novel markers, Cystatin C (CysC) and Neutrophil Gelatinase Associated Lipocalin (NGAL), to predict renal impairment in 80 patients following LT. Methods CysC and NGAL were collected within the first 24 hours of following LT and compared with standard markers of renal function up to 3 months post transplantation. CysC was measured by nephelometry and NGAL by sandwich ELISA (AntibodyShop®). Biochemical and physiological data were collected prospectively and patients were followed for 3 months. Renal dysfunction was classified using either need for renal replacement therapy (RRT) or by the Chronic Kidney Disease (CKD) staging system. Results are expressed as median and interquartile range (IQR). Results: 61 patients underwent LT for chronic liver disease with 19 undergoing LT for acute liver failure or urgent retransplantation. Median pre-operative serum creatinine (sCr) was 99 µmol/L (81-129); MELD 15.5 (12-27); Estimated GFR (eGFR) 66(50-81). Admission NGAL correlated with day 7 sCr (r = 0.6, p <0.001) as did to CysC (r =0.6, p=0.001). There was an association between higher admission NGAL and longer intensive care stay (r=0.5, p<0.01). Both CysC and NGAL were significantly higher in those patients requiring haemofiltration on day 1 when compared with those not needing RRT [CysC 2.84mg/L (2.26-3.43) vs 1.67mg/L (1.23-2.18), p=0.001; NGAL 420ng/mL (290-500) vs 158ng/mL (100-260), p<0.001]. These findings persisted up to day 5 post LT, and admission NGAL was significantly higher in those that had renal impairment at day 14 (classified by eGFR <60). Using receiver operator characteristic curve analysis, area under the curve (AUC) was higher for NGAL when compared with CysC, sCr and eGFR at both day 7 and day 14 post LT, especially in patients with lower eGFRs. Admission NGAL was better at predicting more severe renal impairment on day 7 post LT than admission sCr (NGAL >347ng/ml AUC 0.94, 0.85 – 0.979, p<0.0001, sensitivity 100%, specificity 86%; sCr > 125µmol/L AUC 0.82 (0.715 – 0.897), p=0.001 sensitivity 87.5%, specificity 66.7%). By day 14, all markers performed less well than at day 7 although NGAL retained high sensitivity and specificity (86%, 80% respectively). Conclusion. Cystatin C and NGAL are good predictors of renal dysfunction following LT.
Further studies are needed to assess their clinical utility, especially in relation to manipulation of nephrotoxic immunosuppression.

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GB VIRUS-C INFECTION IS ASSOCIATED WITH BETTER 10-YEAR-SURVIVAL OF LIVER TRANSPLANT RECIPIENTS

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Background & Aims: We earlier demonstrated a beneficial influence of the GB virus C (GBV-C, or hepatitis G virus) on the long-term course of HIV infection. An influence of GBV-C on the short-term survival of liver transplant recipients was not observed. Here we describe the long-term outcome of patients with GBV-C after liver transplantation (OLT).

Methods: We studied overall survival rates of 200 patients transplanted at our center between 1992 and 1996. GBV-C envelope antibodies (anti-E2) and RNA were tested directly before transplantation. 63/200 patients had anti-E2, 17 patients were viremic and in 8 patients both GBV-C antibodies and RNA were found. There were no differences regarding diagnosis, age or sex between the different groups. Survival rates were assessed in 2006, thus 10 years after OLT, by using Kaplan-Meier and Cox’s regression analysis.

Results: Overall 1-, 3- and 10-year patient survival rates were 75%, 69% and 56%. Mean patient and graft survival was 7.8±5.5 and 7.1±5.5 years, respectively. A worse outcome was found for patients requiring retransplantation (p<0.0001) and those with tumor disease (p<0.0001). If patients surviving at least 3 years were analysed, those who were GBV-C RNA positive had a significantly longer survival than RNA-negative patients (figure 1; p<0.03). In Cox’s regression including patient age and sex, need of retransplantation and presence of tumor disease, GBV-C viremia (p=0.006) and absence of retransplantation (p=0.04) were independently associated with patient survival.

Conclusions: Patients with active GBV-C infection had an improved long-term outcome after liver transplantation, actually in line with similar data from renal transplant recipients.

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BLOOD GROUP A2 DONORS FOR BLOOD GROUP O RECIPIENTS IN PEDIATRIC OLT: AN 18-YEAR CASE-CONTROL MULTI-INSTITUTIONAL ANALYSIS

Natasha S. Becker, Thomas A. Aloia, Joel A. Rodriguez, Rise Stirling, John M. Vierling, Norman L. Sussman, Christine A. O’Mahony, John A. Goss; Michael E. DeBakey Department of Surgery, Baylor College of Medicine, Houston, TX

Introduction: The shortage of pediatric liver allografts has necessitated expansion of the donor pool, including the utilization of blood group A2 liver allografts for blood group O recipients (A2-O). Aim: To determine pediatric patient and allograft survival rates after A2-O orthotopic liver transplantation (OLT).

Methods: The UNOS database for the years 1987-2005 was used to identify 23 pediatric patients who underwent A2-O OLT. These patients were matched 2:1 with controls who underwent ABO-Identical (ABO-I) OLT. Matching variables included: recipient age, donor age, ventilator status, Status 1 categorization, PELD/MELD score, donor type (living vs. deceased), indication for transplantation (acute liver failure vs. other), and era of OLT (prior to 2000 vs. 2000-2005). Similarity of cases and controls was confirmed through chi-squared (for categorical variables), and Mann-Whitney-U tests (for continuous variables). Survivals were calculated using Kaplan-Meier analysis and compared with log-rank tests. Results: The study cohort included 23 A2-O patients. Median recipient age was 2 years (range 0-18 years), 65% of patients were male, and 52% were Caucasian. Median donor age was 3 years (range: 0-56). Seven study patients were ventilated at time of OLT (30%) and 13 were Status 1 (57%). Median PELD/MELD score was 20 (range: 15-22). One patient received a living donor allograft (4%). Acute liver failure was the final diagnosis in 7 OLTs (30%), and 14 OLTs (61%) were performed prior to 2000. 46 controls matched for all eight variables were selected from the 5,904 patients transplanted with ABO-I liver allografts. Similarity of cases and controls was confirmed (all comparisons: p > 0.50). One, 3-, and 5-year patient survivals were 71%, 71%, and 64% for A2-O cases; and 84%, 82%, and 75% for ABO-I controls (p=0.4587; Figure 2).
were 67%, 62%, and 56% for A2-O cases, and 80%, 78%, and 71% for ABO-I controls (p=0.1802). One A2-O patient died within 30 days of OLT, yielding a 30-day operative mortality of 4.3%. This was similar to the 7% 30-day mortality for ABO-I patients (p=0.511). Six patients in the A2-O group and 8 patients in the ABO-I group died after 30 days. Analysis of cause of death showed no difference in the percentage of patients dying from graft failure (33% vs.13%, p=0.347) or dying from infection (17% vs. 25%, p=0.707). Discussion: In pediatric patients, A2-O OLT yields patient and allograft survivals similar to those experienced after ABO-I OLT. The use of this alternative will allow for better utilization of available donor organs in the current era of liver allograft shortage.

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The following people have nothing to disclose: Natasha S. Becker, Thomas A. Aloia, Joel A. Rodriguez, Rise Stribling, John M. Vierling, Norman L. Sussman, Christine A. O'Mahony, John A. Goss

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RENAL FAILURE FOLLOWING LIVER TRANSPLANTATION

Alex Aspinall1,2, Robert J. Hilsden1, Bridget K. Gunson2, Barbara L. Aspinall1,2, James M. Neuberger1,2; 1UCMC Area 2, Division of United Kingdom

Background: Following liver transplantation there is long term morbidity and high mortality due to renal failure. Risk factors associated with renal failure include female gender, age, calcineurin use, diabetes and hypertension. While this provides the clinician with a context in which to be vigilant for renal failure, a predictive tool would be more useful. Methods: Baseline and post-transplant data was analyzed from the University of Birmingham liver transplantation database for 587 consecutive patients 1998-2004. Risk factors for the development of chronic renal failure were recorded. The MDRD creatinine clearance was calculated up to 24 mo after transplantation. Renal impairment was defined as a CrCL <60 and renal failure as <30 mL/min or the need for dialysis. Results: Two yr after transplantation, 2.3% of patients had renal failure and 65.2% had renal impairment. Increasing age was a risk factor for renal failure (OR = 1.08, CI 1.05-1.11) and male gender was protective (OR = 0.29, CI 0.17-0.52). When compared to patients transplanted for acute (fulminant) liver disease, patients with chronic liver diseases had greater likelihoods of developing renal failure (OR for alcohol, hepatitis B/C, PBC and other diseases, 2.67 CI 0.82-8.69), 1.94 CI 0.63-5.97, 5.78 CI 1.84-18.2), and 1.4 CI 0.49-4.06 respectively. Importantly, 89.3% of patients with a CrCL of <60 mL/min 6 mo after transplantation continued to have renal impairment 2 yr after transplantation (OR 10.20, CI 5.9-18.2) (Graph). Conclusions: Our data confirm previously identified risk factors associated with developing renal failure. Six mo after transplantation a CrCL <60 mL/min is predictive of future renal impairment and is an important early tool for clinicians treating liver transplant recipients to use in decreasing long term morbidity and mortality following liver transplantation.

Disclosures:
The following people have nothing to disclose: Alex Aspinall, Robert J. Hilsden, Bridget K. Gunson, Barbara L. Aspinall, James M. Neuberger
liver function tests and with portal hypertension. It seems to occur more commonly in patients with hypertension, diabetes and renal insufficiency. Liver biopsy is required to make the diagnosis and it should be included in the differential of causes of portal hypertension post solid organ transplant.

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The following people have nothing to disclose: Shahid M. Malik, David A. Sass, Jaideep Behari, Rajagopal Chadalavada, A Jake Demetris, Jawad Ahmad

597 EVALUATION OF PATIENTS WITH ALCOHOLIC LIVER DISEASE FOR LIVER TRANSPLANTATION

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Alcoholic liver disease (ALD) is a common indication for liver transplantation (LT) in the United States. Many transplant centers require patients with ALD to be abstinent for 6 months before being listed for LT. Evidence for the ‘6 month rule’ to prevent relapse is conflicting and some patients too sick to wait 6 months may die before being listed for LT. Alternative models to evaluate patients with ALD for LT are lacking. Since 2003 we have evaluated patients with ALD for LT by stratifying them into relapse risk categories (low/medium/high) depending on their probability of relapse after LT based on predetermined criteria such as duration of abstinence, completion of a treatment program, family history of alcoholism and other factors derived partly from the model of Gish et al. AIM. To determine the feasibility and outcome of evaluating patients with ALD for LT by stratifying them into relapse risk categories. METHODS. Patients evaluated for LT between September 2003 and September 2006 meeting DSM IV criteria for alcohol abuse or dependence were included in the study. Patients were stratified into relapse risk categories following addiction psychiatry assessment. Low and medium risk patients were generally considered suitable for listing and high risk patients were deferred or declined until they fulfilled the requirements of the addiction psychiatry team. In addition to the risk score, final listing was based on other medical and social conditions as well. We undertook a chart review to determine the psychosocial and demographic characteristics of the patients and their outcome after evaluation for LT. RESULTS. 220 patients were included (139 high risk, 50 medium risk, 31 low risk) in the study. During a median follow-up period of 11 months (range 0-41) 54% (n=119) of the patients were listed, comprising 67% of the low risk, 82% of the medium risk and 41% of the high risk patients. Of these listed patients 42% (n=50) underwent LT, 10% (n=12) died, 3% (n=3) was lost to follow-up. 45% (n=54) are waiting for LT. Among the 101 patients not listed, 35% died and 25% were lost to follow-up. During a median post-transplant follow-up of 18 months (range 0.4-39 months) 12% (67% were high risk) of patients relapsed to alcohol use. CONCLUSION. 1. Evaluating patients with ALD for LT by using this relapse risk stratification system is feasible with greater than 50% of evaluated patients being listed and may prevent exclusion of candidates too sick to wait 6 months before being considered for LT. 2. Further studies are needed to determine ways to prevent death and loss to follow up of patients with ALD following initial evaluation for liver transplantation.

Disclosures:
The following people have nothing to disclose: Elske Sibma, Gregory Gores, Russell H. Wiesner, Walter Kremer, Sheila G. Jowsey, Terry D. Schneekloth, Julie K. Heimbach, Michael R. Charlton, Charles B. Rosen, KV Narayanan Menon

598 HEPATOCELLULAR CARCINOMA (HCC) IN PATIENTS WITH HCV VERSUS NON-HCV CIRRHOSIS: GRAFT AND PATIENT SURVIVAL AFTER LIVER TRANSPLANTATION (LT) IN PRE-MELD AND MELD ERA

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HCC in the presence of HCV may have a worse outcome after LT because of the confounding effects of recurrent HCV and higher HCC recurrence. Our objective was to assess the survival of patients with HCC in those with HCV and non-HCV cirrhosis using UNOS database from 1994-2006. Methods: Patients with HCC were stratified into HCV (HCC-HCV) and non-HCC (HCC-non HCV) and also into MELD and pre-MELD era because of the potential impact of MELD on outcomes. In the pre-MELD era, there were 1,886 HCC patients (639 HCV, 1,079 non-HCV, HCV status unknown in 168) and during MELD era, there were 798 patients (283 HCV, 431 non-HCV and 84 unknown). For the purpose of this study, we analyzed patients with unknown HCV status separately (not shown). Comparative analysis was made of HCV (HCV) and non-HCV (non-HCV) patients without HCC after stratification as discussed above. We calculated unadjusted graft and patient survival rates (%) at 1, 3 and 5 years for pre-MELD and 1 and 3 years for MELD patients. Results: Pre-MELD era – graft survival rates for HCC-non HCV was higher than HCC-HCV patients at 1-yr (77.7 vs. 73.8), 3-yr (63.3 vs. 58.2) and 5 years (56.8 vs. 48.7); pre-MELD era patient survival was similar at 1-yr (80.7 vs. 79.4) and 3-yr (66.2 vs. 64.5), and higher at 5-yr (59.9 vs. 55.2) for HCC-non HCV. Pre-MELD era graft survival rates for patients without HCC in non-HCV at 1-yr (80.6 vs. 79.7), 3-yr (74.4 vs. 69.3) and 5-yr (69.2 vs. 61.8) was also higher than HCV patients as expected; similar trend was also seen for patient survival at 1-yr (85.2 vs. 84.1), 3-yr (79.7 vs. 74.5) and 5-yr (74.8 vs. 67.3). MELD era – graft survival rates were similar for HCC-non HCV and HCC-HCV patients at 1-yr (83.7 vs. 82.7), but was higher at 3-yr (72.2 vs. 65.7); MELD era patient survival was similar at 1-yr (87.1 vs. 86.6) and was higher at 3-yr (73.7 vs. 71.9). MELD era graft survival rates for patients without HCC in non-HCV at 1-yr (82.1 vs. 82.7) and 3-yr (74.5 vs. 69.7), and patient survival 1-yr (86.7 vs. 84.3) and 3-yr (81.3 vs. 76.5) showed similar trend. Survival differences showed similar trends as shown in the table. Conclusions: There has been an improvement in 3-year graft (8.9% for non-HCV, 7.5% for HCV) and patient (14.8% for non-HCV, 13.1% for HCV) survival in MELD era compared to pre-MELD era for HCC patients with similar trends in survival rates in HCV and non-HCV patients with and without HCC suggesting that lower survival in patients with HCV and HCC is mainly due to the confounding effect of HCV.

<table>
<thead>
<tr>
<th></th>
<th>Graft Survival Difference (%)</th>
<th>Patient Survival Difference (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Year</td>
<td>1 yr</td>
<td>3 yr</td>
</tr>
<tr>
<td>Pre-MELD Non-HCV vs. HCV (no HCC)</td>
<td>81.3 vs. 79.4</td>
<td>74.5 vs. 67.3</td>
</tr>
<tr>
<td>Non-HCV vs. HCV (no HCC)</td>
<td>80.7 vs. 79.4</td>
<td>66.2 vs. 64.5</td>
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<tr>
<td>HCV vs. HCC</td>
<td>83.7 vs. 82.7</td>
<td>74.5 vs. 69.7</td>
</tr>
</tbody>
</table>

Disclosures:
The following people have nothing to disclose: Paul J. Thuluvath, Dorry L. Segev
EFFECTIVENESS OF PERCUTANEOUS LASER ABLATION AS BRIDGE TREATMENT TO LIVER TRANSPLANTATION IN CIRRHOTIC PATIENTS WITH HEPATOCELLULAR CARCINOMA

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Percutaneous laser ablation (PLA) is an effective ablative procedure of hepatocellular carcinoma (HCC) and metastatic liver lesions. To the best of our knowledge, this procedure has never been used as bridge treatment in cirrhotic patients with HCC listed for liver transplantation (LT). In this study, the data of 10 patients (all males, mean age 52.8 yrs, Child-Pugh class A 6 cases, B 4 cases, C 1 case) with HCC complicating liver cirrhosis treated by PLA and undergoing LT between 2002 and 2006 were retrospectively reviewed. At the moment of the evaluation for LT, all the patients but one had no more than 3 nodules each smaller than 3 cm, and 6 of them showed a single tumor nodule. Twelve biopsy-proven HCC nodules (mean diameter 2 cm, range 1.5-3 cm) underwent PLA carried out by inserting 300 nm optical fibers through 21-g needles (from two to four) positioned under US guidance into the target lesions. A continuous wave Nd:YAG laser operating at a wave length of 1,064 nm was used. Seven tumors underwent a single PLA session. No procedure-related major side effects were recorded. Transarterial chemoembolization (TACE) prior to LT was performed in three tumors resulted to be incompletely ablated on imaging studies. The mean waiting time to LT after PLA was 1.1 months. Complete necrosis at histological examination of the explanted livers was found in 7 nodules (58%, all treated exclusively with PLA), partial necrosis > 50% in 3 nodules (25%, 2 treated with a combination of PLA and TACE), partial necrosis < 50% in 1 nodule, absent necrosis in 1 nodule (treated with both PLA and TACE). In the latter case the final histological diagnosis was that of an hepatic yolk sac tumor. Mean follow-up length after LT was 22 months: 5 patients are alive without tumor recurrence. The remaining 5 patients died; in only one case HCC recurrence was detected and resulted to be the cause of death. Our preliminary data suggest that PLA is an effective bridge treatment in cirrhotic patients with HCC smaller than 3 cm waiting for LT.

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FOLLOW-UP OF PATIENTS AFTER LIVER TRANSPLANTATION USING DOMINO GRAFTS

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Introduction: In recipients of domino grafts retrieved from patients with hereditary amyloidosis symptoms of the disease transmitted by the graft have been reported. Therefore, we implemented a special follow-up in these patients. The results are reported herein. Patients and Methods: In our center 17 patients underwent a domino liver transplantation (DLT) since 09/98. The longest follow-up was 9 years after transplantation (median 31 months, range 6 - 107 months). The domino-transplanted patients were enrolled in a special post-transplantation protocol, beginning 3 months after transplantation. In addition to physical examination and blood tests, 5-day electrocardiogram (ECG), 24-hour blood pressure measurement, echocardiography, cardiac MRI, cardiac electrophysiological examinations, rectoscopy with microendoscopic technique and biopsy, and extensive neurological examination including quantitative sensory testing, autonomic testing (sympathetic skin response, heart rate variability, orthostatic function) as well as sensory and motor electroneurography were performed. This workup was repeated 3 years and 5 years after DLT and thereafter annually. Results: Six of the 17 transplanted patients died after median 20 months (range 6 – 39 months), due to recurrence of HCC (n=4), recurrence of hepatitis C (n=1) and cardiac arrest (n=1). Since the fatal cardiac complication occurred 3 years after transplantation, deposition of amyloid is unlikely in this short time. So far none of our surviving 12 patients shows a definite onset of amyloidosis after DLT, with a follow-up of the surviving patients between 6 months and 9 years. After 5 years one patient developed isolated neurologic symptoms presenting as painful sensory neuropathy with predominant loss of small nerve fibers, however without any motor or autonomic signs, obviously caused by calcineurin inhibitors. All cardiological examinations were normal. All blood tests were within the common range. Conclusion: From our data, there is no evidence that patients after DLT develop clinical signs of amyloidosis even if the follow-up time is limited. In the face of organ shortage, the use of domino grafts for elderly patients seems to be justified so far. Nevertheless, a close follow-up including physical, neurological, cardiologic, and laboratory examinations is deemed advisable in order to exclude a manifestation of the disease in the recipient of the domino graft and to learn more about the pathophysiology of amyloidosis.

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601 ASSESSMENT OF LIVER TRANSPLANT CANDIDATES BY AN ADDICTION MEDICINE SPECIALIST: A PROSPECTIVE STUDY

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It is important, but not always performed, to ascertain an alcohol history in all patients undergoing liver transplantation (LT). However, the points of view of addiction specialists and LT specialists differ about their consideration of alcoholic disease. Aim: Assessment of alcohol behavior in LT candidates by an addiction specialist in order to appreciate this specific approach. Methods: All patients were seen during the pre-transplant evaluation by an addiction specialist. The diagnostic systems used to assess alcohol disorders were WHO classification, DSM-IV and Babor’s classification. The following characteristics were considered as predictive of severe alcohol relapse after LT: alcohol dependence, young age, number of withdrawals, family history of alcohol abuse, associated drugs and alcoholism. During the evaluation, a brief intervention about alcohol disorders was performed, and a specific management of alcohol abuse was proposed, not imposed, to all patients with predictive factors of severe relapse after LT. Results: 80 patients have been studied. The primary indication for LT was alcoholic liver disease (ALD) in 39 patients, HCV in 20 patients, HBV in 11 patients, others in 10 patients. Abnormal alcohol behavior was present in 57 of the 80 patients: the 39 ALD patients, 13 of 20 HCV patients, 5 of 11 HBV patients. Among these 57 alcohol abusers, 28 were excessive drinkers without dependence and 29 were alcohol-dependent: 19 of 39 ALD patients, 6 of 13 HCV patients, 4 of 5 HBV patients. Predictive factors of severe alcohol relapse after LT were found in 19 patients, 17 of alcohol-dependent patients and 2 of excessive drinkers. Only one of these 19 patients had accepted a pre-LT management of alcohol disorder. During the follow-up, 3 patients died, 1 was withdrawn from the waiting list, 41 are still on the waiting list and 35 have been transplanted: 20 ALD, 8 HCV, 7 others. With a mean follow-up of 6 months (3-16), no severe relapse was observed. Conclusion: A specific assessment of LT candidates by an addiction specialist has detected 71 % of alcohol abusers and 36 % of alcoholic dependant patients. Moreover, among patients with viral liver disease, 58 % were alcohol abusers and 32 % were alcohol-dependant. Predictive factors of severe alcohol relapse after LT were found in 23,7 % of patients. Among these, the majority did not adhere to a rehabilitation program. The impact of the pre-LT brief intervention is under evaluation. These preliminary results are in favor of the intervention of an addiction specialist during the evaluation of LT candidates.

Disclosures:
The following people have nothing to disclose: Hélène Rigole, Pascale Perney, Michaël Bismuth, Francis Navarro, Dominique Larrey, François Blanc, Georges-Philippe Pageaux

602 THE READABILITY OF HEALTH EDUCATION MATERIALS FOR LIVER TRANSPLANT PATIENTS

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The listing process for liver transplantation (LT) is complex and requires patients to comprehend their disease and aspects of LT care. However, millions of adult Americans are illiterate and may not benefit from printed LT educational tools. Laws require many public documents to be written at an eighth-grade level or lower to ensure readability. Aims: (1) To determine the readability of printed LT educational brochures, (2) to quantify educational levels of patients listed for LT, and (3) to determine if demographic factors are associated with patient educational level. Methods: We used the United Network for Organ Sharing database to identify U.S. citizens listed for LT from 1987 to 2005. Chi-squared analysis and Hests determined the relationship between patient demographic factors and educational level. Readability of brochures was determined by using computer software that measured Flesch Reading Ease scores and Flesch-Kincaid grade levels. Reading ease scores range from 0 to 100, with lower scores indicating increased difficulty in readability. Results: Of 60,222 patients, 22,533 (37.42%) were female and 37,689 (62.58%) were male; 3043 (5.05%) had less than high school education; 57,179 (94.95%) had a high school education or above. Patients with less than high school education were more likely to be female, of older age, have Medicare or Medicaid, and be of Hispanic, Asian, or other race (table). Calculated Flesch-Kincaid grade levels and Reading Ease scores for brochures were: American Society of Transplantation: Getting a New Liver (6.2, 74.7), American Liver Foundation (ALF) Liver Transplant (6.6, 71.9), National Institutes of Health: What I Need to Know About Liver Transplantation (7.2, 67.5), American Liver Society LT brochure (7.3, 67.3), and ALF Facts on Liver Transplantation (11.4, 42.1). Conclusions: Most LT patients have completed at least the eighth grade and are likely to understand LT educational brochures. However, the LT evaluation process may exclude many patients with poor literacy skills, and current brochures may be too advanced for those who are beginning to consider LT.

<table>
<thead>
<tr>
<th></th>
<th>Less than high school</th>
<th>High school or above</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender (%) Male Female</td>
<td>1702 (4.52) 1341 (5.95)</td>
<td>35,987 (85.48) 21,192 (45.04)</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Age (mean ± SD)</td>
<td>55.03 ± 9.58</td>
<td>49.79 ± 10.16</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Race (%) White Black Hispanic Asian Other</td>
<td>1456 (3.15) 151 (3.26)</td>
<td>44,176 (86.85) 4453 (86.74)</td>
<td>&lt; 0.0001</td>
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<td>Primary Pay (%) Private Medicare Other government/Charity Other</td>
<td>1269 (2.53) 135 (0.28)</td>
<td>38,983 (68.63) 7523 (75.19)</td>
<td>&lt; 0.0001</td>
</tr>
</tbody>
</table>

Disclosures:
The following people have nothing to disclose: Noel M. Lee, Andrew Muir, Carla Brady

603 LONG TERM OUTCOMES OF LIVER TRANSPLANTATION IN A HIGH-MELD COHORT

Jason Lee1,2, Maximillian Lee1, Lynn Shapiro1, Alice L. Yang1, Alex S. Lapasaran1, Sanah Parvez1, Ahmad Kamali3, Emmet B. Kefee1, Charles O. Esquivel1, Aijaz Ahmed1; 1Liver Transplant Program, Stanford University School of Medicine, Stanford, CA; 2Center for Primary Care and Outcomes Research, Stanford University School of Medicine, Stanford, CA; 3Division of Gastroenterology, Santa Clara Valley Medical Center, San Jose, CA

BACKGROUND: MELD scores predict both pre-transplant and post-transplant mortality. Diabetes mellitus has also been shown to predict post-transplant mortality, but the relative importance of these two factors remains unknown. METHODS: We reviewed all adult liver transplants performed at our institution between February 1995 and June 2006. Of 530 transplants performed, information regarding MELD score and the pres-
ence or absence of diabetes was available for 431. Patients with acute liver failure (n=17), those undergoing kidney-liver transplant (n=28), and those who died prior to discharge (n=51) were excluded. The remaining 370 patients were divided into four groups, based on MELD score (<30 or ≥30) and the presence or absence of diabetes. RESULTS: As expected, survival was poorer in the high MELD group (log-rank statistic 23.2, p<0.001). However, patients with a MELD score ≥30 and without diabetes had a similar outcome as patients with a MELD score less than 30 and with diabetes (log-rank statistic 0.51, p =0.47). The outcome was poor in patients with diabetes and MELD ≥30, but the observation in this group was limited by a small sample size and needs to be studied further. CONCLUSIONS: Our results suggest that patients with MELD score ≥30 in the absence of diabetes have a favorable post-transplant outcome. The presence or absence of diabetes appears to limit the reliability of MELD score as an allocation system for liver transplantation and its ability to predict post-transplant survival outcomes. Further validation studies are needed to assess the impact of diabetes on MELD score.

<table>
<thead>
<tr>
<th>MELD ≤ 30</th>
<th>MELD ≥ 30</th>
</tr>
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<tbody>
<tr>
<td>Number of Patients</td>
<td>254</td>
</tr>
<tr>
<td>Mean Age (yr)</td>
<td>50.1</td>
</tr>
<tr>
<td>Mean BMI (kg/m²)</td>
<td>27.1</td>
</tr>
<tr>
<td>Hepatitis C (%)</td>
<td>48</td>
</tr>
<tr>
<td>Overall Survival (%)</td>
<td>95</td>
</tr>
<tr>
<td>Mean Follow-up (yr)</td>
<td>4.99</td>
</tr>
</tbody>
</table>

Disclosures:
The following people have nothing to disclose: Jason Lee, Maximilian Lee, Lynn Shapiro, Alice L. Yang, Alex S. Lapazaran, Samah Parvez, Ahmad Kamal, Emmet B. Keefe, Carlos O. Esquivel, Ajaz Ahmed

### 604 CORONARY ARTERY DISEASE SCREENING IN LIVER TRANSPLANTATION CANDIDATES

Daniela Fili¹, Giovanni Vizzini¹, Domenico Biondo², Riccardo Volpes¹, Giada Pietrosi¹, Marzia Montalbano¹, Ioannis Petridis¹, Adele D'Antoni¹, Cesare Scardulla¹, Cesar Hernandez Baravoglia², Angela Luca³, Bruno Gridelli³, ¹Hepatology, ISMETT, UPMC Italy, Palermo, Italy; ²Surgery, ISMETT, UPMC Italy, Palermo, Italy; ³Office of Research Health and Biomedical Sciences, ISMETT, UPMC Italy, Palermo, Italy

Background/Aim: In order to identify coronary artery disease in patients with end stage liver disease candidates to liver transplantation (LT), pre LT cardiac evaluation and instrumental screening for coronary artery disease is usually performed. We reviewed through a retrospective analysis accuracy and utility of our coronary artery disease screening protocol in LT candidates. Methods: LT candidates undergo a detailed clinical history with detection of coronary artery disease clinical predictors and definition of risk profile. Patients with medium/high risk profile (1 or more risk factors) perform stress test and, if positive, selective coronary angiography (SCA). Results: Between July 1999 and January 2006, 627 patients with end stage liver disease due to liver cirrhosis were evaluated for LT. 569 were listed and 235 transplanted. 16 patients had a previous diagnosis of coronary artery disease (2.5%). Risk profile analysis showed that 360 male were aged more the 49 years (58.7%), 83 female were aged more than 54 years (13.2%); 229 patients (36.5%) were smokers, 161 (25.7%) had diabetes, 71 hypertension (11.3%), 83 obesity (13.2%), 20 hypercholesterolemia (3.2%), 75 family history (12%). Analysis of the group of patients without a known coronary artery disease (611) showed: 101 patients without risk factors did not have any stress test. 510 patients with medium/high risk profile underwent stress test: 480 resulted negative and 30 positive. 29 patients with positive stress test underwent SCA: we found 2 patients with not critical coronary artery disease not requiring angioplasty. No coronary events occurred during the waiting time (f-up 32 months, SD 24, median 30) in all the population of 611 patients, and no peri-operative mortality related to CAD occurred in the 235 transplanted patients. Conclusions: The observed prevalence of silent myocardial ischemia in this study was extremely low, as well as the positive predictive value of the stress test. Patient’s clinical history and careful identification of risk factors gave the major contribution to the screening process.

### 605 EARLY HEPATOCYTE C4D EXPRESSION AND FAILURE TO RESTORE BASAL HEPATIC TOTAL PROTEIN CONTENT AFTER ISCHEMIA REPERFUSION INJURY ARE ASSOCIATED WITH ONE YEAR GRAFT LOSS IN HUMAN LIVER TRANSPLANTATION

Paola Barsotti¹, Maria Siciliano², Rosanna De Marco², Antonio Molinaro², Andrea Onetti Muda¹, Novella Gualtieri¹, Manuela Merli², Adolfo Francesco Athili², Pasquale Bertolo², Massimo Rossi², Stefano Ginanni Corradini², ¹Experimental Medicine, Universita’ La Sapienza Roma, Roma, Italy; ²Clinical Medicine, Universita’ La Sapienza Roma, Roma, Italy; ³General Surgery Paride Stefanini, Universita’ La Sapienza Roma, Roma, Italy

Hepatic expression of several proteins, including albumin, is reduced during ischemia and restored upon reperfusion in human liver transplantation [J Pathol 2005;207:111–118]. Complement activation during ischemia reperfusion injury has been reported in renal, liver, intestine and heart allografts. A link between proteolysis and complement activation has been demonstrated in myocardial ischemia reperfusion damage [Proteomics 2002;2:988–995]. To better elucidate the mechanism and prognostic significance of complement activation in hepatic ischemia reperfusion injury we measured early changes of liver graft C4d expression and total protein content during liver transplantation. Liver graft samples were obtained from heartbeating donors before the aorta was clamped (T1) and 2 hours after reperfusion in the recipient (T2) during primary whole organ liver transplantation. Total protein content was measured by the Bio-Rad protein assay method in freeze-dried liver homogenates from 47 grafts. In a subgroup of 29 grafts C4d immunohistochemistry was performed on paraffin sections and positive hepatocytes were scored on a semiquantitative scale. The data were evaluated comparing grafts which were lost for liver–related reasons or surviving at one year follow up. Intergroup differences were assessed by the Mann-Whitney U test. Correlations were analysed by the Spearman rank test. Lost grafts [n=10] had a significantly [P<0.05] higher difference between total protein content in T1 and in T2 than surviving grafts [n=37] [31.55±31.51 vs 2.41±33.16 µg/mg dry liver, respectively]. C4d hepatocyte immunohistochemistry was negative in both lost and surviving grafts T1 samples. Lost grafts [n=9] showed a significantly [P<0.05] higher T2 C4d hepatocyte-score than surviving grafts [n=20] [4.44±1.67 vs 2.55±2.28 C4d, respectively]. When lost and surviving grafts
were considered together, a positive correlation was found between T2 C4d hepatocyte score and the difference between total protein content in T1 and in T2 (r= 0.547, P<0.01). Early complement activation and impaired restoring of graft protein content after reperfusion are linked events in hepatic ischemia reperfusion injury and are associated with graft loss within the first year after human liver transplantation.

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607 RESECTION OF HEPATOCELLULAR CARCINOMA: LONG-TERM OUTCOME

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Partial liver resection and liver transplantation are potential curative therapies for hepatocellular carcinomas (HCC). Only few patients are suitable for these therapies. The aim of our study was to investigate the long-term outcome for patients who underwent liver resection for HCC. Patients: In a retrospective study data of 67 HCC patients, who had partial liver resection, were analyzed. The mean age at time of resection was 63±12 yrs (56±9w). Forty-one patients had a liver cirrhosis Child A, 26 patients had no cirrhosis. Patients underwent either resection of liver segments or extended hemihepatectomy. Alpha-fetoprotein of >400 ng/ml was found in 11 of 67 patients. The mean time of follow-up after liver resection was 29±30 months. We analyzed duration of stay in hospital, complications after surgery, age, size of tumor, CLIP-stages, tumor grading at time of operation and incidence and localization of tumor recurrence. Results: The 1- and 4-yr survival time of our patients amounted to 69% and 37%, respectively. The tumor-free survival time was 62% and 37%, respectively. The mean stay in hospital was 17±13 days. After surgery 7 patients (sepsis n=6, myocardial infarction n=1; thereof 5 were cirrhotic patients) died. Tumor size, presence of cirrhosis, CLIP-stage and tumor grading did not significantly influence long-term survival. In contrast, patients with an age <65 yrs at time of resection had a significantly better 4-yr-survival (54%) than older patients (16%; p=0.026). Forty-six percent of patients suffered from tumor recurrence within 4 months to 8 yrs. In 30% of patients the first tumor recurrence occurred in the liver. Time of tumor recurrence was nearly the same in cirrhotic versus non cirrhotic patients (15±14 vs. 18±25 months, respectively). Metastases occurred in lung, bone, adrenal gland, lymph nodes, cardio and peritoneal carcinomatosis. Conclusion: Resection of HCC is a promising therapeutic option in well selected patients. An age over 65 years was a negative predictive factor for long-term outcome after HCC resection.

Disclosures:
The following people have nothing to disclose: Stefan Grune, Hans-Juergen Schlitt, Reiner Wiest, Frank Klebl, Aiman Obed, Juergen Schoelmerich, Gabi I. Kirchner

608 MINIMISING BILIARY COMPLICATIONS AFTER TRANSPLANTATION OF LIVERS FROM DONORS AFTER CARDIAC DEATH

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Background: Liver transplantation (LT) using grafts from donors after cardiac death (DCD) is troubled by a high rate of biliary complications and ischemic cholangiopathy (IC). We report a low rate of biliary complications and IC in the largest liver DCD programme in Europe. Methods: From April 2001 131 livers were retrieved from DCD. Of these, 74 were transplanted. The
ALLOGRAFT SURVIVAL IS DECREASED IN PATIENTS WITH RECURRENT PRIMARY SCLEROSING CHOLANGITIS

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Background: Recurrence of primary sclerosing cholangitis (r-PSC) is a well-recognized complication after orthotopic liver transplantation (OLT). However, its prevalence, predictors and impact on graft and patient survival remain poorly characterized. Aims: The goals of this study were (1) to estimate the prevalence of r-PSC, (2) to determine the predictors of r-PSC and (3) to determine the impact of r-PSC on allograft and patient survival. Methods: All 54 patients who underwent OLT at the University of Florida between 1990 and 2006 for primary sclerosing cholangitis were considered for the study. Those with follow-up less than 4 months (n=5) were excluded. Associations between potential predictive variables and r-PSC were measured using appropriate statistical tests. Variables explored as potential predictors of r-PSC were recipient age/gender, donor age/gender, gender mismatch, cold and warm ischemic times, CMV infection, rejection, use of OKT3, concomitant inflammatory bowel disease, prior colectomy, duration of PSC and immunosuppressive agent. Logistic regression was performed to identify independent predictors of r-PSC post OLT. The Log-rank test was applied to test the difference between stratified survival curves. Similar methods were used to test an independent group of 41 patients transplanted between 1995 and 2004 at the Cleveland Clinic and with follow-up of at least 4 months. Results: Forty nine patients, 78% males, with mean follow-up of 60.7 ± 43.6 months constituted our original cohort. Mean age at the time of OLT was 41.9 ± 13.4 years. r-PSC was diagnosed in 12 (24.5%) cases. The mean time to recurrence was 35.2 ± 26.2 months. Variables found to predict r-PSC were CMV infection (p=0.013) and multiple, but not single, rejection episodes (p=0.037). On multivariate analysis, CMV infection was a strong predictor of r-PSC in patients without history of multiple episodes of rejection (Relative Risk=17, p=0.046). There were 4 re-transplantations, all among patients with r-PSC. Overall patient survival was not different between patients with and without r-PSC, but allograft survival was shorter in those with r-PSC (p=0.001). In the Cleveland Clinic cohort, 6 of 41 patients had r-PSC (15%); CMV infection was the only independent predictor of r-PSC (p=0.035). Six patients underwent re-transplantation, 4 had r-PSC. Allograft survival was decreased in patients with r-PSC (p=0.0006). Conclusion: In two independent cohorts from 2 large transplant centers, r-PSC occurred in 15% and 25% of patients. CMV infection and multiple episodes of rejection appear to increase the risk of r-PSC. Development of r-PSC significantly impacts graft survival.

Disclosures: The following people have nothing to disclose: Cynthia Levy, Claudia O. Zein, Chaoru Chen, David R. Nelson
611 DEVELOPMENT OF A UK SCORE FOR PATIENTS WITH END-STAGE LIVER DISEASE

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Background: Although the MELD score is widely used to predict mortality on the liver transplant list, it has not been validated for use in the UK. A score for UK patients with end-stage liver disease has therefore been developed. Methods: The study cohort comprised adult elective patients registered for their first liver transplant at the 7 UK centres between 1 April 2003 and 31 March 2006. Patients were followed-up until death, liver transplantation or last known follow-up. Patients with any type of cancer recorded as their primary liver disease were excluded. The resulting cohort comprised 1,103 patients. Cox proportional hazards models were used in modelling the hazard of death on the list. The clinical components considered for inclusion in the UK specific score, termed UKELD, were INR, serum creatinine, bilirubin and sodium, and the UKELD score was expressed as a linear combination of the logarithms of the clinical components. The UKELD score was compared to MELD and MELD-Na by computing the change in the log likelihood (2logl) statistic on adding each of the three scores to a Cox model that included relevant patient-specific factors. The UKELD score was validated using data from the 7 UK centres on adult elective patients registered for their first liver transplant between 1 April 2006 and 31 March 2007. The resulting cohort comprised 452 patients. The patients in the validation cohort were divided into 3 groups based on their risk score, with patients having a high, medium and low score. The Kaplan-Meier estimate of the survivor function for each group was then compared to the model based estimates of the survivor function, adjusted for patient-specific factors. Results: The UKELD score is calculated using the following formula: UKELD=[(5.395xln(INR))+{1.485xln(creatinine)}]+{3.13xln(bilirubin)}-{81.565xln(sodium)}]-435. The results from the validation cohort confirmed this score is an appropriate measure of disease severity for UK patients. As UKELD increases the risk of death on the transplant list also increases. UKELD was found to be a superior predictor of mortality on the transplant list compared to MELD and MELD-Na. Conclusion: The UKELD score is deemed appropriate to use to predict mortality on the transplant list for UK patients with end-stage liver disease. Patients with cancer and those with other variant diseases may need to be awarded additional UKELD points to avoid disadvantaging them.

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612 PROPOSAL OF NEW SELECTION CRITERIA FOR LIVING DONOR LIVER TRANSPLANTATION CANDIDATES FOR HEPATOCELLULAR CARCINOMA

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Introduction: The Milan criteria have been accepted as the selection criteria for hepatocellular carcinoma (HCC) patients. However, many patients who did not meet the Milan criteria have survived after undergoing living donor liver transplantation (LDLT). As a result, different patient selection criteria are thus considered to be necessary in LDLT in order to save as many individuals as possible with advanced HCC. Patients and Methods: Of 214 adult-to-adult LDLT patients, 90 with concurrent HCC were included in the study. The mean age of the patients was 56.8 years old (range: 21 to 70). Sixty-five patients (72.2%) suffered from a hepatitis C virus infection. The overall survival rates according to various factors were compared to identify the risk factors for survival and to establish new selection criteria for LDLT candidates for HCC. Results: Fifty-three patients (58.9%) preoperatively exceeded the Milan criteria. The 1-, 3- and 5-year overall survival rates of the patients who fulfilled the Milan criteria were 100%, 100%, and 100%, respectively, whereas those for patients who exceeded the criteria were 85.5%, 82.8%, and 85.8%, respectively. In a multivariate analysis, both the tumor diameter (p=0.0187) and the des-gamma-carboxy prothrombin value (DCP; p=0.026) were found to be favorable independent factors of survival after LDLT. Therefore, the authors devised new selection criteria for HCC patients (which are named the KU criteria; a tumor diameter of less than 5 cm or a DCP of less than 300 mAU/ml). The 1-, 3- and 5-year overall or recurrence-free survival rates of the 85 patients who met the KU criteria were 92.1 / 85.2 / 85.2% or 90.6 / 87.1 / 87.1%, respectively, which were significantly different from those of the 5 patients who did not meet the KU criteria (80 / 20 / - % or 20 / 0 / 0 %; p< 0.0001). Conclusions: A combination of two factors, namely the tumor size and the DCP level, was found to be useful for predicting survival after LDLT. These new selection criteria (the KU criteria) might therefore be useful for expanding the selection of LDLT candidates for HCC.

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613 C4D IMMUNOHISTOCHEMICAL (IHC) REACTIVITY AS AN ADJUNCT IN DISTINGUISHING BETWEEN ACUTE CELLULAR REJECTION (ACR) AND RECURRENT HEPATITIS C VIRAL (HCV) INFECTION IN LIVER TRANSPLANTS

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End stage liver disease due to Hepatitis C is the most common indication for liver transplant in the US. Distinguishing between acute cellular rejection (ACR) and recurrent hepatitis C virus (HCV) infection is notoriously difficult since these entities lack specific morphological features. In this study we evaluated the
value of immunohistochemical stain for C4d in facilitating the distinction between ACR and rHCV infection. Material and methods: Fifteen (15) cases with the histologic diagnosis of ACR, 16 cases with the histologic diagnosis of rHCV infection, and a control group of 16 cases of non-transplanted liver (9 cases of normal liver, 7 cases of chronic hepatitis C) were studied. Each case was stained with a polyclonal antibody for C4d (American Research Products. Inc, Belmont, MA) and evaluated for C4d immunoreactivity. Positive staining was evaluated in the portal veins, portal stroma portal capillaries, portal arteries and central veins. The intensity of stain was semi-quantitatively reported as 1+, 2+ and 3+. Clinical information including time after transplant, medication, viral status, treatment after the biopsy, and outcome were retrospectively reviewed and correlated with the pathologic diagnosis and C4d reactivity. Results: Ten of 15 cases with pathologic diagnosis of ACR, 8 met clinical criteria and were treated. Of these 8, 6(75%) were C4d positive. Of the remaining seven cases (46.6%) histologically interpreted as ACR, 4 were treated as rHCV infection. In this second group, C4d was positive in only 1/4 cases (25%). The remaining 3 cases, showed hepatic artery thrombosis in 2 (with 1/2 cases C4d +) and hepatic vein thrombosis in 1 case (C4d +). In summary, C4d was expressed in 75% of cases histologically and clinically proven as ACR, and showed a specificity of 71%, a PPV of 75% and a NPV of 71%. In the group of 16 cases with histological and clinically proven rHCV infection, C4d was positive in 3/16 cases (18.7%). In the control group, only 1/16 cases was C4d +. Difference in detection of C4d was statistically significant when comparing rejection cases to controls (p<0.001) as well as between rejection and rHVC-cases (p<0.021). Conclusion: IHC stain for C4d is expressed in 75% of cases of histologically and clinically proven ACR; in 18.7% of cases of liver transplant with rHCV infection, and 6% of the controls. In conclusion, this study suggests that C4d may be helpful in differentiating ACR from rHCV infection in liver transplants. Our findings suggest that C4d could be used as an adjunct to complement the conventional histopathological criteria in the distinction between rHCV infection and ACR.

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615 OUTCOME DIFFERENCES AFTER LIVING DONOR LIVER TRANSPLANTATION (LDLT) IN ADULTS WITH AUTOIMMUNE AND CHOLESTATIC LIVER DISEASES: SRTR ANALYSIS

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Introduction: Autoimmune hepatitis (AIH), Primary Biliary Cirrhosis (PBC) and Primary Sclerosing Cholangitis (PSC) all lead to progressive liver disease needing liver transplantation. Living Donor Liver Transplantation (LDLT) has been used in adults with complications linked to these conditions as an alternative to deceased donor liver transplantation (DDLT). There are no comparative data between autoimmune and cholestatic liver diseases in terms of outcomes after LDLT. Our objective was to determine outcome differences between AIH, PBC and PSC after LDLT using the SRTR database and to explain these differences based on the known natural history of these diseases and recurrence after liver transplantation. Methods: We reviewed all LDLTs done in the US for autoimmune (AIH) and cholestatic liver disease (PBC and PSC) between 1995 and 2006. The results were analyzed using SPSS vs. 14.0. Results: 502 LDLTs were performed for PSC (34%), PBC (31%) and AIH (12%) in this time period. The overall female to male ratio was 1.4:1. The median age of the recipients was 47 yrs. The median MELD scores at time of transplant for patients in all 3 groups were similar (however 60% of all transplants were done after MELD score started being utilized). The median age of the recipients...
at time of transplant was PBC (53.3yrs) being the oldest compared to those with AIH (42.9yrs) and PSC (44.7yrs). The mean survival of all the 3 conditions was as follows: PBC 1786 days; PSC 2228 days and AIH 2136 days. The 1, 3 and 5 yrs survival were as follows: AIH (84.7%,84.7%, 77.6%), PBC (81%,76.6%,66.4%) and PSC (87.4%,86.8%,74.3%) respectively. We found that patients with PBC had a worse outcome compared to AIH and PSC after LDLT (p value 0.024).

**Discussion:** LDLT is a very favorable alternative to DDLT in patients with autoimmune liver disease awaiting liver transplantation. In this analysis, patients with PBC had a worse survival as compared to PSC and AIH after LDLT. We found that PBC patients were older at the time of transplantation compared to AIH and PSC recipients. An earlier study reported 5 yr survival after LDLT for PBC to be 80% 1. PBC has the highest rate of recurrence after liver transplantation. The poorer outcome of PBC compared to AIH and PSC may be linked to older recipient age at time of LDLT and higher rate of recurrence. This sheds light on a previously unreported aspect of autoimmune and cholestatic liver diseases with regards to living donor liver transplantation. Reference: Hasegawa et al. Living Donor Liver Transplantation for Primary Biliary Cirrhosis- retrospective analysis of 50 patients at a single center. Transplant International Vol.18:7,794-799 July 2005

The following people have nothing to disclose: Adel Bozorgzadeh, Parvez S. Mantry, Randeep Kashyap

**616 TRANSPLANT COSTS OF ADULT LIVING DONOR LIVER TRANSPLANTATION AT AN EXPERIENCED TRANSPLANT CENTER ARE SIMILAR TO DECEASED DONOR LIVER TRANSPLANTATION DESPITE INCREASED SURGICAL COSTS**

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**BACKGROUND:** The growing deficit of organs available for transplantation warrants continued evaluation of the application of adult living donor liver transplantation (LDLT). Several studies have reported the costs of deceased donor liver transplantation (DDLT), but data regarding the costs of LDLT are limited by including early experience, which is associated with increased complication rates, and small sample sizes. AIM: To provide a comparison of transplant hospitalization costs of LDLT vs DDLT after the initial experience. METHODS: This was a retrospective cohort of consecutive patients who received liver transplants at our institution from 1/1/00-12/31/06 for whom financial data were available. Our primary outcome was cost, estimated from total charges of the transplant hospitalization (converted into relative cost units), including donor charges for LDLT. All charges were classified into cost categories, including: floor services, diagnostics, laboratory, surgical, pharmacy, and other services. Our independent variable was type of transplant (LDLT/DDLT). Covariates included age, sex, race, insurance status, MELD, and length of stay (LOS).

Any variable associated with total costs (p<.2) in bivariate analysis was included in multivariate analysis (MVA) with and without LOS as a covariate. RESULTS: This study included 469 patients (n=84 LDLT vs n=385 DDLT). Baseline characteristics differed substantially between LDLT and DDLT, including age (50 vs 54 yrs, respectively), %male (51% vs 74%), pre-transplant MELD (20.6 vs 23), %white (62% vs 48%), %black (1% vs 12%), %Hispanic-white (30% vs 18%), and %other (7% vs 22%). LOS and insurance status were similar. Total charges for LDLT were 5% lower than for DDLT, but this was not statistically significant (Table). Cost category analysis revealed increased surgical charges for LDLT, but decreased charges for labs and pharmacy (Table). In MVA, LDLT was similar in cost to DDLT after adjustment for age, black race, private insurance, and MELD score, both with (p=0.53) and without (p=0.31) adjustment for LOS. Age, MELD, and black race did predict higher cost.

**CONCLUSION:** In a comparison of the financial costs of LDLT vs DDLT at an experienced transplant center, overall transplant costs of LDLT were similar to DDLT, despite increased surgical costs.

**Table: Comparison of total and select cost category charges, in relative cost units**

<table>
<thead>
<tr>
<th>Description</th>
<th>LDLT (n=84)</th>
<th>DDLT (n=385)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>160,553</td>
<td>168,081</td>
<td>0.58</td>
</tr>
<tr>
<td>Surgical</td>
<td>35,117</td>
<td>20,992</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Laboratory</td>
<td>20,054</td>
<td>28,159</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Pharmacy</td>
<td>12,591</td>
<td>23,272</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

Disclosures: The following people have nothing to disclose: Jennifer C. Lai, Jean C. Emond, Robert S. Brown

**617 SUCCESSFUL ALGORITHM FOR SELECTIVE LIVER BIOPSY IN THE RIGHT LOBE LIVER DONOR (RLD)**

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**Background:** Routine vs. selective pre-donation liver biopsy (LBx) remains controversial for assuring the safety of RLD. We report the results of our selective algorithm for LBx in RLD. Methods: Between 12/99 and 3/07, 380 potential RLD were evaluated and 131 donated. Indications for selective LBx were: abnormal liver function tests, abnormalities noted on imaging studies, BMI>28, and a genetic relation to a recipient with immune mediated liver disease. All donors had a LBx at the time of surgery. The same pathologist reviewed all LBx. Results: Of the 380 potential RLD evaluated (53.9% male, mean age 37.6), 139(36.6%) were accepted as donors, 140(36.8%) were rejected for either donor or recipient reasons, 68(17.9%) withdrew from the process, 23(6.1%) had their recipient receive a deceased donor graft, and 10(2.6%) are currently completing evaluation. 81(21.3%) met criteria and had a selective LBx. 7 results are still pending. 58(78.4%) had either normal (n=21) or mild macrosteatosis of 0-5%(n=37) and 40 of these went on to LD right hepatectomy (LDRH). 12 RLD were eliminated as donors as a result of abnormal LBx. Of the 89 who did not have LBx, none had abnormal liver histology at LDRH. 3/81 (3.7%) had complications from the LBx resulting in overnight admission (2 for pain, 1 for bleeding, transfusion not required).Conclusions: Use of this algorithm for selective pre-donation LBx in the RLD resulted in 78.7% of potential donors avoiding biopsy and potential complications. No significant liver pathology was identified in donors not meeting criteria for LBx. Routine pre-donation LBx is unnecessary in potential RLD.

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618 HIGH MELD SCORES DO NOT PREDICT ONE-YEAR SURVIVAL RATE OF PATIENTS WITH A SMALL-FOR-SIZE GRAFT IN ADULT LIVING DONOR LIVER TRANSPLANTATION

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Adul living donor liver transplantation (ALDLT) has shown comparable outcome to the deceased donor recipients because of being better risk candidates. However, outcome of very ill patients with high MELD score is fairly elusive especially in small grafts (< 0.8% of graft-to-recipient weight ratio, GRWR) in ALDLT. Between 1999 and 2005, consecutive 167 hepatitis B virus-infected recipients underwent ALDLT at our center. Based on pre-transplant MELD, recipients were stratified as low risk (<25, Group L, n=105) and high risk (>25, Group H, n=62). To analyze the risk of graft size in very ill patients, patients were divided HR (high risk group; MELD score >25 and GRWR < 0.8%), LR (low risk group; MELD >25 or GRWR <0.8%), and NR (no risk group; MELD ≤25 and GRWR ≥ 0.8%) group. Primary end points were one-year patient survival. Post-transplant follow-up was 32.6 months. Mean MELD scores were 17.1 in Group L and 32.6 in Group H. In pre-transplant recipients' data, Group H more frequently had uncontrolled ascites (50.0%), encephalopathy (64.5%), bacterial peritonitis (48.4%), and UNOS status 2A (38.7%) than Group L (27.6%, 30.5%, 27.6% and 4.8%) (p<0.05). Accompanying hepatocellular carcinoma (HCC) was more common in Group L (61.0%) than in Group H (22.6%) (p=0.000). Mean hospital stay were 33 days in Group L and 47 days in Group H (p=0.000). One-year patient survival rate (1-YSR) was 86.7% in Group L and 83.8% in Group H, respectively (p=0.48). There was no significant difference in 1-YSR among HR (72.7%), LR (84.6%), and NR (88.5%) group, respectively (p=0.278). Multivariate analysis revealed accompanying HCC and early transplant year to be risk factors of poor outcome (1-YSR) (p<0.05). In conclusion, high MELD scores (>25) do not predict 1-YSR of recipients with small-for-size grafts in ALDLT for hepatitis B related liver disease. Because, factors associated with 1-YSR were accompanying HCC and early transplant year, accumulation of center’s experience may improve the early post-transplant survival even in very ill patients.

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619 IMPACT OF ISCHEMIC PRECONDITIONING IN GRAFT FUNCTION AND INFLAMMATORY MEDIATORS IN ORTHOTOPIC LIVER TRANSPLANTATION

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INTRODUCTION: Ischemic preconditioning (IP) has been proposed to improve graft function in orthotopic liver transplantation (OLT). The aim of this study was to determine whether IP in OLT improves graft function, to study inflammatory mediators implicated in IR injury and to correlate the liver biopsies. Material and methods: this is a randomized and prospective study, this includes 12 patients with OLT and their cadaveric donors, we made 2 groups, with and without IP. Ischemic preconditioning was performed in 5 donors for 10 min. followed by 10 reperfusion min. Seven patients were transplanted with the classic technique. The etiologies of the liver cirrhosis were alcohol and autoimmune 25% each one, virus C 17%, virus B and hepatocellular carcinoma, cryptogenic, secondary biliary cirrhosis and Budd Chiari 8.3% each one. The myeloperoxidase (MPO), p-selectin (PS), leuocriinin B4 (LTB4), and ICAM1 were measured by ELISA in the different phases of the transplant (prepararatomy (phase R1), preperfusion (phase R2) and postreperfusion (phase R3). Liver biopsies were performed before procurement in the 3 phases of the receptor mention above. RESULTS: The PS in the group with IP in the donor (D) phase showed high levels (24 ng/mL), while in the other phases the levels diminished, the R3 phase of the group without PI showed higher levels (33 ng/mL) with respect to the other phases. The ICAM1 in the group with IP were diminished in the R3 phase (391 ng/mL), the patients with IP maintained similar levels. LTB4 levels were increased in the group with IP in the R3 phase (29 ng/mL), and diminished in the same phase of the group without IP (8 ng/mL). MPO showed similar levels in both groups. The AST in the group with IP vs without IP in the phase R3 was 26 vs 11.8 times above the upper normal limit (TAUNL). The ALT was similar in both groups. Histopathology: the introtoclasticm vacuolisation, confluent necrosis and nuclear focal picnosis were similar in both groups, the neutrophilic infiltration was observed in all patients with IP vs 2/7 of the group without IP. One patient with more than 50% steatosis (group with IP). There were no cases of primary dysfunction of the graft. The mortality was 17% (2 without IP). CONCLUSIONS: The inflammatory mediators didn’t show a characteristic pattern, there was higher elevation of AST in the group with IP. Neutrophilic infiltration was more common in liver biopsies with IP, however MPO did not show differences. This is an ongoing study, a larger population should be included before drawing definite conclusions on IP effects in OLT.

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The following people have nothing to disclose: Linda E. Muñoz Espinosa, Homero Zapata, Paula Cordero, Miguel Escobedo, Liliana Torres, Belia Garduño, Edelmiro Perez, Magadalena Cepeda, Marcela De Luna, Amanda Mercado, Briceluid Garza, Eloy Caballero, Joan Roselló

620 INFLUENCE OF DIFFERENT LABORATORY METHODS ON MELD CALCULATION IN THE BELGIAN LIVER TRANSPLANT CENTRES

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Introduction: Liver allocation in Eurotransplant is based on the MELD score only including so called “objective” parameters (INR, bilirubin, creatinine). This score, theoretically, permits no
subjective centre dependent variation in priority for liver transplantation. It was recently shown that the laboratory methods, used to determine the MELD score may introduce significant and clinically relevant variation in this score between different laboratories. The aim of the study was to investigate whether significant differences in MELD score are obtained in patients with liver cirrhosis between the Belgian liver transplant centres. Patients and methods: Sixty five patients with biopsy proven cirrhosis (15 Child A, 25 Child B and 25 Child C) in 2 centres were enrolled. From each patient 6 aliquots were drawn, each aliquot containing samples for analysis of creatinine, bilirubin and INR. Analyses were performed in the 6 liver transplant centres. Control aliquots from 30 healthy volunteers and 30 patients receiving oral anticoagulants were collected for measurement of the same parameters at the same 6 centres. Multivariate analysis (within factor: 6 different centres and between factors: 5 different patient groups) was used to compare means, p<0.05 was considered statistically significant. Results: Significant differences (p<0.001) in MELD score were noted between the 6 centres as well for Child A (5.3 ± 1.1 - 10.1 ± 0.8), B (10.3 ± 0.8 -14.2 ± 1.0) and C (14.6 ± 1.5 -20.0 ± 1.3) patients. The highest difference in MELD score between centres was 4 points. In these centres INR and bilirubin values were significantly different, where as no differences were noted for creatinine. Differences in INR were also noted in normal controls and patients receiving oral anticoagulants in all 6 centres. Conlusion: Significant differences in MELD score were observed between the 6 Belgian liver transplant centres in patients with liver cirrhosis, mainly related to differences in INR and bilirubin values. These differences are explained by laboratory methodologies. The differences in MELD score might lead to significant differences in allocation priority on the waiting list for liver transplantation. We could demonstrate these differences not only in Child C patients, but for the first time also in Child A patients.

Disclosures: The following people have nothing to disclose: Jeffrey Schouten, Sven M. Francque, Hans Van Vlierberge, Isabelle Colle, Frederik Nevens, Jean DeWaele, Michael Adler, Peter Stäerkel, Dirk Ysebaert, Alain Gadisseur, Peter F. Michielsen compared to the 30 non-DCD LKT. RESULTS: In the LKT recipients 41.0% were diabetic, 48.7% required renal replacement therapy (RRT) pre-transplant and 33.3% required RRT post-operatively. None of the transplants utilized HBCAb-positive grafts and 1 non-DCD recipient received a HCVAb + graft. There was no significant difference in patient characteristics or transplant outcomes other than initial hospital length of stay (LOS). * The one-year patient survival for DCD LKT was 88.9% compared to 83.3% with non-DCD LKT. The one-year liver and kidney graft survival for the DCD group was 77.8% and 88.9%, respectively. Glomerular Filtration Rate at one year after LKT was 55.0 ± 30.4 and 46.8 ± 18.3 ml/min for non-DCD (n=20) and DCD (n=4) grafts, respectively. CONCLUSION: The use of DCD organs for solitary liver transplant or solitary kidney transplant is well described. The combination of the two has not been well defined. Despite the higher incidence of need for RRT and longer hospital stay the utilization of DCD donors provides comparable graft and patient survival and should be considered for patients requiring combined liver and kidney transplants.

Pt Characteristics

<table>
<thead>
<tr>
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<th>DCD (n=9)</th>
<th>non-DCD (n=30)</th>
<th>p-value</th>
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<tr>
<td>Age at Tx (yrs)</td>
<td>56.3 ± 10.2</td>
<td>55.3 ± 8.9</td>
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<tr>
<td>Donor Age (yrs)</td>
<td>32.4 ± 13.4</td>
<td>34.9 ± 16.4</td>
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<tr>
<td>MELD</td>
<td>25.2 ± 6.7</td>
<td>22.4 ± 6.2</td>
<td>0.24</td>
</tr>
<tr>
<td>RRT post-Tx (%)</td>
<td>5 (50%)</td>
<td>8 (27%)</td>
<td>0.11</td>
</tr>
<tr>
<td>Liver CIT (hr)</td>
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<td>7.0 ± 1.3</td>
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<tr>
<td>Kidney CIT (hr)</td>
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<td>LOS (d)</td>
<td>23.4 ± 14.3</td>
<td>12.7 ± 9.6</td>
<td>0.02*</td>
</tr>
</tbody>
</table>

Disclosures: The following people have nothing to disclose: Winston R. Hewitt, John Stauffer, Martin Mai, Hani Grewal, Lisa Arasi, Barry Rosser, David Kramer, Juan Canabal, Justin H. Nguyen, Hani Waeedi, Nasimul Ahsan, Darrin Willingham, Andrew Keaveny, Jaime Aranda-Michel, Denise M. Harnois, Raj Satyanarayana, Rolland C. Dickson, Thomas Ganwa, Christopher B. Hughes

622 PRIMARY LIVER RESECTION FOR CHILD-PUGH-A HCC WITHIN MILAN CRITERIA: FEASIBILITY OF RESECTION FOLLOWED BY SALVAGE TRANSPLANTATION

Takaaki Sugimoto, Junichi Yamanaka, Shinichi Saito, Tadamihiro Hirano, Toshiihiro Okada, Nobukazu Kuroda, Yui Imuro, Jiro Fujimoto, Surgery, Hyogo college of medicine, Nishinomiya, Japan

AIM: The 5-year survival after liver resection (LR) for Child-Pugh A HCC is comparable with that after liver transplantation (LT). But the recurrence rates of Child-Pugh A HCC after LR is high. The aim of this study is to investigate long-term survival and pattern of recurrence after LR in patients with preserved liver function, otherwise eligible for salvage transplantation. MATERIALS & METHODS: Curative liver resection was performed for HCC in 600 patients from 1984 to 2006. Retrospective data collection analysis was carried out on pattern of recurrence of these patients according to Milan criteria. Clinico-pathologic parameters at the time of the primary resection were analyzed to determine the factor(s), for which patients became ineligible for salvage transplantation. Treatments for recurrence and transplantable period were also analyzed. RESULTS: Overall 1, 3 and 5-year survival rates of the patients within Milan criteria after primary LR were 94, 74 and 56%. Child-Pugh A accounted for 88.6% of the patients, whose 1, 3 and 5-year survivals were 96, 77 and 60%. With a median follow up of 30 months, 66.7% developed tumor recurrence, of which 37.9% were within Milan criteria allowing salvage transplan-
tation. Microvascular invasion was the only significant factor for development of recurrence exceeding Milan criteria on univariate and multivariate analysis. Transplantable period after primary resection of the patients with microvascular invasion was 1511±1485 days and shorter than that (2088+1269 days) of the patients without microvascular invasion. CONCLUSIONS: Five-year survival after resection for Child-Pugh A HCC within Milan criteria was comparable with that after LT. Primary LR is ideal for Child-Pugh A HCC within Milan criteria. However, the patients with microvascular invasion are likely to exceed Milan criteria at the early stage after relapse. Therefore, their high-risk patients should have closer follow-up postoperatively to detect recurrence and receive salvage transplantation at earlier stage.

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ASSESSMENT OF REPRODUCIBILITY OF CREATININE MEASUREMENT AND MELD SCORING IN FOUR LIVER TRANSPLANT UNITS IN THE UK

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It has been proposed that the MELD scoring system be introduced in the UK and Ireland in order to improve equity of access to liver transplantation. Previous papers showed significant variation in creatinine results when different laboratory techniques were used, particularly in those with high bilirubin. This study aimed to evaluate the reproducibility of creatinine measurements and therefore MELD scores in four liver transplant units in the UK. Methods: Six transplant units in the UK and Ireland were invited to participate in the study. The centres were coded as centres ‘A-D’, and only the co-ordinator of the study knew the code. A total of 35 samples, up to 10 from each centre participated in the study. The participants were either on the liver transplant waiting list or undergoing assessment for transplant. In a pre-selected week 2x10ml of blood was collected in a lithium heparin tube from each of the participants, this was divided into 2ml aliquots. These 2ml aliquots were then sent to all of the participating centres, so creatinine and bilirubin were measured in every centre for every patient. All centres used measurement techniques based on the kinetic Jaffe technique except for centre C which used the Dade dimension technique. We then analysed these results for agreement between centres on creatinine values and MELD scores were significantly different between all centres (p<0.05) except for MELD A vs. MELD B and MELD B vs. MELD D. The difference between MELD score was 2 points in 15% of samples between MELD A vs. MELD C, and in 9% samples between MELD C vs. MELD D. Conclusion: This important study demonstrates lack of reproducibility in creatinine measurement and MELD scoring between 4 UK liver transplant centres. The fact that there was good agreement between creatinine values from centre B and C, but not in MELD score also suggests the impact of variation in bilirubin results between the centres. A difference in 2 MELD points could have significant impact on patient out-come and these factors will have to be addressed if a UK wide transplant list is to be initiated.

Disclosures:
The following people have nothing to disclose: Carol Goulding, Evangelos Cholongitas, Devaki Nair, Andrew Kerry, Murat Akyol, Simon Walker, Derek Manus, David McClure, Neville Jamieison, David Cartwright, David Patch, Andrew K. Burroughs

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DOES HEALTH RELATED QUALITY OF LIFE CORRELATE WITH THE MODEL FOR END STAGE LIVER DISEASE SCORE BEFORE LIVER TRANSPLANTATION?

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Introduction: With minimal existing data our primary aim was to correlate the model for end-stage liver disease (MELD) score pretransplant and quality of life after liver transplantation (LT). Methods: A single institution prospective study of all patients undergoing first time LT over a 4-year period was conducted. Patients were given the SF-36 at 1, 3, 6, or 12 months post-LT. MELD was calculated the day of LT. Simultaneous multiple regression-based path analysis was used to test the effects of MELD, diabetes, diagnosis, length of stay (LOS), time post-LT, rejection, and the contemporaneous Karnofsky performance score (KPS), on the physical component (PCS) and mental component (MCS) summary scales of the SF-36. Results: There were 209 participants. Diabetes, LOS, and rejection were associated with worse KPS (p=0.02) whereas time post-LT was associated with an improved KPS (p=0.01). Factors influencing quality of life measured by PCS and MCS of the SF-36 are shown in the table. Conclusions: Increasing MELD score was associated with improvement in PCS, MELD did not affect MCS. Functional performance, measured by KPS, had the largest effects on post-LT quality of life. Since good quality of life can be expected even with higher MELD scores, they should not discourage transplantation.

Factors Influencing Quality of Life

<table>
<thead>
<tr>
<th>Variable</th>
<th>PCS</th>
<th>MCS</th>
</tr>
</thead>
<tbody>
<tr>
<td>MELD</td>
<td>β=0.16, p=0.01</td>
<td>NS</td>
</tr>
<tr>
<td>Diabetes</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Cholestatic Cirrhosis</td>
<td>β=0.12, p=0.05</td>
<td>NS</td>
</tr>
<tr>
<td>Acute Hepatic Failure</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Neoplasm</td>
<td>β=0.23, p=0.01</td>
<td>NS</td>
</tr>
<tr>
<td>Autoimmune Disease</td>
<td>β=0.18, p=0.01</td>
<td>β=0.16, p=0.02</td>
</tr>
<tr>
<td>LOS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Time post-LT</td>
<td>β=0.16, p=0.01</td>
<td>NS</td>
</tr>
<tr>
<td>Rejection</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>KPS</td>
<td>β=0.49, p=0.01</td>
<td>β=0.28, p=0.01</td>
</tr>
</tbody>
</table>

Values shown are standardized correlation coefficients. Note: NS = non significant.

Disclosures:
The following people have nothing to disclose: Eric T. Castaldo, Robert T. Russell, Irene D. Feurer, C Wright Pinson
625

SHOULD DONOR ORGANS AND RECIPIENTS BE MATCHED IN LIVER TRANSPLANTATION? AN ANALYSIS OF THE UNITED KINGDOM AND IRELAND LIVER TRANSPLANT DATABASE

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BACKGROUND The prevailing practice of matching liver transplant donors and recipients according to their respective risks of graft failure is predicated on the tacit assumption that the adverse effects of a high-risk graft are minimized in a low-risk recipient. The evidence-base supporting this practice is currently unclear. METHODS Using the UK and Ireland National Transplant Database, we examined the effect of the interaction between donor characteristics and recipient characteristics on the risk of graft loss (defined as graft failure or death whichever occurred first) among adult first single-organ liver transplant recipients in the two countries between 1 March 1994-31 October 2006 (n=6,658). Donors were categorised as high- or low-risk using the 75th centile of the UK and Ireland Donor Risk Index (UKDRI) as a threshold. Recipient risk was categorised as high, medium or low using the highest, middle two and lowest quartiles of the MELD score, respectively. Cox regression models including UKDRI and MELD with and without their interaction terms were fitted and compared using the likelihood-ratio test to determine whether the increased relative risk (RR) of graft loss for high-risk donors was consistent across the MELD categories. RR estimates by UKDRI in high, medium and low MELD categories were derived from Cox models including the interaction terms, stratified by transplant centre, with and without adjustment for other recipient risk factors and year of transplantation. RESULTS Recipients in the lowest MELD score quartile, respectively, of all recipient risk categories (table 1). The magnitude of the risk increase was similar in the first year but was modestly greater in high-risk recipients thereafter. CONCLUSIONS Both high- and low-risk recipients experience a significantly greater, albeit unequal, risk of graft loss when receiving high-risk organs. Given their substantially different mortality risks without transplantation, these results question the wisdom of the current practice of allocating high-risk organs to low-risk recipients in preference to high-risk recipients.

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LIVER TRANSPLANTATION FOR HEPATOCELLULAR CARCINOMA: VALIDATION OF A NEW PROGNOSTIC SCORE PREDICTING OVERALL SURVIVAL

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Aim: To provide a new prognostic score for refining the prediction of disease-free survival after LT for HCC, using variables assessable pre-operatively and to compare the prognostic value of this model with Milan criteria. Patients and Methods: The prognostic model was derived from the multivariate Cox model analysis of a series of 373 patients (training cohort (TC)) without portal thrombosis, transplanted for HCC (1998-1999) in 14 centers. 3 independent predictors were identified: maximal diameter (p<0.0001), tumor differentiation (p<0.0001) and number of nodules (p=0.001). Regression coefficients were estimated for each class of the variables, and a discrete prognostic score was derived. The score was subsequently simplified by linear transformation of the regression estimates. Two different prognostic groups were identified on the TC: group A < 3 points and group B > 4 points. The score was subsequently
Background: Hepatocellular carcinoma (HCC) is associated with a very high morbidity and mortality especially in the presence of portal hypertension. Our objectives were to characterize predictors of in-hospital mortality and to determine whether surgery at a liver transplant center (LTC) was associated with improved survival. Methods: We queried the Nationwide Inpatient Sample, the largest all-payer dataset of hospital discharges in the U.S., from 1998 to 2003, to identify patients who had undergone hepatectomy for a diagnosis of HCC using International Classification of Diseases, 9th Revision, Clinical Modification (ICD-9-CM) procedure and diagnosis codes. Multivariable logistic regression, accounting for survey design, was used to assess the independent effect of portal hypertension and surgery at a LTC while adjusting for demographic, clinical, and hospital factors. Results: There were an estimated 7,841 hepatectomies for HCC in the U.S. during the 6-year period. Since 2000, there has been an average 17% annual increase in the rate of HCC-related hepatectomies. The average age at surgery was 57 years, 63% were males, and 7% had clinical manifestations of portal hypertension. The overall in-hospital mortality was 8.2%. Patients with portal hypertension experienced higher mortality than those who did not (22% vs. 7%, p < 0.0001). Undergoing hepatectomy at a LTC compared to a non-transplant center was associated with lower mortality in both the portal hypertensive group (14% vs. 31%, p = 0.03) and non-portal hypertensive group (6% vs. 9%, p = 0.01). After multivariate adjustment, the odds ratio of in-hospital death for those with portal hypertension was 3.1 (95% CI: 1.9 – 5.1) and for those who underwent hepatectomy at a LTC was 0.61 (95% CI: 0.42 – 0.89). The median number of HCC-related hepatectomies performed at liver transplant and non-transplant centers was 50 and 6, respectively. The protective effect of hepatectomy at a LTC was eliminated after adjustment for hepatectomy volume (OR 0.93; 95% CI: 0.56 – 1.52). Mortality benefit from higher hepatectomy volumes plateaued at > 10 surgeries per year. Hepatectomies performed at centers that performed > 10 per year had 52% lower odds of death than those performed at centers with < 10 per year. The average length of stay was 2 days shorter at a LTC compared to non-LTC (9.3 days vs. 11.1 days, p = 0.003). Conclusions: Hepatectomy rates appear to be increasing as HCC incidence continues to rise. In-hospital mortality associated with hepatectomy is greatest in HCC patients with portal hypertension. Hepatectomy at a LTC confers survival benefit due to higher volumes of hepatic resection.

Disclosures: The following people have nothing to disclose: Geoffrey C. Nguyen, Dorsey L. Segev, Warren R. Maley, Paul J. Thuluvath

628 TREATMENT OUTCOMES OF TRANSCATHETER ARTERIAL CHEMOINFUSION (TACI) IN PATIENTS WITH UNRESECTABLE HEPATOCELLULAR CARCINOMA (HCC) PRIOR TO ORTHOTROPIC LIVER TRANSPLANTATION

Whitney de Luna, Bo Yoon Ha, Aijaz Ahmed, Daniel Sze, Emmet B. Keefe, Mindie H. Nguyen; Stanford University, Stanford, CA

Purpose: Transcatheter arterial chemoembolization (TACE) is a common therapy for unresectable HCC. While both TACE and TACI are generally well tolerated by patients with compensated liver disease, those with poor hepatic tend to have higher rates of complications. Compared to TACE, TACI without embolization may have similar efficacy with fewer side effects and may allow us to include patients with less hepatic reserve. Our purpose is to examine outcome of TACI in HCC patients awaiting orthotopic liver transplantation (OLT) and its efficacy as a bridge towards transplantation. Methods: We performed a retrospective study of 160 TACI cases in 82 HCC patients between 4/98 - 3/07 at single U.S medical center. We examined 30 and 90 day-morbidity following TACI. We also measured overall patient mortality while waiting on transplantation list. Results: Mean age was 55 ± 7, 84% were male, 44% were Asian and 41% were white. Most had cirrhosis (88.6%), and either chronic hepatitis C (62.0%) or B (32.9%). TNM staging of I, II, III and IV were 25%, 57%, 13% and 5%, respectively. Mean MELD score was 10.1 ± 4.1 and mean CTP score was 6.5 ± 1.7. Number of patients with Child A, B, C was 45, 25, 7, respectively. 40 patients underwent OLT. The average waiting period to OLT is 6 months. Among 42 patients on waiting list, 7 patients died. The cause of death for the seven patients include multigain failure, metastasis, recurrent hepatitis C and infection. Only one patient died in 90 days following TACI and the other 6 patients died in the one or two years following TACI. Among the remaining 35 patients, 23 patients are alive and remained on transplantation list and 12 patients are lost for follow up. Only 7 patients were hospitalized for > 24 hours (6.25% = 10/160 cases) The cause of hospitalization for > 24 hours include nausea, vomiting, fever, variceal bleeding and encephalopathy. One patient (0.63% = 1/160 cases) had worsening of liver function at the same day following first TACI procedure. Conclusion: TACI is an effective and safe treatment for unresectable HCC prior to OLT. Most of our patients required less than 24 hours of hospitalization, while patients undergoing TACE generally require longer hospitalization. Rate of worsening of liver function following was only 0.63%. Rate of mortality related to TACI was 1.43% (1/70). TACI may be used as an effective bridge towards transplantation.
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INOS AND COX-2 SYNERGISTICALLY ENHANCE PGE2 PRODUCTION THROUGH S-NITROSYLATION OF CYTOSOLIC PHOSPHOLIPASE A2α IN HUMAN CHOLANGIOCARCINOMA CELLS
Lihong Xu, Chang Han, Kyu Lim, Tong Wu; Department of Pathology, University of Pittsburgh, School of Medicine, Pittsburgh, PA
Cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS) have recently been implicated in cholangiocarcinogenesis. The COX-2-mediated production of prostaglandin E2 (PGE2) requires concomitant activation of cPLA2α, which releases arachidonic acid (AA) from membrane phospholipids providing substrate for COX-2. This study describes a novel interplay among iNOS, cPLA2α and COX-2, which synergistically regulate the production of PGE2, a key lipid mediator perpetuating inflammation and driving carcinogenic process. Specifically, we show that iNOS-derived nitric oxide (NO) enhances PGE2 synthesis through S-nitrosylation of the cPLA2α enzyme in human cholangiocarcinoma cells and this effect is dramatically augmented by COX-2. Treatment of human cholangiocarcinoma cells (CCLP1 and SG231) with NO donor (GSNO) or transfection with iNOS adenoviral expression vectors in cholangiocarcinoma cells (CCLP1 and SG231) with NO donor (GSNO) or transfection with iNOS adenoviral expression vector induced S-nitrosylation of cPLA2α, resulting in a 2-3 fold increase of cPLA2 activity and AA release. These effects were blocked by the iNOS inhibitor, 1400W. Interestingly, iNOS overexpression in combination with COX-2 induction or overexpression induced a much more remarkable cPLA2α S-nitrosylation than either iNOS or COX-2 alone. Accordingly, overexpression of iNOS in combination with COX-2 induction or overexpression dramatically stimulated AA release by approximately 20 fold, whereas COX-2 induction alone caused only 1.5 fold increase of AA release. While COX-2 induction alone resulted in 20 fold increase of PGE2 production, combined COX-2 induction and iNOS overexpression enhanced PGE2 synthesis by approximately 100 fold. Furthermore, cPLA2α bound iNOS in human cholangiocarcinoma cells and their association was dramatically increased by overexpression of COX-2. Taken together, our data provide the first evidence that iNOS binds and S-nitrosylates cPLA2α and this effect is enhanced by COX-2 through formation of the iNOS-cPLA2α-COX-2 binding complex. The close proximity of these molecules in cell compartment provides a unique spatial advantage for iNOS-mediated S-nitrosylation and activation of cPLA2α, which cleaves AA for efficient PGE2 synthesis via COX-2. Therefore, therapy aimed at disrupting this interplay may represent a promising strategy to effectively inhibit PGE2 production and prevent cholangiocarcinogenesis.

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ROLE OF IKKβ/NF-κB ACTIVATION FOR DEVELOPMENT OF LIVER METASTASIS
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Liver metastasis is one of critical factors for determination of prognosis in cancer patients. Activation of NF-κB plays an important role in regulation of innate immune responses, inflammation and oncogenesis. Intrasplenic administration of lung carcinoma cells, LLC, and melanoma cells, B16F10, activate NF-κB through IκB kinase (IKK)-dependent phosphorylation, induces metastasis in the liver. To explore the roles of IKKβ, which is the critical kinase for NF-κB activation in metastatic process, we administered cancer cells into two kinds of conditional IKKβ knockout mice, hepatocyte-specific (Δhep) and whole liver knockout (Δl+h), and control (F/F) mice. The Δl+h mice developed liver metastasis with significantly lower numbers of metastatic foci compared to the Δhep and F/F mice. The intrasplenic tumor injection induced mRNA expressions of IL-6, cyclooxygenase (COX)-2, and matrix metalloproteinase (MMP)-9 in Δhep and F/F mice, whereas in Δl+h mice these genes were poorly expressed. These observations indicate that IKKβ/NF-κB signals in non-parenchymal cells such as Kupffer cells play critical roles in liver metastasis. In IL-6 knockout mice, number of liver metastasis was marked decreased compared with controls. We also tested whether NEMO-binding domain (NBD) peptide, which was shown to block association of NEMO with IKKβ and inhibit NF-κB activity, reduces liver metastasis in mice. NBD peptide reduced tumor foci through downregulating proinflammatory cytokines such as IL-6. Collectively, these observations suggest the IKKβ/NF-κB signaling pathway is an attractive target for the development of anti-metastatic drugs.

Disclosures:
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A PROTECTIVE ROLE FOR ADIPONECTIN IN HUMAN HEPATOCELLULAR CARCINOMA: ACTIVATION OF AMPK INHIBITS HEPG2 PROLIFERATION IN VITRO AND STUNTS TUMOR GROWTH IN HEPG2-NUDE MICE XENOGRAFTS
Neeraj K. Saxena1, Xiaokun Ding1, Dipali Sharma2, Frank A. Anania1; 1Medicine, Division of Digestive Diseases, Emory University School of Medicine, Atlanta, GA; 2Haematology and Oncology, Winship Cancer Institute School of Medicine, Atlanta, GA
Adiponectin, secreted by white adipose tissue, has been proposed to possess therapeutic properties and is inversely correlated with obesity. Low levels of serum adiponectin are associated with a higher relative risk for cancer of the breast, endometrium, stomach, colorectum, pancreas, and hepatocellular carcinoma (HCC). The purpose of the present study was to elucidate the mechanism whereby adiponectin negatively modulates HCC growth. METHODS: mRNA transcripts and protein expression of adiponectin receptors, Adipor1 and Adipor2, were detected in the human HCC cell line HepG2 by RT-PCR and immunoblot analysis, respectively. Adiponectin dose and time studies were conducted to determine cell viability and cell proliferation by BrdU incorporation. Metastatic properties, including the effect of adiponectin on cell migration, were determined, by Electric Cell-substrate Impedance Sensing (ECIS). Adiponectin signal-transduction was assessed by immunoblot assay for phosphorylated S5-AMP-activated protein kinase (AMPK), PCNA, Cyclin-D1, p21/WAF, caspase-3 and cleaved caspase-3. Five-week old athymic nude-mice were injected with 5 x 10⁶ HepG2 cells into the right gluteal region. Two weeks later mice were subjected to either intraperitoneal saline injections or adiponectin (84µg/g/day) for four weeks. Tumor growth was monitored regularly by caliper measurements. RESULTS: Adipor1 and Adipor2 transcripts and respective proteins were identified in HepG2 cells. Optimization from dose and time course studies revealed that full-length
adiponectin treatment [10µg/ml] reduced HepG2 cell proliferation by 50% and increased apoptosis by six-fold as compared to serum-free (SF) treatment (p<0.01). Adiponectin significantly increased both phosphorylation of AMPK and cleaved caspase-3 production by three-fold (p<0.01). Adiponectin reduced PCNA expression and down-regulated cyclinD1 while up-regulating p27KIP2. Adiponectin decreased the rate of HepG2 cell migration in the wound-healing assay compared to SF conditions. None of the effects of adiponectin in HepG2 were observed in primary cultured hepatocytes. HepG2-xenograft-nude mice resulted in large tumors. Adiponectin treatment dramatically reduced the tumor burden as compared to saline-treated control mice. **CONCLUSIONS:** These data indicate that adiponectin has significant potential to inhibit malignant properties of HCC by decreasing cell proliferation and increasing apoptosis of HepG2 via AMPK activation. Taken together with the in vivo data, adiponectin may be an attractive therapy for human HCC.

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The following people have nothing to disclose: Neeraj K. Saxena, Xiaokun Ding, Dipali Sharma, Frank A. Anania

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**CANCER TARGETED AND TISSUE SPECIFIC GENE THERAPY OF IN VIVO HEPATOCELLULAR CARCINOMA MODEL BY HTERT-TARGETING TRANS-SPlicing RIBOZYMES AND LIVER SPECIFIC PROMOTER**

**Jin S. Jeong**, Sang Y. Han, Min S. Song, Seong W. Lee, In H. Kim, Dipali Sharma, Frank A. Anania

Hepatocellular carcinoma (HCC) is one of the most frequent human cancers worldwide. Effective treatment, surgical resection is available for only a small number of patients. Advanced HCC, multiple tumors, distant metastasis, recurrent tumors and even small HCC with impaired liver function have been required to develop new therapeutic modalities. In this study, we investigate a specific and efficient approach to HCC treatment that is based on a HCC-specific trans-splicing ribozyme. We constructed adenoviral vectors containing hTERT targeting trans-splicing ribozyme with downstream lacZ gene (Adv-PEPCK.Ribo.LacZ) or therapeutic suicide gene HSVtk (Adv-Ribo.Tk) under the control of liver-specific PEPCk or CMV promoter. We maintained hTERT(+)- Hep3B and HepG2 HCC cell lines, and hTERT(-) THLE3 non-tumorigenic normal liver cell line. For carcinoma peritonei model, we injected 2x 107 of Hep3B cells, intraperitoneally (i.p.) in BALB/C male nude mice and observed 95% of success rate within 21 days in the pilot work. For the specific transgenic expression, X-gal staining in removed abdominal organs and tumors in toto, and frozen tissue sections was done. For antitumor effect, we injected 1×109 pfu of Adv-PEPCK.Ribo.Tk (n=10), Adv-CMV.Ribo.Tk (n=10) and Ad-PEPCK-LacZ (n=10, as control) on 18th day, intraperitoneally, following injection of gancyclovir (50mg/kg), twice per day for 10 days. Eighteen days after virus injection, the total mass weights of removed tumors were measured. For the hTERT expression levels, RT-PCR and immunohistochemistry were performed. We observed tumor specific expression of B-galactosidase in Adv-PEPCK.Ribo.LacZ group and both liver and tumor in Adv-PEPCK.LacZ group. Twenty eight mice (no tumors in one mouse injected with Adv-PEPCK.Ribo.LacZ and Adv-PEPCK.LacZ, each) showed carcinoma peritonei. The mean tumor weights (mg) of control, Adv-PEPCK.Ribo.Tk and Adv-CMV.Ribo.Tk groups were 8.26±2.97, 3.91±1.66 and 2.36±1.39, showing significantly reduced tumor growth in treated groups, compared to control (Mann-Whitney [U] test, PEPCK; 0.0016, CMV; 0.0006). In addition to the hTERT-dependent therapeutic gene induction, significant reduction of the levels of hTERT RNA (> 75%) were observed in HCC mouse model by the specific ribozyme. These results showed that hTERT targeting TSR with PEPCk promoter represents a powerful dual targeting and dual efficacy agent for tumor targeted and liver specific gene therapy of HCC.

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634 RELEVANCE OF IGF SIGNALING PATHWAY IN HUMAN HEPATOCELLULAR CARCINOMA, AND PRE-CLINICAL ASSESSMENT OF NOVEL TARGETED THERAPIES

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BACKGROUND: Aberrant activation of IGF signaling has been involved in cell transformation, proliferation and survival in cell cultures and cancer mouse models, and has been implicated in human cancer. Targeted therapies abrogating this pathway have scarcely been tested in hepatocellular carcinoma (HCC).

AIMS: We aimed to explore the role of IGF signaling in a large cohort of HCV-induced HCC and to assess the impact of abrogating this pathway by blocking IGF-1R activity. METHODS: We evaluated the IGF pathway in 105 paired HCV-related HCC/non-tumoral cirrhotic liver and 10 healthy liver tissues assessing the activation status of six proteins (IGF-II, pIGF-1R, pIRS-1, pAkt, pERK, and pS6) by immunohistochemistry, and western blot, the expression levels of 7 genes (IGF-I, IGF-II, IGF-1R, IGFBP-3, IRS-1, IRS-2, IGFBP-3 and PTEN) by quantitative real-time PCR and the structural status using SNP-array (Affymetrix STY Mapping Array®). Liver cancer cell lines (Huh7, Hep3B and HepG2) were incubated with increasing concentrations of a monoclonal antibody against IGF-1R (A12, ImClone). We assessed cell viability (MTT assay), cell proliferation (3[H] Thymidine Incorporation Assay, FACS) for cell cycle analysis and immunoblot to evaluate protein expression. Experimental studies with HCC xenograft testing this molecular therapy were conducted. RESULTS: Activation of IGF signaling (defined by immunohistochemical analysis of pIGF-1R) was detected in 26% of cases, which significantly correlated with gene expression levels of IGF-II and IGFBP-3 (p=0.043 and p=0.038, respectively). Expression of IGF-II was up-regulated (>10 fold change) in 12% of HCCs in comparison to normal samples, whereas the tumor suppressors IGFBP-3 and PTEN were significantly down-regulated, >10 fold change in HCCs (both in 25% of cases). Increase copy number changes of IGFBP-3 by SNP array analysis was significantly associated with pIGF-1R (p=0.017). Abrogation of the IGF1R activation with the monoclonal antibody A12 [0.1 – 200 nM] decreased cell viability by 48h by 35%, and was able to decrease cell proliferation by 15-30% without inducing significant apoptosis, as revealed by flow citometry analysis. CONCLUSION: IGF pathway is dysregulated in a subgroup of HCCs patients, as a result of an increase of both IGFBP-3 ligand and IGFBP-3 copy number changes. Abrogation of IGF1R signaling in vitro reduces cell viability and proliferation. There is rationale for testing A12 monoclonal antibody against IGF-1R in experimental HCC models.

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IFNγ IS CRITICALLY REQUIRED FOR IL-12-MEDIATED NK CELL ACTIVATION AND ANTI-TUMOR EFFECT IN THE LIVER
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Background and Aim: The liver is the most common site of metastatic malignancy and the status of this organ is an important determinant of survival in patients with advanced disease. Since the liver contains an abundance of immune cells, the cytokine-mediated activation of these cells may be a promising approach toward this end. IL-12 is a pleiotropic cytokine and a strong inducer of IFNγ. IL-12 has been shown to elicit anti-tumor effects in various murine models of cancer. But the mechanisms have not been fully elucidated. The aim of this study was to examine the role of IFNγ in anti-tumor effect of IL-12 by using IFNγ knockout mice (GKO mice). Methods: We hydrodynamically injected naked plasmid DNA encoding IL-12 (pCMV-IL-12) into mice 2 days after intrasplenic injection of CT-26 cells. The serum IL-12 and IFNγ levels were measured using ELISA. The NK activity of mononuclear cells was assessed with standard 4-hour 51Cr-releasing assay using Yac1 cells as targets. Mononuclear spleen cells of wild type mice were injected via tail vein into GKO mice 1 days before hydrodynamic injection of plasmid DNA. Expression of phospho-STAT1 of whole cell lysate separated from mononuclear cells was analyzed by Western blotting. Results: Single injection of pCMV-IL-12 efficiently enhanced the NK activity of hepatic mononuclear cells, induced serum IFNγ elevation and led to complete rejection of tumors in the liver. All effects of pCMV-IL-12 were abolished in GKO mice. NK cells were critically required for IL-12-mediated rejection of hepatic metastasis, because their depletion by injecting anti-asialo GM1 antibody completely abolished the anti-metastatic effect. When wild-type splenocytes were adoptively transferred into GKO mice before pLl2 injection, anti-tumor effect and NK cytolysis were restored. Upon IL-12 stimulation splenocytes from wild-type mice showed enhanced NK activity and phosphorylation of STAT1, but those from GKO mice did not. When splenocytes from GKO mice cultured with recombinant IL-12 and recombinant IFNγ, NK cytolysis was restored and the expression of phospho-STAT1 was detected. Conclusion: NK cells play critical roles for IL-12-mediated initial rejection of hepatic metastasis of micro-disseminated tumors. The IFNγ production from mononuclear cells is significantly important for the NK activation and the anti-tumor effect of IL-12.

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LIBRARY OF GENETIC ASSOCIATIONS: CONNECTING COMPLEX LIVER DISEASES AND GENETICS
Stefan Buchkremer, Jasmin Hendel, Markus Krupp, Arndt Weinmann, Kai Schlamp, Peter R. Galle, Andreas Teufel; Department of Medicine I, Johannes Gutenberg University, Mainz, Germany

A genetic basis has been established for almost all common liver diseases. However, the genetic basis of most of these diseases must be assumed to be rather due to a complex genetic interaction of multiple genes than to an individual, single gene. Thus, future efforts to unravel the genetic basis of common liver diseases will necessarily have to look into genetic networks and signaling pathways. However, at present no database is available providing a complete overview of genetic associations in common liver diseases. Therefore, we have established a Library of Genetic Associations providing these genetic informations for HCC, NASH, AIH, PBC, and PSC. In order to establish this database, the complete Pubmed database, currently containing more than 15 million publications, has initially been searched by means of MeSH terms and text mining algorithms. These tools suggested 57800 abstracts to provide evidence for genetic associations. Subsequently, these remaining publications were than individually and manually validated for genetic associations, leaving approximately 500 confirmed genetic associations for these diseases. Finally, validated informations were stored in a publicly available database (www.medicalgenomics.org/databases/LoGA). This database is searchable via a web interface by diseases, pathways or genes. Furthermore, the database output contains information on the genetic association and publications providing the respective genetic information. We are currently further extending this genetic resource to additional diseases such as viral hepatitis, fibrosis, and cirrhosis. Together, this novel database provides the complete current knowledge on genetic associations of HCC, NASH, AIH, PBC, and PSC. As it is publically and automated searchable, it will be a valuable resource for the analysis of complex genetic networks and clusters contributing to the development of chronic liver disease.

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INHIBITION OF HEPATIC TUMOR ANGIogenesis WITH MET INHIBITORS
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Background: Aberrant function of the tyrosine kinase Met receptor has been associated with numerous types of human cancers including hepatocellular carcinoma (Kaposi-Novak, JCI 2006). Met disregulated activity correlates with local invasion and metastasis. Met signalling stimulates VEGF production and promotes tumor angiogenesis. Therefore, we evaluated the potential of the Met inhibitors SU11274 and PHA665752 to prevent angiogenesis in an orthotopic liver tumor model.

Methods: NIH3T3 cells stably expressing Met constitutively activated mutated variant receptors, were injected subcutaneously into the liver of SCID mice. Two mutations were used, M1268T and Y1248H that exhibit respectively sensitivity and resistance towards the Met tyrosine kinase inhibitors PHA665752 and SU11274. One week after implantation, animals were randomized for PHA665752 (25mg/kg/day i.p.) or vehicle treatment. Following 7 days of treatment, tissues were harvested and tumor size was measured. Microvessel density was assessed by CD31 immunostaining. In vitro, the production of VEGF by M1268T (sensitive) and Y1248H (resistant) cells was determined by ELISA. The angiogenic effect of the media was assessed with the rat aortic ring assay. Means±standard deviations, Mann-Whitney test.

Results: M1268T (sensitive) tumors measured 716±164 mm³
PEGYLATED INTERFERON ATTENUATES WNT/β-CATENIN SIGNALING IN HEPATOCELLULAR CANCER

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Pegylated-Interferon (Peg-IFN) is used in patients with chronic Hepatitis C virus (HCV) infection. An observed consequence of such treatment is decreased occurrence of hepatocellular carcinoma (HCC). HCC remains the most common form of liver cancer, with poor prognosis. Wnt/β-catenin activation contributes to the development of a significant subset of HCC. Furthermore, activation of this pathway has been noted in many cases of HCV infection. We explored the possibility that anti-HCC activity of Peg-IFN might be through the inhibition of Wnt/β-catenin signaling. We treated two human hepatoma cell lines (HepG2 & Hep3B) with Peg-IFN or Ribavirin. Western blot and transcriptional activity of β-catenin was measured via the TOPFLASH luciferase reporter assay. Transgenic mice overexpressing a stabilized form of β-catenin (Serine-45-mutated) were exposed to Peg-IFN, which may be the mechanism of delayed HCC in treated HCV patients. In addition, this observation might have chemopreventive and chemotherapeutic implications in the scenario of aberrant canonical Wnt/β-catenin signaling.

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EXPLOITING THE MULTIPLE MECHANISMS OF SORAFENIB: TUMOR GROWTH INHIBITION WITH A NOVEL COMBINATION OF SORAFENIB AND RAPAMYCIN TARGETING BOTH RAS AND MTOR PATHWAYS

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Background: Sorafenib has a proven survival benefit in advanced HCC. It is a potent inhibitor of Raf-1, a serine/threonine protein kinase, and has activity against several receptor tyrosine kinases, including VEGFR, PDGFR, and c-kit. Because of the heterogeneity of HCC, however, combination with other therapies targeting additional pathways may be more effective in these advanced tumors. Rapamycin is an mTOR inhibitor approved for immunosuppression post transplantation, with known anti-tumoral activity, whose efficacy in HCC is unknown. Aims: 1) To characterize Ras and mTOR pathway activation in human HCV-hepatocarcinogenesis, and 2) to assess the impact of their antagonism using Sorafenib in combination with Rapamycin, in culture and in a xenograft model. Methods. We investigated the expression of several key genes of the Ras pathway in 77 human HCC samples by qRT-PCR and used immunohistochemistry to localize expression of phosphorylated mTOR. Mutation analysis for k-Ras mutations was performed using direct sequencing. Liver cancer cell lines were incubated with increasing concentrations of ICG-001 to assess cell viability (MTT assay), proliferation (3[H] Thymidine Incorporation Assay), and apoptosis (flow cytometry). Xenograft nude mouse models were administered both agents alone and in combination. Immunoblotting was performed to demonstrate abrogation of downstream protein targets, including phosphorylated ERK and S6. Results: H-RAS was upregulated 4-fold in advanced HCC human samples compared to normal liver. Membranous staining of phospho-mTOR was lost in HCC (p<0.001). There was an additive effect of Sorafenib and Rapamycin in vitro, leading to decreased cell viability (p<0.001) and inhibition of proliferation (p<0.001). Flow cytometry confirmed apoptosis of HCC cell lines with Sorafenib alone and in combination with Rapamycin. Xenograft models are showing an early trend toward increased survival in all three treated groups compared to controls. Conclusions: Sorafenib and rapamycin synergize to inhibit tumor growth. Aberrant signaling through both the Raf/MEK/ERK pathways and the Akt/mTOR pathways has been demonstrated in human HCC, and this study provides compelling evidence that the combination of Sorafenib and Rapamycin merits consideration in human trials of HCC.

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in vehicle treated mice and 125±37 mm³ in PHA665752 treated mice (n=4, p<0.05). This was accompanied by a reduction by 35% of the microvessel density in the treated tumors. Y1248H (resistant) tumors measured respectively 178±52 mm³ and 346±227 mm³ (p>0.05) and microvessel density in drug treated tumors was increased by 47%. Treatment of M1268T (sensitive) cells with PHA665752 led to a 4 fold reduction in culture medium VEGF levels (p<0.05) whereas VEGF levels in medium of Y1248H (resistant) cells remained unchanged. In the rat aortic ring assay, vessels number was reduced by 44% after addition of medium from 2µM SU11274-treated cells compared to medium from untreated M1268T (sensitive) cells (p<0.05). Exposure to the medium of the Y1248H (resistant) cells treated with SU11274 or PHA665752 increased the number of vessels by 32% and 76% respectively. Conclusions: Tyrosine kinase inhibitors of Met receptor prevent Met-dependent VEGF production and hepatic tumoral angiogenesis in tumors expressing a drug sensitive receptor. In contrast, the same inhibitors seem to promote tumoral growth and angiogenesis in tumors expressing a drug-resistant receptor. These data emphasize the angiogenic role of Met signalling in hepatic tumor formation and stress the therapeutic importance to determine the nature of the Met receptor expressed.

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641 ACTIVATION OF THE WNT/B-CATENIN PATHWAY IN HEPATOCELLULAR CARCINOMA, AND IN VITRO GROWTH INHIBITION WITH A NOVEL SMALL MOLECULE ICG-001

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Background: Aberrant Wnt signaling has been implicated in the pathogenesis of HCC. There are no drugs approved that target this pathway in any tumors. ICG-001 is a small molecule which specifically inhibits the interaction between b-catenin and Cbp-binding protein (CSP), causing repression of a subset of TCF/b-catenin-mediated transcription. This small molecule has been shown to reduce growth in vitro of colon cancer but has not been evaluated in HCC. Aims: 1) To characterize Wnt pathway activation in hepatocarcinogenesis in a large cohort patients with HCV-related HCC; and 2) to test the effect of Wnt pathway abrogation with a novel small molecule inhibitor, ICG-001. Methods: Expression of Wnt pathway genes in 104 human HCV+ HCC samples was measured by qRT-PCR, and immunohistochemistry was used to localize b-catenin and E-cadherin. B-catenin gene mutations in exon 3 were assessed by direct sequencing (N=93 cases). Integrative genomic analysis of samples from the same cohort was performed using 250K SNP arrays and genome-wide microarrays (Affymetrix, Genechip Human Genome-U133 Plus 2.0). HCC cell lines were incubated with increasing concentrations of ICG-001 to assess cell viability (MTT), proliferation (3[H] Thymidine Incorporation), and apoptosis (flow cytometry). Luciferase reporter plasmids were used to measure TCF/b-catenin-mediated transcriptional activity. Results: Nuclear staining of B-catenin was observed in one-quarter of HCCs, but not in cirrhotic tissues, and was trendly correlated with tumor recurrence (p=0.09). Among 93 HCCs, point mutations in B-catenin were identified in 16 (17%), which correlated with recurrence (p=0.02). There was a significant correlation between B-catenin point mutations and nuclear staining (p=0.007). B-catenin was upregulated >2 fold in 34% of HCC samples across progressive stages of HCC. Interestingly, nuclear staining of B-catenin significantly correlated to lower levels of mRNA. ICG-001 decreased cell viability by 40-60% and proliferation by 80% in vitro in HCC cell lines, demonstrating a dose-dependent effect. ICG-001 also sensitized cells to apoptosis. Finally, ICG-001 reduced TCF/b-catenin-mediated transcriptional activity as measured by a luciferase reporter (p<0.00001). Conclusions: Wnt signaling is clearly activated in a subset of Hepatic C-induced HCCs, and correlates with tumor recurrence. The small molecule ICG-001 significantly decreased cell viability and proliferation by abrogating the Wnt pathway. These results provide the rationale for testing this compound in experimental models.

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642 CLOSE RELATIONSHIP BETWEEN CYTOKERATIN 19 EXPRESSION AND SIDE POPULATION PHENOTYPE DURING HUMAN HEPATOCELLULAR CARCINOGENESIS

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Aims: There is increasing evidence that stem cells might participate in the development of various tumors, including hepatocellular carcinoma (HCC). Cytokeratin 19 (CK19) expression is one of the most reliable phenotypic markers of hepatic stem/progenitor cells. The side population (SP) phenotype is a recently discovered functional marker of stem/progenitor cells. It is now believed that the SP phenotype is maintained by the expression of ATP-binding cassette transporter G2 (ABCG2) or multidrug resistance-1 (MDR-1). In this study, we examined relationships between CK19-positive cells and the SP phenotype during carcinogenesis of human HCC. Methods: We performed dual fluorescent immunostaining of CK19/ABCG2 and CK19/MDR-1 on pathological specimens (15 HCCs and 10 dysplastic nodules) and three HCC cell lines (HepG2, HuH7 and PLC5). Next, we tried to identify SP cells in each cell line, and sort into SP and non-SP fractions. Expressions of CK19, ABCG2 and MDR-1 in SP and non-SP fractions were examined by RT-PCR and dual fluorescent immunostaining. Results: In the non-neoplastic livers, expressions of CK19, ABCG2 and MDR-1 were closely co-localized in bile ducts, bile ductules and parenchymal oval cells. In dysplastic nodules, small dysplastic cells around entrapped portal tracts were positive for these three molecules. In addition, carcinoma cells dual positive for CK19/ABCG2 and CK19/MDR-1 were also identified in histological specimens of HCCs. We could isolate and sort into SP and non-SP cells from HuH7 and PLC5, but not HepG2. SP cells of HuH7 and PLC5 could generate SP and non-SP progenies, whereas non-SP cells generated only non-SP in sustained culture. This result suggested that tumor cells with SP phenotype are located at the higher level of tumor-cell hierarchy, and cancer stem cells might exist in SP cells. SP cells more intensely expressed CK19 than non-SP, and CK19 expression in SP cells decreased time-dependently during sustained culture. In addition, ABCG2 and MDR-1 were commonly expressed in CK19-positive cells in SP fractions. Conclusion: This study revealed that CK19-positive cells were closely related to expressions of ABCG2 and MDR-1 during carcinogenesis of human HCC in vivo. In addition, in vitro studies revealed that carcinoma cells double positive for CK19/ABCG2 or CK19/MDR-1 were enriched in SP fractions of human HCC cell lines. This study suggested that CK19 expression and SP phenotype might be closely related during carcinogenesis of human HCC.

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643 THE INTRONIC P53 BINDING SITE IS ESSENTIAL FOR ACTIVATION OF THE CD95 GENE BY THE P53 FAMILY NETWORK

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Introduction: p53 is a key regulator of cell-cycle control, apoptosis and genomic stability. The p53 family members p63 and...
p73 give rise to proteins that have p53-agonistic as well as p53-antagonistic functions. However, like p53, they possess the ability to induce apoptosis after DNA damage. p63 and p73 express two main classes of isoforms: isoforms which contain the transactivation domain (TaP73 and TaP63) executing transcrip tional activity and dominant negative isoforms which are truncated at the NH2-terminus (DeltaNp63 and DeltaNp73) acting as operant inhibitors of TaP73, TaP63 and wild-type p53, and thus containing oncogenic potential. Aim: The aim of our study has been to provide insight into the molecular mechanisms of the regulation and transactivation of the CD95 gene. Methods: 1. Identification of the p53 binding site in intron 1 and three p53 binding sites in the promoter of the CD95 gene by immunoselection assay and computer analyses. 2. Establishment of CD95-Luciferase constructs with mutations and deletions of the p53 binding sites of the CD95 gene. 3. Performance of chromatin immunoprecipitation (ChIP) to show the ability of wt p53, TaP63 and TaP73 protein to bind directly the p53 binding site in the first intron of the CD95 gene. 4. Assessment of the p53/TaP63/TaP73-dependent transactivation of the CD95 gene, using different established CD95-Luciferase constructs. Results: Wt p53, TaP63 and TaP73 transactivate the CD95 gene via binding to the p53 binding site of intron 1 and three p53 binding sites in the promoter of the CD95 gene. Transcriptional activation of the CD95 gene by all p53 family members involves cooperation between the p53 binding sites of the CD95 promoter and the intronic p53 binding site. By mutational analyses, we can clearly identify the intrinsic p53 response element as the most important binding site for the transactivation of the CD95 gene by wt p53, TaP63 and TaP73. The oncogenic potential of the dominant negative isoforms of p63 and p73 is evidenced by the fact that deltaNp63 and deltaNp73 dramatically inhibit the wt p53-, TaP63- and TaP73-mediated transactivation of the CD95 gene. Discussion: Deregulated dominant negative p63 and p73 isoforms play an oncogenic role in human cancer and contribute to chemoresistance. Here we show the molecular mechanisms of the regulation of the CD95 gene by the full-length TA isoforms of p63 and p73. Furthermore, we show the inhibition of the transactivation of the CD95 gene by deltaNp63 and deltaNp73. This leads to resistance towards apoptosis. Interfering with the expression of deltaNp63 and/or deltaNp73 in tumor cells may render such tumors more responsive to therapy.

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EXPRESSION OF MULTIDRUG RESISTANCE-ASSOCIATED PROTEIN 1 IN HEPATOCELLULAR CARCINOMA IS ASSOCIATED WITH AGGRESSIVE TUMOR PHENOTYPE AND CAN REFLECT PROGENITOR CELL ORIGIN

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Patients with hepatocellular carcinoma (HCC) often have poor response to treatment with chemotherapy due to intrinsic or acquired multidrug resistance (MDR). This MDR can be caused by over-expression of ATP-binding cassette transporters in the tumor cells. The transcriptional profile of ABC transporter genes was assessed in a series of 65 solitary untreated HCCs that were earlier subjected to global gene expression analysis. The multidrug resistance-associated protein 1 (MRP1, ABCC1) appeared as the only ABC transporter whose expression level was significantly up-regulated in HCCs in comparison with surrounding normal tissue and its expression level is well associated with a more aggressive phenotype. Recent studies showed that HCC could be of progenitor cell origin. In view of the specific expression of MRP1 in hepatic progenitor cells (HPCs) and not in hepatocytes, expression of this transporter in HCC can be a reflection of progenitor cell origin and provide the tumor cells with a MDR phenotype. To confirm this micro-array data we performed real-time RT-PCR and immunohistochemistry for MRP1 (ABCC1), MRP3 (ABCC3), MDR1 (ABCB1), BCRP (ABCG2) and biliary/ progenitor cell markers cytokeratin 7 (CK7) and CK19 on an independent set of 23 primary untreated hepatocellular carcinomas and surrounding non-tumoral liver (available in 11 cases). Data were correlated with clinical pathological data of the HCCs. In 11 of 23 HCCs we observed a basolateral staining pattern of MRP1 in the tumor cells. In 9 of the HCCs, we observed MRP1 positivity in more than 30% of tumor cells. MRP1 protein expression is associated with differentiation grade, tumor diameter and microvascular invasion. Expression of CK7 and/or CK19 in more than 5% of tumor cells was seen in 9 of 23 patients (34%). Expression of CK19 only was seen in 5 of 23 patients (22%) of which 4 were MRP1 positive in the same areas of the tumor. MRP1 mRNA levels were significantly up-regulated in HCCs in comparison with surrounding non-tumoral liver and associated with invasion, differentiation grade and tumor diameter. All other transporters, including MDR1 showed a trend towards decreased protein and mRNA expression probably as a sign of dedifferentiation. The strong basolateral MRP1 expression can result in more growth potential and a more aggressive phenotype of the tumor by pumping toxic substances out of the tumor cells and can be the reflection of a progenitor cell origin. Given the existence of specific MRP1 inhibitors, this finding may be of therapeutic relevance. (1) Lee JS et al. Nature Medicine 12(4): 410; (2) Durnez A et al. Histopathology 49(2):138

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SELECTIVE HOMING OF ENDOTHELIAL PROGENITOR CELLS WITH CYTOSINE DEAMINASE CDNA TO TUMOR TISSUES SUPPRESSES GROWTH OF HEPATOCELLULAR CARCINOMA BY 5-FLUOROURACIL SECRETION

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Background/Aim: Cytosine deaminase (CD) is the enzyme that transforms nontoxic 5-fluorocytosine (5-FC) into the highly toxic 5-fluorouracil (5-FU). Recently, it has been proved that endothelial progenitor cells (EPC) are selectively recruited into tumor tissues. Stromal cell-derived factor-1 (SDF-1) and its receptor CXCR4 interactions tightly regulate the homing of stem and progenitor cells. In the present study, we investigated the anti-tumor effect of a CD/5-FU system with CD cDNA transfected EPC for hepatocellular carcinoma (HCC).

Methods: Human hepatoma cell line (HuH-7) and rat EPC cell line (TR-BME) were

Aims: Identification of the p53 binding site in intron 1 and three p53 binding sites in the promoter of the CD95 gene by immunoselection assay and computer analyses. 2. Establishment of CD95-Luciferase constructs with mutations and deletions of the p53 binding sites of the CD95 gene. 3. Performance of chromatin immunoprecipitation (ChIP) to show the ability of wt p53, TaP63 and TaP73 protein to bind directly the p53 binding site in the first intron of the CD95 gene. 4. Assessment of the p53/TaP63/TaP73-dependent transactivation of the CD95 gene, using different established CD95-Luciferase constructs. Results: Wt p53, TaP63 and TaP73 transactivate the CD95 gene via binding to the p53 binding site of intron 1 and three p53 binding sites in the promoter of the CD95 gene. Transcriptional activation of the CD95 gene by all p53 family members involves cooperation between the p53 binding sites of the CD95 promoter and the intronic p53 binding site. By mutational analyses, we can clearly identify the intrinsic p53 response element as the most important binding site for the transactivation of the CD95 gene by wt p53, TaP63 and TaP73. The oncogenic potential of the dominant negative isoforms of p63 and p73 is evidenced by the fact that deltaNp63 and deltaNp73 dramatically inhibit the wt p53-, TaP63- and TaP73-mediated transactivation of the CD95 gene. Discussion: Deregulated dominant negative p63 and p73 isoforms play an oncogenic role in human cancer and contribute to chemoresistance. Here we show the molecular mechanisms of the regulation of the CD95 gene by the full-length TA isoforms of p63 and p73. Furthermore, we show the inhibition of the transactivation of the CD95 gene by deltaNp63 and deltaNp73. This leads to resistance towards apoptosis. Interfering with the expression of deltaNp63 and/or deltaNp73 in tumor cells may render such tumors more responsive to therapy.

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used. In vitro; E. coli CD cDNA was transfected into TR-BME with a retroviral system (CD-TR-BME). The expression of CXCR4 on CD-TR-BME was assessed by RT-PCR. The inhibitory effect of 5-FU secreted by CD-TR-BME with 5-FC on the proliferation of co-cultured HuH-7 was evaluated by a tetrazolium-based assay. In vivo; Nude mice received subcutaneous injection of HuH-7 (5 x 10^6). After tumor formation, the mice received CD-TR-BME (5 x 10^7) injection via tail vein for 5 days and then received intraperitoneal injection of 5-FC (500 mg/Kg body weight) for 10 days. During the course of experiment, tumor volume was measured every 3 days. SDF-1 expression in tumor tissue was evaluated by RT-PCR. The localization of CD-TR-BME in tumor tissues was investigated. Serum and tumor tissue S-FU levels were measured. Results; In vitro; Transfection of CD cDNA in TR-BME was confirmed by RT-PCR. CD-TR-BME expressed CXCR4. CD-TR-BME secreted 5-FU and inhibited the proliferation of HuH-7 in a dose dependent manner of 5-FC addition. In vivo; Tumor tissues expressed SDF-1. CD-TR-BMEs were selectively recruited into the tumor tissues and incorporated into endothelial cells of tumor vessels. Tumor growth was significantly suppressed in the period of 5-FC administration. 5-FU level in tumor tissue was 26.4 ± 11.3 ng/g tissue weight. However, 5-FU was not detected in the mouse serum. Body weight loss and bone marrow suppression were not observed. Conclusion; These results suggest that the new CD/S-FU system with CD cDNA transfected EPC will be an effective and safe strategy to suppress the growth of HCC. Selective homing of endothelial progenitor cells with cytokine deaminase cDNA to tumor tissue suppresses the growth of hepatocellular carcinoma by 5-fluorouracil secretion.

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MAPK7 IDENTIFIED AS A PROBABLE TARGET FOR AMPLIFICATION AT 17P11 IN HEPATOCELLULAR CARCINOMA

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(Background and aims) Amplification of chromosomal DNA is one mechanism capable of activating genes whose over-expression contributes to the development and progression of cancer. The recent introduction of high-density single nucleotide polymorphism (SNP) arrays enable high-resolution and genome-wide identification of DNA copy-number aberrations in cancer. The aim of the present study is to detect novel amplification in HCC cell lines using SNP arrays that have been missed in a conventional analysis, such as via comparative genomic hybridization (CGH), and to investigate the role of the target gene within the amplicon in HCC cells. (Methods) The GeneChip Mapping 100K array set and GeneChip Mapping 250K Sty array (Affymetrix) were used in this study. Genomic DNA and mRNA of MAPK7 were quantified using real-time PCR. MAPK7 expression was down-regulated by small interfering RNA (siRNA). Cell viability was determined by MTT assay. (Results) Two of the twenty HCC cell lines exhibited amplification at chromosomal region 17p11 via SNP array analysis. We were able to define the smallest commonly affected region in the 17p11 amplicon. This region includes seven known or predicted protein-coding genes, GRAP, EPN2, EPPB9, MAPK7, MFAp4, ZNF179, and FLJ10847. Of all seven genes including the region, we found that mitogen-activated protein kinase 7 (MAPK7), which encodes extracellular-regulated protein kinase 5 (ERK5), was over-expressed in cell lines in which the gene was amplified. ERK5 is a member of the mitogen-activated protein kinase (MAPK) subfamilies that transmit extracellular signals from cell surface receptors to specific targets within cells. Several lines of evidence have suggested that ERK5 is implicated in tumorgenesis. In our studies, copy-number gain for MAPK7 was observed in 35 of the 66 primary HCC tumors (53%). We next investigated the effects of MAPK7 over-expression on HCC cells. PD98059, an inhibitor of the MEK5/ERK5 and MEK1/ERK1/2 pathways, caused concentration-dependent growth inhibition of a HCC cell line with the most remarkable amplification and over-expression of MAPK7. Because we were not able to find a specific chemical inhibitor of the MEK5/ERK5 pathway, we knocked down MAPK7 expression via RNAi. siRNA-mediated down-regulation of MAPK7 suppressed growth of the HCC cells. (Conclusion) Our results suggest that MAPK7 is likely to be a target of 17p11 amplification and may play a role in the growth of HCC cells and thus, that the ERK5 protein product of the MAPK7 gene may be an optimal target for development of novel therapies for this widespread type of cancer.

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NEIGHBOR OF PUNC E11 (NOPE): A NOVEL MARKER FOR MURINE HEPATOCELLULAR CARCINOMA

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Hepatocellular carcinoma (HCC) is the 5th common malignancy worldwide and the incidence has almost doubled within the last 40 years. As established markers fail to detect up to one third of HCC, the identification of new markers is particularly relevant. We have recently identified Neighbor of Punc E11 (Nope) as a surface marker of murine fetal liver stem cells. In analogy to established HCC markers like α-Fetoprotein (AfP) and Glypican-3 (Gpc-3), we here investigate Nope as a potential onco-fetal marker of HCC and its expression during hepatocarcinogenesis. Methods: We induced HCC in 8 to 12 week old, Cre-inducible SV40 T-antigen transgenic mice (Alb-SV40TAgind). Animals were sacrificed at different time points after tumor induction to obtain specimens from all stages of HCC development. RNA and protein fractions were extracted. Expression levels of Nope, AfP and Gpc-3 were analyzed by quantitative RT-PCR using Gapdh and β-Actin as internal controls. Western blot studies were performed to analyze protein expression of Nope and AfP with β-Actin as internal control. Sections from different stages of tumor development were stained with hematoxylin/eosin, and cryosections were costained for Nope and AfP or E-cadherin for identification of epithelial tumor cells. Results: In livers of our mice, hyperplastic nodules develop at 2 weeks and diffuse cellular dysplasia was detected at 3 weeks after tumor induction. HCC start to develop at 8 weeks after tumor induction. We detected expression of Nope neither in the normal liver nor at early stages of HCC development by quantitative RTPCR. In contrast, we found a significant increase in the expression of Nope at 8 weeks after...
tumor induction and at all later time points when HCC were identified. All samples were negative for Afp, but expression of Gpc-3 closely correlated with Nope expression. Results were confirmed on protein level in Western Blot studies. In immunohistochemistry, Nope was specifically detected in HCC as phenotypically diagnosed in H/E stainings and all Nope positive cells coexpressed E-cadherin. Conclusion: We identified Nope as a novel surface marker for murine HCC by quantitative RTPCR, Western Blot and immunohistochemistry. Nope was specifically expressed by epithelial tumor cells of HCC but not in normal liver or at earlier stages of tumor development. Further investigations will concentrate on the functional and prognostic significance of Nope as a novel marker of murine and human HCC.

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648 HSP90 INHIBITION ABROGATES HUMAN HCC GROWTH THROUGH CDC2-MEDIATED G2/M CELL CYCLE ARREST
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Heat shock protein 90 (Hsp90) is a molecular chaperone which can promote human cancer growth by stabilizing client proteins. 17-(demethoxy), 17-allylamino geldanamycin (17-AAG), the specific inhibitor of Hsp90, can decrease growth in some cancers. We examined 1) the potential effects of 17-AAG-mediated Hsp90 inhibition on human HCC growth, and 2) the possible mechanisms of these effects. HepG2, Hep3B and HuH7 cells were exposed to 17-AAG or DMSO (control) for different time periods and compared. (1) Hsp90 expression was detected by Western blots; (2) Viabilities were measured by MTT assays and IC50 values were calculated. (3) Cell death (necrosis and apoptosis) were determined by fluorescence microscopy of Hoechst 33342/PI-stained cells. (4) Cell cycles were analyzed by flow cytometry of PI-stained cells. (5) The G2/M cell cycle checkpoints Cdc2 (total and phosphorylated Tyr15)) and Cyclin B1 were examined by Western blots. Each of the three HCC cell lines showed the presence of Hsp90 by Western blot. Compared to their respective controls, each HCC cell line treated with 17-AAG showed (1) decreased relative viability at 24, 48 and 72 hours (1.0 vs 0.2, P<0.05), (2) increased apoptosis and necrosis (28% vs 4%, and 5% vs 1%, P<0.05) and decreased total cell numbers (1 x 105 vs 1.3 x 106 cells/well), (3) increased G2/M cell cycle arrest at 24 h (39.2±14.2% vs 26.0±5.0%), and (4) decreased total and phosphorylated Cdc2 at 24, 48 and 72 hours. 17-AAG-mediated inhibition of Hsp90 abrogates human HCC cell growth in part through Cdc2-mediated G2/M cell cycle arrest. Although further studies using physiologic models are needed to delineate the mechanisms of Hsp90's effects, these data suggest a potential therapeutic strategy against human HCC growth.

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649 LIVER SPECIFIC LDB1 DELETION RESULTS IN ENHANCED LIVER CANCER DEVELOPMENT
Andreas Teufel1, Heiner Westphal2, Yangu Zhao2, Stephan Kanzler1, Peter R. Galle1; 1 Department of Medicine I, Johannes Gutenberg University, Mainz, Germany; 2 National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, MD
Ldb1 has been demonstrated to be essential to embryonic development in diverse tissues as the constitutive Ldb1 knock out in mouse lead to an embryonic lethal and pleiotropic phenotype with impaired development of multiple organs, such as the heart, head and posterior axis as well as severe defects in mesoderm-derived extraembryonic structures, including the allantois, blood islands, and primordial germ cells. Ldb1 was demonstrated to be a ubiquitously expressed LIM- and Homeodomain binding protein. Some of these have already been demonstrated to be involved in cancer development. Furthermore, we suggested earlier, that Ldb1 induced functions through regulating the Wnt signaling pathway. Wnt signaling has also been repeatedly demonstrated to be involved in cancer development. We now analysed the influence of Ldb1 on liver cancer development. Therefore, conditional Ldb1 knock out mice containing a loxP flanked genomic stretch of the exons 1 – 9 were crossed to mice expressing Cre recombinase liver specifically under the control of the albumin promoter. Subsequently, liver cancer development was induced by DEN injection at the age of 10 days followed by continuous phenobarbital administration. Analysing the mice 9 months after cancer induction, Ldb1 deleted mice exhibited an average 10-fold higher rate of liver cancer nodules. We currently furthermore investigate a role of differential Wnt signaling in these tumors. Together, we demonstrate that deletion of Ldb1 results in enhanced liver cancer development and expand the current picture on the genetic basis of HCC development by adding yet another key regulatory gene, Ldb1.

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The following people have nothing to disclose: Andreas Teufel, Heiner Westphal, Yangu Zhao, Stephan Kanzler, Peter R. Galle

650 LIVER AND SPLEEN DENDRITIC CELL FUNCTION MODIFICATION DURING A MURINE MODEL OF HEPATO-CARCINOGENESIS
Antonino Castellaneta, Nicola De Tullio, Francesca I. Gagliardi, Doriania Francioso, Michele Barone, Alfredo Di Leo, Antonio Francavilla; Department of Emergency and Organ Transplantation, University of Bari, Bari, Italy
Background and aims: male transgenic (Tx) mice overproducing HbsAg develop a severe liver inflammation progressing within 8 month to nodules of HCC (1). Dendritic cells (DC), efficient Antigen-Presenting Cells, play a critical role in anti-tumor immunity. Aim of this study is to evaluate, in the Chisari's animal model, costimulatory molecules (CM), MHC class II (IAb) expression and T cell stimulatory activity of liver (L) and spleen (S) DC isolated when liver inflammation injury (3 month) and at all later time points when HCC were identified. Results were confirmed on protein level in Western Blot studies. In immunohistochemistry, Nope was specifically detected in HCC as phenotypically diagnosed in H/E stainings and all Nope positive cells coexpressed E-cadherin. Conclusion: We identified Nope as a novel surface marker for murine HCC by quantitative RTPCR, Western Blot and immunohistochemistry. Nope was specifically expressed by epithelial tumor cells of HCC but not in normal liver or at earlier stages of tumor development. Further investigations will concentrate on the functional and prognostic significance of Nope as a novel marker of murine and human HCC.

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The following people have nothing to disclose: Robin D. Kim, Go Watanabe, Kevin E. Behrns

662 ENHANCED LIVER CANCER DEVELOPMENT DURING A MURINE MODEL OF HEPATO-CARCINOGENESIS
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Ldb1 has been demonstrated to be essential to embryonic development in diverse tissues as the constitutive Ldb1 knock out in mouse lead to an embryonic lethal and pleiotropic phenotype with impaired development of multiple organs, such as the heart, head and posterior axis as well as severe defects in mesoderm-derived extraembryonic structures, including the allantois, blood islands, and primordial germ cells. Ldb1 was demonstrated to be a ubiquitously expressed LIM- and Homeodomain binding protein. Some of these have already been demonstrated to be involved in cancer development. Furthermore, we suggested earlier, that Ldb1 induced functions through regulating the Wnt signaling pathway. Wnt signaling has also been repeatedly demonstrated to be involved in cancer development. We now analysed the influence of Ldb1 on liver cancer development. Therefore, conditional Ldb1 knock out mice containing a loxP flanked genomic stretch of the exons 1 – 9 were crossed to mice expressing Cre recombinase liver specifically under the control of the albumin promoter. Subsequently, liver cancer development was induced by DEN injection at the age of 10 days followed by continuous phenobarbital administration. Analysing the mice 9 months after cancer induction, Ldb1 deleted mice exhibited an average 10-fold higher rate of liver cancer nodules. We currently furthermore investigate a role of differential Wnt signaling in these tumors. Together, we demonstrate that deletion of Ldb1 results in enhanced liver cancer development and expand the current picture on the genetic basis of HCC development by adding yet another key regulatory gene, Ldb1.

Disclosures:
The following people have nothing to disclose: Andreas Teufel, Heiner Westphal, Yangu Zhao, Stephan Kanzler, Peter R. Galle

650 LIVER AND SPLEEN DENDRITIC CELL FUNCTION MODIFICATION DURING A MURINE MODEL OF HEPATO-CARCINOGENESIS
Antonino Castellaneta, Nicola De Tullio, Francesca I. Gagliardi, Doriania Francioso, Michele Barone, Alfredo Di Leo, Antonio Francavilla; Department of Emergency and Organ Transplantation, University of Bari, Bari, Italy
Background and aims: male transgenic (Tx) mice overproducing HbsAg develop a severe liver inflammation progressing within 8 month to nodules of HCC (1). Dendritic cells (DC), efficient Antigen-Presenting Cells, play a critical role in anti-tumor immunity. Aim of this study is to evaluate, in the Chisari's animal model, costimulatory molecules (CM), MHC class II (IAb) expression and T cell stimulatory activity of liver (L) and spleen (S) DC isolated when liver inflammation injury (3 month) and at all later time points when HCC were identified. Results were confirmed on protein level in Western Blot studies. In immunohistochemistry, Nope was specifically detected in HCC as phenotypically diagnosed in H/E stainings and all Nope positive cells coexpressed E-cadherin. Conclusion: We identified Nope as a novel surface marker for murine HCC by quantitative RTPCR, Western Blot and immunohistochemistry. Nope was specifically expressed by epithelial tumor cells of HCC but not in normal liver or at earlier stages of tumor development. Further investigations will concentrate on the functional and prognostic significance of Nope as a novel marker of murine and human HCC.

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TARGETING HISTONE DEACETYLATION FOR TREATMENT OF HEPATOCELLULAR CARCINOMA

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Post-translational modification of histones resulting in chromatin remodelling plays a key role in the regulation of gene expression. Also, it is well established that global hypoacetylation of histone H4 is a common hallmark of human tumors and changes in H4 acetylation may occur early in the tumorigenic process (Gallinari et al., Cell Research 2007) We have recently identified an elevated expression of histone deacetylase 2 (HDAC2) gene in primary human hepatocellular carcinoma (HCC) and HCC derived cell lines (Lee et al., Hepatology 2004). In this study we have examined the effects of HDAC2 gene knockdown on cell growth and apoptosis in human HCC cell lines. For silencing HDAC2 gene expression, Huh7 and HepG2 cells were treated with 5 – 20 nM of three different siRNAs (HDAC2-1, HDAC2-2 and HDAC2-3) directed against HDAC2. Cell growth was then analyzed by MTT and apoptosis was analyzed by ELISA for detection of ssDNA and caspase activation. The expression of HDAC2 target gene was determined by quantitative real-time RTPCR. Among the three tested siRNA molecules, the HDAC2-1 siRNA was the most effective in inhibiting HCC cell growth when compared to control treatments (untreated and treated with a negative control siRNA). Huh7 and HepG2 cells transfected with 15 nM of HDAC2-1siRNA for 4 days showed 68% and 71% growth inhibition, respectively, associated with decreased G2/M cell populations. Inhibition of liver cancer cell growth was due to increased rate of apoptotic cell death which was about 1.9-fold higher than in control cells. The increased level of apoptosis directly correlated with activation of caspase-3 and reduction of target mRNA level. Taken together, these results show that HDAC2 is an important regulator of HCC cell growth and survival, and therefore may represent a potential target for human HCC treatment.

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a subset of HCV-related HCC. Increased copy number change of Rictor (mTOR complex 2) predicts HCC recurrence, and identifies this protein as a novel molecular target.

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DECREASED INTRAHEPATIC CD8 TRAPPING IS ASSOCIATED WITH SUPPRESSION OF HEPATOCELLULAR CARCINOMA: A ROLE FOR THE BETA GLYCOLIPID ACYL CHAIN LENGTH
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Different immune-escape mechanisms are associated with tumor progression. Redistribution of lymphocyte subsets is a possible counter-measure to overcome this phenomenon. It has been suggested that the glycolipid acyl-chain may be important in determining the affinity to the CD1 groove in tumor-antigen presenting cells affecting CD8 and NKT activation. Aims: To determine the effect of beta-glycolipids with different acyl chains on CD8 redistribution and hepatocellular carcinoma progression. Methods: Athymic Balb/c mice (n=8/group) were sublethally irradiated and transplanted with 1x106 human Hep3B HCC, followed by daily intraperitoneal injections of beta-glucosylceramide (GC), or beta-lactosylceramide (LC), with 8 or 12 carbons in the acyl chain (GC8, GC12, LC8, and LC12, respectively. 1.5µg in 100µl PBS/dose) or PBS for 42 days. The effect of the acyl chain length on CD8 redistribution was assessed by FACs analysis of intrahepatic and intrasplenic lymphocytes in CD8, CD4 and NKT, markers. The anti-tumor effect of lymphocyte redistribution was assessed by follow up of tumor volume and alpha-feto-protein (AFP) levels. Results: The beta-glycolipids GC12 and LC8 exerted a profound effect on CD8 lymphocyte distribution, manifested by decreased intrahepatic CD8 T lymphocyte trapping (spleen/liver CD4/CD8 lymphocyte ratio 3, 0.39 vs. 4.68 for GC12, LC8 vs. PBS, respectively). NKT lymphocyte distribution was also affected, shown by an increase in intrahepatic NKT lymphocytes (liver/spleen NKT lymphocyte ratio 2.6, 0.97 vs. 0.35 for GC12, LC8 vs. PBS, respectively, p<0.05). Administration of GC12 and LC8 markedly suppressed HCC growth. Maximal tumor volume was significantly lower for GC12 and LC8 compared with GC8 and LC12, respectively (0.63, 0.19 vs. 1.22, 2.72 mm3, respectively, p<0.05). Conclusions: Length of the beta-glycolipid acyl chain was essential in determining the magnitude of CD8 redistribution, breaking immune tolerance towards tumor antigens, leading to marked suppression of HCC growth. Alteration of the numbers of carbons in beta-glycolipids may serve as an important tool for modulation of CD8 lymphocyte function in anti-tumor immune surveillance.

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SUPPRESSION OF ALPHA-1,6 FUCOSYLTRANSFERASE INHIBITS INVASIVENESS OF HUMAN HEPATOCELLULAR CARCINOMA CELLS
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Background: The proportion of alpha-1,6 fucosylated alpha-fetoprotein (AFP), which is also called the percentage of Lens culinaris agglutinin (LCA)-reactive AFP (AFP-L3), is a useful diagnostic marker for hepatocellular carcinoma (HCC). Furthermore, AFP-L3 is considered as a poor prognostic factor of HCC. The mechanism by which AFP-L3 is related to the malignant potential has not yet been clarified. The factors that participate in the mechanism of increasing the malignant potential may be a promising treatment target. Alpha-1,6 fucosylated glycoproteins (FGPs) are synthesized by alpha-1,6 fucosyltransferase (FucT). We aimed to clarify the role of FucT in the cell proliferation and invasiveness of HCC cells in an in vitro model. Method: HCC cell line, HepG2 was used. (1) For the assay of fluorescence staining to detect FGPs, cells were plated on the chamber slide and treated with FITC-labeled LCA, which recognizes FGP, and analyzed by a confocal laser microscope. To evaluate FGPs quantitatively, lectin blot using HRP-labeled LCA was performed. (2) Short interfering RNA targeted to FucT (FUT8 siRNA, Ambion®) was used to knock down FucT mRNA. After 48 h of FUT8 siRNA transfection, FucT mRNA was measured by real-time PCR. The alteration of the expression of FGP was examined by fluorescence staining and lectin blot. (3) Cells were plated on 96-well plates at 1x104 cells/well after transfection of FUT8 siRNA and cell proliferation was evaluated with the time course. (4) Cells were plated on an 8 µm pore-sized migration chamber coated with or without Matrigel® after transfection of FUT8 siRNA. The numbers of the cells migrated through the membrane were counted. Results: (1) FGP were detected on the cell surface and in the cytoplasm. The bands of lectin blot were quantified by densitometry. (2) After 48 h of FUT8 siRNA transfection, FucT mRNA decreased 85.7 ± 4.2 % compared with the negative control (scramble sequence). The expression of FGP was decreased 54.6 ± 2.2 % by lectin blot. (3) The numbers of proliferated cells after FUT8 siRNA transfection (x104 cells/well vs negative control) were as follows: 24 h, 1.81 ± 0.24 vs 1.89 ± 0.21; 48 h, 2.46 ± 0.18 vs 2.29 ± 0.25; 72h, 3.25 ± 0.11 vs 3.46 ± 0.24. There was no significant difference between them. (4) Cell migration through the membrane and Matrigel® coated membrane was decreased 48.6 ± 2.1 % and 42.6 ± 1.6 % by FUT8 siRNA transfection (p<0.05). Conclusion: Suppression of FucT by siRNA reduced the expression of FGP and inhibited the invasiveness of HCC cells. These results provide the rationale of the mechanism by which AFP-L3 is related to the malignant potential in HCC patients.

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The following people have nothing to disclose: Takayuki Kagure, Yoshiyuki Ueno, Osamu Kimura, Tooru Shimosegawa
INDUCTION OF HEPATOMA-SPECIFIC IMMUNITY BY SUICIDE GENE THERAPY IN CC CHEMOKINE RECEPTOR (CCR) 1- OR CCR5-DEPENDENT MANNER

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BACKGROUND: Immunomodulatory agents including chemokines are believed to enhance the antitumor effects of tumor cell apoptosis induced by suicide gene therapy. We recently found that suicide gene therapy, together with delivery of a chemokine, eradicated hepatoma cells and exerted prolonged antitumor effects by activating innate immune responses (J. Immunol. 178:574, 2007). Inflammatory chemokine receptors such as CCR1 and CCR5 expressed by immature dendritic cells (DCs) are presumed to regulate their migration into inflammatory sites. In order to clarify the roles of these receptors in tumor immunity, we evaluated immune responses during suicide gene therapy, in mice deficient in these chemokine receptors, in comparison with wild-type mice. METHODS: Wild-type (WT), CCR1-/-, CCR5-/-, and CCL3-/- mice received ganciclovir (GCV), after they were injected with a murine hepatoma cell line, BNL 1ME A.7R.1 (BNL) transfected with herpes simplex virus-thymidine kinase gene. We examined tumor-infiltrating lymphocytes and mRNA expression of chemokine in the tumor after GCV treatment. Moreover, we examined cell population of lymphocytes in the draining lymph nodes after GCV treatment. Furthermore, we determined cytotoxicity of lymphocytes in draining lymph nodes against BNL cells. Finally, we re-challenged parental BNL cells into the mice after GCV treatment, to determine tumor growth rates. RESULTS: GCV treatment increased the numbers of DCs, CD4+ T and augmented mRNA expression of CCL3 in the tumor. Moreover, CCR1-, CCR5-, or CCL3-deficient mice, intratumoral and intranodal DC infiltration and subsequent cytotoxicity generation were impaired in these mice. When parental cells were injected again after complete eradication of primary tumors by GCV treatment, wild-type mice completely rejected the re-challenged cells, but these deficient mice exhibited impairment in rejection. CONCLUSIONS: CCR1, CCR5, and their ligand, CCL3, may have a crucial role in regulation of DC accumulation into hepatoma tissues and subsequent establishment of tumor-specific immunity after suicide gene therapy.

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MOLECULAR COMBINED THERAPY IN HEPATOCELLULAR CARCINOMA (HCC) WITH AEE788 (EGFR/HER2/VEGFR INHIBITOR) AND RAD001, EVEROLIMUS (mTOR INHIBITOR): ANTINEOPLASTIC ACTIVITY IN A XENOGRAFT MODEL OF HCC

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Background: The knowledge of the signaling pathways involved in HCC provides the rationale for using new targeted molecular therapies. Aim: To study the effect in vivo of a combined dual-level selective pathway blockade in HCC: EGFR/VEGFR (AEE788, Novartis-Pharma, Basel) and mTOR signalling (RAD001, everolimus, Novartis-Pharma). Methods: We used a xenograft model by injecting 5x10^6 HuH-7 cells subcutaneously in athymic mice. Mice were randomized in 4 groups: placebo (n=9), AEE788 [25 mg/kg 3 times per week] (n=9), RAD001 [5 mg/kg 3 times per week] (n=7), and combination of AEE788+RAD001 (n=9). We assessed tumor growth and animal weight every 48 hours. Distressed mice were euthanized according to IACUC protocol. Tumors were collected for immunohistochemistry analysis. We studied tumoral cell proliferation (Ki-67 staining, Proliferation index: percentage of positive nuclei in 10 high-power fields), and apoptosis (TUNEL staining, Apoptotic index: number of positive nuclei in 10 high-power fields). In order to evaluate the effect of both drugs on their molecular targets, we studied the staining status of phosphorylated EGFR and phosphorylated S6. Results: AEE788 and RAD001 delayed tumor growth: 2-week mean volume in control, AEE788, RAD001 and combination were 2396, 1395, 1039 and 784 cc (p<0.05) respectively. There was also a significant increase in median survival: 15, 20.8, 23.1, and 34 days, respectively (p<0.05). Benefits in survival were significantly higher in combination therapy when compared with both drugs separately. Proliferation index was significantly reduced in tumors from mice treated with RAD001 alone and in combination (52.6% and 57.3% vs 80% in control, p<0.05). Apoptotic index was significantly higher in tumors from mice treated with AEE788 alone and in combination (18 and 16 respectively vs 8 in control, p<0.05). There was 1 partial response in the AEE788 group and 1 complete response in the combination group. Drugs were well tolerated at the doses applied. AEE788 and RAD001 decreased p-EGFR and p-S6 staining, respectively. Conclusions: Blockade of EGFR/VEGFR and mTOR signaling with AEE788 and RAD001 significantly reduce proliferation, increase apoptosis, decrease tumor growth and improve survival in an animal model of HCC. These results establish a rationale to test these drugs in clinical trials in HCC.

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INTEGRATIVE GENOMIC CLASSIFICATION OF HEPATITIS C VIRUS POSITIVE HEPATOCELLULAR CARCINOMAS

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We adopted an integrated genomics approach to characterize molecular alterations in hepatocellular carcinomas that arose in patients with chronic hepatitis C virus infection. METHODS: Genomic copy number alterations were measured with Affymetrix 238K Sry arrays in 102 tumor samples obtained at the time of surgical resection or transplantation. For 81 samples obtained from resection, gene expression changes were also profiled with Affymetrix U133 Plus 2.0 arrays. DNA was extracted from tumor and uninvolved cirrhotic tissue samples paired from the same patients. For each sample, 250 ng of genomic DNA was processed according to Affymetrix protocols, and gene expression estimates were obtained for each sample with the RMA algorithm. RESULTS: Among 81 resected patients, the mean age was 65.1 years, 67% were male, 58% had microscopic vascular invasion, and the mean tumor diameter was 4.4 cm with a standard deviation of 3.1 cm. The most frequent copy number alterations included gains of chromosome arms 1q, 5, 6p, 7, 8q, and 17q, as well as losses of 4q, 6q, 8p, 13q, 16, and 17p. We noted recurrent high-level amplifications at cytoband 6p21 in 4% of samples, as well as 11q13 amplifications in 7% of samples. Significant increases in RNA expression of VEGFA and TMEM63B were found among tumor samples with 6p21 amplification. Significantly overexpressed genes among samples with 11q13 amplification included ORAOV1, CCND1 and FGFR19. Comparison of gene expression profiles with previously published studies supported at least two distinct molecular classes that were significantly associated with copy number alterations. We confirmed that poor differentiation and high AFP levels were associated with a high fraction of alveolocytic type, especially in chromosomes 13q, 14 and 16q. Notably, we found that another molecular class could be distinguished by gains of chromosomes 5 and 7, both of which were strongly associated with recurrence after resection. CONCLUSIONS: We identified candidate oncogenes in regions of high-level copy number amplifications and determined that certain chromosomal gains and losses segregated with independently validated gene expression classes.

EXPRESSION AND ROLE OF STEAROYL COENZYME A DESATURASE (SCD) IN HUMAN HCC

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Hepatocellular carcinoma (HCC), the third most important cause of cancer death worldwide, is highly resistant to currently available chemotherapy. Alterations in lipid metabolism have been recently linked to HCC pathogenesis. However the expression and role of stearoyl coenzyme A desaturase (SCD), the rate limiting enzyme in the biosynthesis of monounsaturated fats, in human HCC remains completely unknown. Thus, the AIMS of our study were to determine the expression of SCD in human HCC and to examine the role of SCD in modulating HCC survival and resistance to chemotherapy. METHODS: SCD expression was assessed by immunoblot analysis and immunohistochemistry in three different human HCC cell lines (HepG2, Hep3B, PLC/PRF5), HCC liver tissues (n = 20) and normal liver (n = 10). Cells were incubated in the absence or presence of different chemotherapeutic agents commonly used for HCC treatment (Staurosporine-STS, 5-FU, Doxorubicin) and a time course of SCD expression, caspase 3 activation and apoptosis was assessed by immunoblot, Apo-1 caspase-3 assay, and cell titer blue assay, respectively. Finally, SCD activity was suppressed using a siRNA approach. RESULTS: SCD was strongly expressed in all three hepatoma cell lines as well as in all 20 surgically resected human HCC tissues. The three HCC cell lines showed low sensitivity to chemotherapy induced apoptosis with levels below 15% at 24 hours for all three drugs (STS, 1µM, 5-FU, 10µg/ml and doxorubicin, 1µg/ml). Exposure of HCC cell lines to this panel of drugs resulted in a similar time-dependent upregulation of SCD expression which parallel the degree of resistance to drug-induced apoptosis despite the presence of significant caspase 3 activation. Finally, SCD-specific siRNA to knock down SCD expression resulted in a significant increase sensitivity to chemotherapy induced apoptosis (% apoptosis at 24 hrs: STS from 15% to 50%, p<0.001; 5-FU, from 5% to 25%, p<0.01). CONCLUSIONS: Our data suggest that increased SCD expression may play an important role in HCC development and resistance to chemotherapy induced apoptosis. These results may have important implications in the understanding of HCC pathogenesis as well as in designing novel therapeutic strategies.

KNOCKDOWN OF MIRNAS ENCODED BY THE POLY-CISTRON, MIr-17-92, CAUSES A PARTIAL REVERSION OF THE MALIGNANT PHENOTYPE OF HEPG2 CELLS

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Erin Connolly, Tatiana Tchaikovskaya, Leslie E. Rogler, and Charles E. Rogler. Liver Research Center, Department of Medicine, Albert Einstein College of Medicine, Bronx, NY, MicroRNAs (miRNAs) have emerged as a general regulator of tissue specific gene expression and differentiation. Certain miRNAs have been implicated in oncogenesis including the miRNA encoded by the microRNA polycistron mir-17-92, which includes six miRNAs (mir-17-5p, mir-17-3p, mir-18a, mir-19a, mir-20a, mir-19b-1 and mir-92). mir-17-92 is regulated by c-myc and last year we reported that the mir-17-92 cluster was over expressed in 100% of primary woodchuck HCCs and 95% of
human primary HCCs tested. This year we have carried out functional testing to determine the effect of knocking down the levels of miR-17-92 miRNAs on the malignant characteristics of HepG2 hepatoma cells in culture. We have used a transfection approach with 2′O-methyl antisense oligonucleotides against the six miRNA in the miR-17-92 polycistron. Transfection of HepG2 cells with the 2′O-methyl mix led to a loss of miR-17-92 miRNAs as assayed by quantitative RT/PCR. FACs analysis of HepG2 cells 48 hours after 2′O-methyl transfection revealed a significant increase in cells in G1 compared to a scrambled control, suggesting a block in cell cycle progression. An apoptosis assay that measures the levels of Caspases 3 and 9, showed no increase in apoptosis in cells subjected to the 2′O-methyl transfection. HepG2 cells transfected with 2′O-methyl mix were planted on soft agar and the extent of colony formation measured five days later. A significant reduction in colony growth was observed down to the level of 5 nM per 2′O-methyl. Additional experiments with individual miRNAs from the miR-17-92 cluster are ongoing. In summary, these data are the first of their kind to link over expression of miR-17-92 miRNAs with the maintenance of the malignant phenotype of HCC cells.

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HEPATOCELLULAR PROLIFERATIVE RESPONSE TO PARTIAL HEPATECTOMY IS MARKEDLY IMPAIRED IN TIRAP KNOCKOUT MICE
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Introduction. Partial hepatectomy induces a well-orchestrated cascade of signaling events necessary for the cell cycle progression and proliferation of hepatocytes which culminates in liver regeneration. Proinflammatory cytokines and innate immune system play important roles in the initiation of liver regeneration, yet individual roles of distinct members of the Toll-like receptor (TLR) signaling pathways remains to be fully identified. TIRAP (Toll/IL-1 receptor domain containing adaptor protein) is an adaptor protein that mediates intracellular signaling from TLRs 2 and 4 and plays important roles in the induction of cytokine response during inflammation. However, it is unknown if TIRAP-mediated signaling plays any role in the induction of cytokine response and influence hepatocyte proliferation during liver regeneration. Therefore, the purpose of this study was to test the hypothesis that TIRAP plays a key role in the induction of hepatocyte proliferation in response to partial hepatectomy. Methods. To test our hypothesis, 70% partial hepatectomy (PH) was performed on adult (12 weeks) male wide-type (WT) and TIRAP knock-out mice (KO). Resected lobes and remnant livers obtained after PH (1, 3 hrs) were analyzed by TaqMan real-time PCR for the expression of cytokines and the activation of cytokine-mediated signaling pathways by electromobility shift assays, and western blotting. Mice subjected to PH were injected with BrdU (50 mg/kg) 2 hrs prior to the harvest of remnant livers (24, 45, 72 hrs PH) and the hepatocyte proliferative response was analyzed by immunohistochemistry for the incorporation of BrdU and Ki67 expression, established markers of cell proliferation. Results and discussion. The early proliferative response was analyzed by immunohistochemistry for the incorporation of BrdU and Ki67 expression, established markers of cell proliferation. Results and discussion. The early proliferative response was analyzed by immunohistochemistry for the incorporation of BrdU and Ki67 expression, established markers of cell proliferation. Results and discussion. The early proliferative response was analyzed by immunohistochemistry for the incorporation of BrdU and Ki67 expression, established markers of cell proliferation. Results and discussion. The early proliferative response was analyzed by immunohistochemistry for the incorporation of BrdU and Ki67 expression, established markers of cell proliferation. Results and discussion. The early proliferative response was analyzed by immunohistochemistry for the incorporation of BrdU and Ki67 expression, established markers of cell proliferation. Results and discussion. The early proliferative response was analyzed by immunohistochemistry for the incorporation of BrdU and Ki67 expression, established markers of cell proliferation. Results and discussion. The early proliferative response was analyzed by immunohistochemistry for the incorporation of BrdU and Ki67 expression, established markers of cell proliferation. Results and discussion.
INTRACELLULAR INTERLEUKIN-2 IN CYTOTOXIC CD8+ T-LYMPHOCYTES CORRELATES TO THE BANFF SCORE DURING ACUTE ORGAN REJECTION IN LIVER TRANSPLANT RECIPIENTS

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Liver transplantation is a highly effective treatment for end-stage liver disease. Acute liver rejection is a rare complication compared to other organ systems in transplantation medicine. Therefore a noninvasive method to detect this complication could be beneficial in long term aftercare. The aim of our study is to compare the histological grading of acute organ rejection according to the Banff score compared to the noninvasive detection of intracellular IL-2 in cytotoxic CD8+ T-Cells from blood samples. Methods: 95 Patients were included into this study from march 2005 until december 2006. (66 liver transplant recipients and 29 healthy controls). The blood samples of liver transplant recipients were collected beside routine lab testing or in case of suspected organ rejection by elevated liver enzymes. Immediate liver biopsy in Menghini technique was arranged within 36h including confirmation from our pathologist. For cytometry the blood samples were collected in our outpatient clinic and prepared for the Flow Cytometer (FACSscalibur). After Isolation of white blood cells, the cells were stained with fluorochromes for CD3+(FITC), CD8+(PE-Cy5) and intracellular IL-2(PE). The cells were double-gated (CD3+ and CD8+) for analysis of percentage for intracellular IL-2. Results: The average Banff score in patients during acute organ rejection was 5.8±1.8. The percentage of cells with detectable intracellular IL-2 was significantly increased in patients with acute rejection (p<0.001, ANOVA) compared to recipients without rejection or healthy individuals. The mean detectable intracellular IL-2 percentage (%±SEM) was in rejection patients (7.7±0.91), in stable liver transplant recipients (2.1±0.32) and healthy controls (1.5±3.12). There is a good correlation in rejection patients for intracellular IL-2 and the Banff score (Spearman's rho=0.81, two tailed p<0.05). This cytometric method shows a good sensitivity (71%) and an excellent specificity (95%) for histological proven organ rejection. Conclusion: This flow cytometric method correlates very well to the histological grading according to the Banff score and shows a good sensitivity and excellent specificity. Preparation and analysis is fast, non invasive and cost effective.

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INCREASED MORTALITY IN MMP-9 KNOCKOUT MICE FOLLOWING 75-PERCENT PARTIAL HEPATECTOMY

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Partial hepatectomy (PH) is routinely performed for liver tumors or for transplantation. Mediators of liver regeneration following PH are poorly understood. Recent data suggest that MMP-9 plays an important role in liver regeneration. We hypothesized that MMP-9 is critical for the survival of small liver remnant following massive PH. Method: We performed 75-percent PH in MMP-9 gene knockout mice with C57BL/6 background by removing right and left posterior and anterior lobes of the liver. The age-matched wild-type (WT) C57BL/6 mice were purchased from Jackson laboratory. Following PH, the mice were recovered in heated-support beddings. The extent of PH was estimated by the ratio of the weight of resected liver lobes to the estimated liver weight (ELW). From 10 WT mice, ELW was correlated to the body weight (BW): LW=0.077*BW - 0.7977, with a correlation coefficient of 0.918. Results: 9 MMP-9 KO and 10 WT mice had PH of 70.9±7.6 percent versus 76.4+/−6.5 percent, respectively (p=0.11). As shown in the Figure, only 1 of 9 MMP-9 KO mice survived by 3 days after PH. In contrast, 5 of 10 WT survived more than 3 days after PH. The mortality at 24 hours after PH was 10% versus 70% in WT versus MMP-9 KO mice, respectively (p=0.023). Conclusion: These results corroborate that MMP-9 plays an important role in posthepatectomy liver regeneration and survival.

DOWN-REGULATION BY 1,25 DIHYDROXYVITAMIN D3 OF CD40L-INDUCED IMMUNOREGULATORY CYTOKINES PRODUCTION AND CO-STIMULATORY ACTIVITY IN MONOCYTE-MACROPHAGES FROM LIVER TRANSPLANT RECIPIENTS

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Background. In transplant, rejection depends on proper T cell arm activation of the immune system. In turn, T cell activation relies on the CD40L-induced co-stimulatory effect of antigen presenting cells (APC) such as monocyte-macrophages. 1,25 dihydroxyvitamin D3 (VitD3) has been previously shown to affect several functions of APC. In search for new non-toxic, anti-rejection compounds, we examined whether VitD3 may interfere with the ability of CD40L to induce co-stimulatory activity in monocyte-macrophages from liver transplant recipients. Patients and Methods. Twelve consecutive patients transplanted >2 years earlier for end-stage liver disease (8 males; median age 58 years, range 46 to 65) were investigated. Peripheral blood mononuclear cells were obtained by centrifugation over Ficoll-Hypaque density gradient solution. The effect of VitD3 (10nM) to affect the ability of CD40L to induce co-stimulatory activity in monocyte-macrophages from liver transplant recipients. The age-matched wild-type (WT) C57BL/6 mice were purchased from Jackson laboratory. Following PH, the mice were recovered in heated-support beddings. The extent of PH was estimated by the ratio of the weight of resected liver lobes to the estimated liver weight (ELW). From 10 WT mice, ELW was correlated to the body weight (BW): LW=0.077*BW - 0.7977, with a correlation coefficient of 0.918. Results: 9 MMP-9 KO and 10 WT mice had PH of 70.9±7.6 percent versus 76.4+/−6.5 percent, respectively (p=0.11). As shown in the Figure, only 1 of 9 MMP-9 KO mice survived by 3 days after PH. In contrast, 5 of 10 WT survived more than 3 days after PH. The mortality at 24 hours after PH was 10% versus 70% in WT versus MMP-9 KO mice, respectively (p=0.023). Conclusion: These results corroborate that MMP-9 plays an important role in posthepatectomy liver regeneration and survival.

Survival

WT

MMP-9 KO

p=0.0232

664

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AASLD ABSTRACTS
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labeled by immobilized anti-CD3. Surface markers expression and cytokine production was evaluated by FACS. Data were analyzed using Student’s test (paired two tailed) and results are means±SD. Results: VitD3 strongly reduced the production of IL-12 (4.4±5.3% vs 1.1±1.6%, p<0.05) and TNF-α (5.2±3.8% vs 1.0±1.2%, p<0.05) by CD40L-stimulated monocyte-macrophages. VitD3 also significantly reduced the expression of CD80 (74.9±13.1% vs 31±27.2%, p<0.05) but not that of CD40 (14.5±7.4% vs 14.6±7.4%, p>0.05). Finally, the addition of VitD3 to T lymphocytes cultured in the presence of immobilized anti-CD3 plus autologous CD40L-stimulated macrophages resulted in a marked decrease in the production of IFN-γ (6,9±7,1% vs 2,9±2,8 %, p<0.05). Conclusion. VitD3 interferes with the co-stimulatory activity of macrophages and cytokines production induced by CD40L. Therefore this compound has a potential role in controlling rejection in liver transplanted patients.

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665 PERIPHERAL BLOOD MONONUCLEAR CELLS GZB POSITIVE INCREASE AFTER THE FIRST WEEK FROM LIVER TX IN PATIENTS EXPERIENCING HCV RECURRENCE

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Background: It has been demonstrated that the Granzyme B (GZB) ELISPOT assay might be used as an alternative marker for the cytotoxic T-lymphocyte (CTL). It has also been demonstrated that GZB plays a role during HCV infection as marker of CD8+ activation to clear the virus. We aimed to verify whether GZB could be used as marker to early predict HCV recurrence after liver transplantation. Material and Methods: We enrolled 26 patients (pts) undergoing LTx for end stage liver disease HCV related. On this subset of subjects we assayed the GZB spot forming clonies (SFCs) on PBMCs by means of ELISPOT assay stimulated with tethanic toxoid (TT), before (T0) and one week after liver TX (T1). Furthermore, we also performed a GZB ELISPOT assay when HCV clinical recurrence was diagnosed (high HCV-RNA viral load and histological evidence). All patients underwent the same immunosuppressive treatment schedule (Calcineurin Inhibitors + Steroids +Myophenonol) and were divided in two groups on the basis of HCV recurrence: (Group A – 16 pts with HCV recurrence; Group B 10 pts – no HCV recurrence) statistical analysis (U Mann whitney Test) has been managed once the two groups were settled. Results: A T0 we had 132 ± 42 GZB SFCs in group A and 118 ± 36 GZB SFCs in group B without any statistical significant difference. At T1 we had 270 ± 89 SFCs in Group A; and 120 ± 58 SFCs in Group B, with statistical significant increase in group A respect Group B (U Mann-Whitney Test, p < .0001). Moreover, at the onset of HCV clinical recurrence ( 4th month in mean), GZB SFCs were 256 ±68 in group A, without any statistical significant difference respect to T1. Conclusion: Granzyme B, markers of CTL activation during viral infection, has been found increased one week after OLTx in those patients who experienced a HCV recurrence and to remain triggered at the onset of HCV clinical recurrence. These results would suggest GZB ELISPOT assay as an useful tool to assess the early HCV recurrence and to candidate those patients to a pre-emptive interferon plus ribavirin treatment.

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666 HEPATITIS C VIRUS COULD BE ERADICATED WITH STRONG IMMUNE RESPONSES AFTER WITHDRAWING INTERFERON TREATMENT POST LIVING DONOR LIVER TRANSPLANTATION

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Background and aim: Hepatitis C virus (HCV) specific immune response is believed to control hepatitis activity and clinical course. Recently several reports revealed that strong HCV specific immune response was seen in recurrent hepatitis C patients with mild hepatitis activity after liver transplantation. These data were mostly in patients who underwent cadaveric liver transplantation. The cadaveric liver and living donor transplanted liver are different since living donor partial liver shows aggressive proliferation of hepatocytes after transplantation. The immune response might also be different in these two different conditions. To investigate the HCV specific immune responses after living donor liver transplantation, we examined the HCV specific interferon (IFN) gamma production by ELISPOT assay using CD4 positive T cells with in vitro established dendritic cells (DC) coculture systems. Methods: Subjects comprised 20 patients with hepatitis C virus (HCV)-related end-stage liver cirrhosis who underwent living donor liver transplantation (LDLT) and survived longer than 6 months. Seven of the HCV-related patients received IFN treatment. Peripheral blood mononuclear cells (PBMC) of 10 of these patients were separated and CD14 positive cells were enriched using CD14 microbeads. These CD14 positive cells were cultured with media containing GM-CSF and IL-4. On day 5, the cells, proved to be immature DC were harvested and pulsed with 5 recombinant HCV proteins (core, NS3, NS4, NS5A, and NS5B). These HCV treated iDC were matured with lipopolysaccharide (LPS). After maturation, mature DC and CD4 positive T cells were mixed and IFN gamma ELISPOT assay was performed. Results: In all patients, the spots were very small amount after one month of LDLT. The amount of the spots in all HCV proteins was 19.2 in average. The amount of the spots of 7 patients who showed active hepatitis was reduced to 12.4 during 3 months to 12 months after LDLT. However, the amount of the spots of 3 patients who showed normal ALT levels increased to 36.3. One patient received one year interferon therapy with unsuccessful outcome. However, 2 months after cessation of interferon the patient showed HCV RNA negative and the interferon gamma spots amount increased to 34 from 13 before interferon administration. Conclusion: In LDLT patients, IFN gamma production increased in patients with no signs of active hepatitis or patients who eradicated the virus after cessation of interferon, although active hepatitis patients showed reduced HCV specific IFN gamma production 6 months after operation.

Disclosures:

The following people have nothing to disclose: Akinobu Takaki, Takahito Yagi, Yoshiaki Iwasaki, Hiroshi Sadamori, Kazuko Koike, Masashi Tatsukawa, Haruhiko Kobashi, Kohsaku Sakaguchi
ISCHEMIC PRECONDITIONING INCREASES MN-SUPEROXIDE DISMUTASE AND PREVENTS FREE RADICAL-DEPENDENT MITOCHONDRIAL DEPOLARIZATION IN SMALL-FOR-SIZE LIVER GRAFTS

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**Background:** Our previous studies showed that small-for-size liver transplantation causes a free radical-dependent mitochondrial permeability transition. Ischemic preconditioning (IP) renders tissues more tolerant to subsequent longer episodes of ischemia. Therefore, the **Aim** of this study was to test if IP attenuates injury of small-for-size liver grafts by preventing free radical production and mitochondrial dysfunction.

**Methods:** IP was induced by clamping the portal vein and hepatic artery for 9 min. Livers were harvested from donor rats 5 minutes after releasing the clamp. Half-size livers were implanted into recipients of about twice the donor weight, resulting in quarter-size liver grafts. Mitochondrial polarization and cell death were assessed by intravital confocal and multiphoton microscopy of liver grafts. Mitochondrial polarization and cell death were assessed by intravital confocal and multiphoton microscopy of liver grafts. Mitochondrial polarization and cell death were assessed by intravital confocal and multiphoton microscopy of liver grafts. Mitochondrial polarization and cell death were assessed by intravital confocal and multiphoton microscopy of liver grafts.

**Results:** After quarter-size liver transplantation, alanine aminotransferase, serum bilirubin and necrosis all increased. IP blocked these increases by >58%. Brdu labeling and graft weight increases were only ~3% and 0.2% in quarter-size grafts without IP, respectively, but increased to 32% and 60% after IP, indicating better liver regeneration. Eighteen hours after implantation, viable cells with depolarized mitochondria in quarter-size grafts detected by intravital confocal microscopy were 15 per high power field (hpf), and non-viable cells were <1/hpf, indicating that depolarization preceded necrosis. IP blocked mitochondrial depolarization by 66%. Hsp10, Hsp27, Hsp32, Hsp60, Hsp70, Hsp72, Hsp75 and cytosolic Cu/Zn-SOD (SOD1) before and after transplantation of quarter-size liver grafts but enhanced the expression of Hsp90, a chaperone that facilitates protein import into mitochondria, and increased Mn-SOD (SOD2), a mitochondrial antioxidant enzyme that is synthesized in the cytosol and then imported into mitochondria. A 6-line ESR free radical adduct signal was detected from bile after implantation of quarter-size grafts detected by intravital confocal microscopy.

**Conclusion:** Taken together, IP decreases injury and improves regeneration of small-for-size liver grafts, most likely by increasing mitochondrial SOD2, thus protecting against free radical-dependent mitochondrial dysfunction (NIDDK).

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The following people have nothing to disclose: Hasibur Rehman, Henry D. Conner, Venkat K. Ramshesh, Tom Theruvath, Ronald P. Mason, John J. Lemasters, Zhi Zong

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ADENOVIRAL GENE DELIVERY OF INTERLEUKIN-10 REDUCES HEPATIC ISCHEMIA-REPERFUSION INJURY IN RATS THRU INHIBITION OF KUPFFER CELL ACTIVATION

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**Background and aims:** Ischemia-reperfusion injury to the liver occurs in trauma, hemorrhagic shock, and after hepatic surgery, including tumor resection and transplantation. Reactive oxygen species, produced by activated macrophages (in particular Kupffer cells) and neutrophils, play a critical role in the injury caused by ischemia-reperfusion. The multifunctional cytokine Interleukin-10 (IL-10) is known for its ability to inhibit activation and effector function of T cells, monocytes, and macrophages. Therefore, the aim of this study was to investigate the effect of adeno viral gene delivery of IL-10 in an acute model of hepatic oxidative stress. **Methods:** Kupffer cells (KC) were isolated from Sprague Dawley rats and infected with adenoviral vectors expressing (human) Interleukin-10 (Ad5.IL-10) applying different multiplicities of infection (moi: 0.1-1000 pfu/cell). Adenoviral vectors expressing beta-Galactosidase (Ad5.LacZ) served as control. In some experiments KC were stimulated with LPS (10 µg/ml) and cytokine release was analyzed with IL-10, IL-6, and TNFα ELISAs, respectively. Oxidative stress was established using a warm ischemia-reperfusion model. Three days prior to the experiments, animals were infected i.v. with 3 x 10⁹ plaque-forming units (PFU) of Ad5.IL-10 or Ad5.EGFP. IL-10 expression was analyzed using quantitative PCR analysis and ELISA. **Results:** Transduction of isolated KC with Ad5.IL-10 resulted in a dose-dependent induction of IL-10 secretion with a maximum at moi = 50 pfu/cell. In functional assays, transduction of KC with Ad5.IL10 blunted LPS-induced IL-6 and TNFα secretion as compared to Ad5.LacZ-infected and control cells. In vivo infection with Ad5.IL-10 caused a significant increase of hepatic IL-10 mRNA expression and IL-10 serum levels as compared to Ad5.EGFP-treated control animals. The increase of serum transaminases (ALT, AST) observed after hepatic ischemia-reperfusion was significantly reduced compared to controls. Furthermore, histopathological hepatic changes as focal necrosis and leukocyte infiltration seen in control animals were almost completely absent in Ad5.IL10 infected animals. **Conclusions:** Increased hepatic IL-10 expression can effectively reduce hepatic oxidative stress caused by hepatic ischemia-reperfusion. These data support the hypothesis that increased IL-10 secretion plays an important antiinflammatory role in liver disease and suggest that KC-targeted therapeutic approaches may be a promising strategy affecting hepatic inflammation.

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URIDINE-5’-TRIPHOSPHATE (UTP) PROTECTS AGAINST HEPATIC ISCHEMIC INJURY IN MICE

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Warm ischemia occurs in liver transplantation, trauma, shock and during partial hepatectomy. Liver failure is the most com-
mon cause of mortality. Adenosine 5' triphosphate (ATP)-depletion from hepatic tissue following warm ischemia causes necrotic and apoptotic cell death. Uridine 5'-triphosphate (UTP) significantly reduced cardiomyocyte death induced by hypoxia via activating P2Y receptors. The role of pyrimidine nucleotides in the hypoxic liver has not been explored. The aim of this study was to investigate the role of UTP on the hepatic injury induced by ischemia in isolated mouse livers. Isolated mouse livers were randomly divided into five groups: (1) control group, perfused for the whole study period (120 minutes); (2) 30-minute perfusion followed by 90 minutes of ischemia; (3) like group 2, but with the perfusion of UTP (1 µM), for 30 minutes before ischemia; (4) like group 2, but with the perfusion of suramin (200 µM), a P2Y antagonist, for 30 minutes before ischemia (5) like group 3 but with the simultaneous perfusion of suramin. Effluent enzyme levels, intrahepatic ATP content and caspase-3 activity were measured. Apoptotic cells were identified by morphological criteria, the terminal deoxynucleotidyl trans-ferase-mediated dUTP nick-end labeling (TUNEL) fluorometric assay and immunohistochemistry for caspase-3. Results: Post-ischemia, there was a statistically significant reduction in liver enzyme levels in the animals pretreated with UTP (p<0.05), the infrahepatic caspase-3 activity was significantly decreased (p<0.05) and the infrahepatic ATP content increased (p<0.05) compared to group 2 (ischemic untreated). The reduction in postischemic apoptotic hepatic injury in the UTP-treated groups was confirmed morphologically, by the significantly fewer apoptotic hepatocytes detected (p<0.05); immunohistochemically, by the significantly weaker activation of caspase-3 compared to the ischemic untreated group 2 (p<0.05); and by the TUNEL assay (p<0.05). The administration of suramin (group 4) aggravated the apoptotic ischemic injury while the simultaneous perfusion of UTP and suramin induced ischemic changes in group 2. Conclusion: The administration of UTP before induction of ischemia can attenuate the postischemic hepatic apoptosis and thereby minimize liver damage. Apoptotic hepatic injury seems to be mediated through caspase-3 activity. Inhibition of endogenous release of UTP aggravates the ischemic hepatic injury. These findings have important implications for the potential use of UTP in ischemic hepatic injury.

Disclosures: The following people have nothing to disclose: Ziv Ben-Ari, Orit Pappo, Smadar Yitzhaki, Yelena Cheporko, Asher Shainberg, Eytan Mor, Edith Hochhauser

671 INTERMITTENT REPERFUSION STRESS ACCELERATES LIVER REGENERATION BY INDUCING ENTRY AND PROGRESSION IN CELL CYCLE OF HEPATOCYTES

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Massive blood loss during liver resection can be prevented by temporary vascular inflow occlusion, consequently leading to ischemia and reperfusion injury in the remnant liver. It has been shown that intermittent clamping was less deleterious than continuous clamping. Our goal was to evaluate the effects of intermittent ischemia-reperfusion stresses on regenerative capacity of the liver. For this purpose, we developed an original rat model of selective lobe occlusion to isolate reperfusion stress from ischemia injury followed by 70% partial hepatectomy. Four animal groups were distinguished: the "reperfusion group", median and left lateral lobes were subjected to selective intermittent clamping before resection so that the remnant liver (caudal and right lobes) did not suffer from ischemia injury but was exposed to the reperfusion effects of resected lobes. In the "control group", simple exteriorisation of median and left lateral liver lobes was performed before resection. These two groups were compared to "standard group" subjected to immediate partial hepatectomy and to "sham group" subjected to laparotomy only. Higher levels of serum transaminases and increased numbers of apoptotic figures were detected in the reperfusion group compared to other groups, demonstrating a transient and greater cellular damage induced by the reperfusion stress. This cellular damage appeared not impairing liver regeneration since DNA synthesis and mitotic index were similar in both reperfusion and control groups. However, cell cycle progression was speeded up in these two groups when compared to the standard hepatectomy group, as DNA synthesis and mitotic index peaked at least 6 hrs earlier. Further analysis of Cyclin D1, CDK1 and Cyclin A expression kinetics evidenced an earlier cross-over of the mitogen-dependent restriction point and G1/S transition in both groups. In addition, we perfusate were analysed. Results: Six livers showed serum biochemical evidence of initial poor function (IF). These livers had significantly more staining for C4d of both lobular and periportal hepatocytes. C4d positive hepatocytes were also found in the liver during cold storage (3/15). These periportal hepatocytes also showed evidence of necrosis and were found to have intracellular neutrophils. Hepatocyte rounding in zone III, necrosis and C4d staining in recipient were also significantly correlated with the degree of lactic acidosis during this phase. Intrahepatic lactic acidosis at all time points was significantly associated with sinusoidal endothelial cell (SEC) injury post-reperfusion. There were no correlations between glucose, pyruvate and glycerol levels and histopathological changes in the liver. Discussion: In the patients studied the degree of C4d staining correlated with IF and was associated with intrahepatic lactic acidosis in the donor, during cold storage and post-reperfusion. Complement activity in the liver during cold storage may be as a result of in situ activation. Intrahepatic lactic acidosis is associated with SEC and hepatocyte injury. The presence of intrahepatic neutrophils is likely to be in response to cell necrosis. Disclosures: The following people have nothing to disclose: Michael A. Silva, Darius F. Mirza, Nick Murphy, Douglas A. Richards, Stephen J. Wigmore, John A. Buckels, Desley A. Neil

670 INTRAHEPATIC COMPLEMENT ACTIVATION, SINUSOIDAL ENDOTHELIAL INJURY AND LACTIC ACIDOSIS IS ASSOCIATED WITH INITIAL POOR FUNCTION OF THE LIVER POST TRANSPLANTATION

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Background: We recently described changes in glucose metabolism in the liver during transplant procurement, storage and post-reperfusion using microdialysis. We now aim to correlate these findings with histopathological, immunohistochemical and ultrastructural changes in liver during pre-harvest, post-storage and post-reperfusion. Methods: Microdialysis catheters were inserted into 15 human livers to monitor metabolic changes that took place during procurement, backtable preparation, and post-reperfusion in the recipient. At each stage menhingi needle biopsies were also taken. Each biopsy was studied using light microscopy and electron microscopy. The microdialysis cannula was perfused with isotonic solution and samples of

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showed that in the reperfusion group, c-fos and IL-6 mRNA were expressed before hepatectomy suggesting that G0/G1 transition occurred before resection in this group. In conclusion, our study provides evidence that the surgical exteriorisation step itself induces cell cycle progression. Moreover it shows that the transient cellular damage related to the reperfusion process might trigger greater cell defence mechanisms and signalling pathways that contribute to liver regeneration acceleration. These observations strongly argue for a positive effect of reperfusion stress on liver regeneration which should be beneficial to clinical practice.

Disclosures: The following people have nothing to disclose: Sasse-Fanie Mbatici, Hélène Duval, Stéphane Grandadam, Catherine Ribault, Claire Piquet-Pellorce, Pascal Loyer, Christiane Guguen-Guilhouzo, Anne Corlu, Karim Boudjema

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OXIDATIVE DAMAGE TO MITOCHONDRIAL DNA AND PROTEINS IN SMALL-FOR-SIZE LIVER GRAFTS
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Background: Small-for-size liver transplantation compromises energy supply. Mitochondrial dysfunction probably plays an important role in graft injury and suppression of regeneration after transplantation of small-for-size liver grafts. The Aim of this study was to investigate mitochondrial injury in small-for-size liver grafts. Methods: After harvest, liver grafts were reduced in size to ~50% ex vivo, stored in UW solution at 0-1°C for 6 h and then implanted into recipients of similar or greater body weight, resulting in a relative graft weight of 50% (half-size) or 25% (quarter-size). Results: Serum transaminase was 3-fold higher after transplantation of quarter-size than of full-size grafts. Cell proliferation detected by BrdU incorporation increased from ~1% in full-size to 17% in half-size grafts but did not increase in quarter-size grafts. ATP content in livers was not significantly altered after transplantation of full-size grafts but decreased to 30% of normal in quarter-size liver grafts. Mitochondrial DNA (mtDNA) contains genes which encode proteins critical for oxidative phosphorylation, and an adequate copy number of mtDNA per cell is required for respiration and oxidative phosphorylation. The ratio of mtDNA/nuclear DNA detected by native gel Southern blotting was not altered at 5 h after transplantation of quarter-size relative to full-size grafts (p<0.05), indicating double-strand DNA damage. Alkaline gel Southern blotting also showed that mitochondrial DNA underwent single-strand damage in quarter-size liver grafts. 8-Oxyguanosine, an indicator of DNA oxidation, increased in both nuclei and mitochondria, but more markedly in mitochondria. Subunit III of respiratory chain Complex I, a protein that is encoded by mitochondrial DNA, decreased substantially in quarter-size grafts. COX IV and ATP synthase-β, mitochondrial proteins encoded by nuclear DNA, also decreased in quarter-size grafts. C. sinenesis polyphenols (20 µg/mL), potent scavengers of reactive oxygen and nitrogen species, prevented mitochondrial DNA damage, blunted decreases of mitochondrial respiration chain components and restored ATP levels to 75%. In Conclusion, small-for-size liver transplantation increases free radical formation which causes mitochondrial and nuclear DNA damage and a decrease of mitochondrial respiratory chain proteins. These alterations may contribute, at least in part, to compromised energetic status and graft failure after small-for-size liver transplantation. These effects can be effectively prevented by free radical scavengers (NIDDK).

Disclosures: The following people have nothing to disclose: Zhi Zhong, Hasibur Rehman, Tom Theruvath, John J. Lemasters

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ALTERNATIVE PRECONDITIONING WITH DEATH LIGANDS TNF-α & FASL PROTECTS THE CIRRHOTIC MOUSE LIVER AGAINST ISCHEMIC INJURY
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Background and Aims: Ischemic preconditioning is the pre-emptive proven strategy to reduce ischemic injury in the liver, but it can be harmful in the elderly or patients with liver diseases. Patients who have underlying liver diseases are increasing, but the chance of transplantation which might be the last choice for the patients is rare. Thus, alternative ways to protect the livers are required. Ischemic preconditioning induces cyto-protective effects via activation of oxidative stress. Low dose application of Fas ligand or tumour necrosis factor can induce a similar response by inducing limited hepatic injury. Therefore, we tested if death ligands could mimic ischemic preconditioning. Methods: Ischemia was performed for 60 minutes in carbon tetra chloride induced cirrhotic mice. The optimal dose of preconditioning was found in a pilot study in cirrhotic animals. Death ligands were given 40 minutes before ischemia by intraperitoneal injection. Ischemic injury was assessed by histology, and biochemical assays after 1, 4, and 24 hours of reperfusion. To elucidate the mechanism of protection, we used zinc-protoporphyrin, an inhibitor of heme oxygenase-1 (HO-1) and gadolinium chloride, an inhibitor of Kupffer cell activation. Results: Compared to the non-treated control group, death ligand preconditioning strongly reduced all markers of injury: serum transaminase levels were reduced; the area of necrosis and the extent of apoptosis were significantly ameliorated. Death ligand preconditioning mimicked the effect of ischemic preconditioning in terms of HO-1 up-regulation, predominantly in macrophages. When zinc-protoporphyrin or gadolinium chloride was applied prior to preconditioning, the beneficial effect of preconditioning was lost. Additionally, death ligand preconditioning induced anti-inflammatory (IL-1 receptor antagonist) and pro-inflammatory (IL-1beta, CXCL2) cytokines prior to ischemia, which are known to be produced by macrophages. Conclusion: These results demonstrate that ischemic preconditioning can be replaced by death ligand preconditioning in the cirrhotic liver to prevent ischemic injury. The protective mechanism is depending on HO-1 induction in macrophages. These results open new doors for novel hepatoprotection strategies in cirrhotic liver surgery.

Disclosures: The following people have nothing to disclose: Jae-Hwi Jang, Wolfgang Moritz, Rolf Graf, Pierre A. Clavien

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P66SHC, AN AGEING PROTEIN, PLAYS A PIVOTAL ROLE IN POST-HEPATECTOMIZED LIVER REGENERATION IN AGED MICE
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Liver regeneration is composed of a series of complicated processes, which are affected by various patho-physiological...
conditions. The present study was designed to investigate the influence of ageing on liver regeneration following partial hepatectomy (PH) in mice. [Materials & Methods] Male C57BL/6j mice (8-10 wks and 13/20 mo old), fed by low fat/protein CR-LPF chow for long breeding (Oriental Yeast Co. Japan), were used as young and aged mice models, respectively. These mice were subjected to simple 70% PH. Adenoviral vectors were introduced intravenously 3 days prior to the experiment (5 x 10^8) pfu/body. The mice were sacrificed and liver specimens were collected and applied for the assays before and after hepatectomy, and the recovery of liver mass was also examined until 14 days post-PH. [Results] Post-PH liver regeneration was significantly impaired following to age (Fig.). Nevertheless, reactive liver cell proliferation after PH was not impaired at all even in 20 mo old mice after PH, assessed by mitotic index and PCNA positivity. Instead, liver cell apoptosis was markedly increased in the aged mice after PH. Many apoptotic cells were observed immediately after PH and continued until 14 days post-PH in the aged mice, though much less apoptosis was observed in the young mice. In support of this observation, serum levels of AST/ALT were also increased until 3 days post-PH in the aged mice. By western blot analysis, signal proteins responsible for cell proliferation (MAPK, STAT3) were expressed and activated after PH in the aged mice liver, equally or even more strongly than the young mice. However, Akt was less activated though its protein was expressed enough in the aged liver tissue. Interestingly, p66Shc, ageing protein known to control 1) cellular redox states, 2) Akt-mediated 'survival' pathway and 3) apoptosis, was expressed moderately in the aged mice, and strongly phosphorylated at Serine36. To examine the effect of p66Shc on liver regeneration in the aged mice, p66Shc was knocked down by introducing adenoviral vector coding p66Shc-siRNA. By the knocking down of p66Shc, liver regeneration was significantly improved in the aged mice, compared to the LacZ-control mice, mainly by suppressing apoptotic cell death in the post-PH liver. [Conclusions] p66Shc may play a pivotal role in liver regeneration in the aged mice, by increasing vulnerability to the stresses such as hepatocyte and/or oxidative stress. Further study must be performed to examine the underlying mechanisms in the impaired liver regeneration in the aged mice. However, this may provide a clue to understand the processes of liver regeneration especially in the aged.

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** 675 METHOXYPOXYETHYLENE GLYCOL MODIFIED-ALBUMIN (PEG-ALB) ENHANCED THE COLD PRESERVATION PROPERTIES OF UW SOLUTION IN RAT LIVER GRAFTS**

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Liver grafts preserved in cold undergo changes mainly manifested by morphological changes of the sinusoidal endothelium. Swollen and fragmented cytoplasm translates into poor portal blood flow, increase release of liver enzymes and low bile production upon liver reperfusion. Studies were performed to determine if the addition of higher molecular weight polyethylene glycol modified albumin to the University of Wisconsin (UW) preservation solution ameliorates the cold preservation injury of liver grafts. Methoxyxypolyethylene glycol 5000 activated with cyanuric chloride was covalently coupled to human albumin (Peg-Alb) at multiple sites. The isolated Perfused Rat Liver model was used (IPRL). Human and Rat hepatocytes cell lines were preserved in cold under similar preservation solutions. Effects were studied after rewarming of cells on Glutathione turn over by mass spectrometry. Apoptosis of SLC's on liver tissue and cell lines were evaluated by Tunel assay and flowcymeter techniques. Preliminary results showed Glutathione turnover was significantly decreased in all groups compared to negative controls. In contrast, apoptosis of SLC was similar in the PEG-Alb group when compared to the negative control group but significantly decreased in the PEG-Alb group when compared to other groups. Conclusions: The addition of high molecular albumin to UW preservation solution appears to ameliorate endothelial injury of cold preserved liver grafts as judged by better portal vein blood flow, increased bile production and decreased SLC apoptosis. PEG-Alb appears to have no effect on hepatocytes preservation.

**IPRL results of grafts preserved with UW solution and UW solution plus PEG- Alb. Values are given after 60 minutes of perfusion with a sanguineous perfusate.**

<table>
<thead>
<tr>
<th>Group <strong>(n=4)</strong></th>
<th>(preservation time in hours)</th>
<th>Portal Blood flow ml/g of liver/minute</th>
<th>AST Units/g of liver</th>
<th>Bile production ml/g of liver</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control neg UW</td>
<td>3(1h)</td>
<td>0.93±0.03**</td>
<td>2.1±1.08*</td>
<td>10.5±5.97</td>
</tr>
<tr>
<td>Control pos UW</td>
<td>3(1h)</td>
<td>0.19±0.010</td>
<td>14.4±0.34</td>
<td>0±0</td>
</tr>
<tr>
<td>PEG-Alb &amp; UW</td>
<td>3(1h)</td>
<td>0.96±0.005**</td>
<td>28.4±1.03</td>
<td>3.3±7.54</td>
</tr>
<tr>
<td>Alb &amp; UW</td>
<td>3(1h)</td>
<td>0.05±0.007</td>
<td>26.9±2.45</td>
<td>0±0</td>
</tr>
</tbody>
</table>

** p<0.05 by ANOVA

Disclosures: The following people have nothing to disclose: Rime Abbas, David Dingam, Deepak Malhotra, Henry Brunengraber, Juan R. Sanabria

**676 HUMAN HEPATIC NRF2 CORRELATES WITH POST-TRANSPLANT IL-8 AND TRANSAMINASES INDICATING A NOVEL PROTECTIVE MECHANISM**

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Ischemia reperfusion (IR) leads to generation of reactive oxygen species (ROS), which, as well as causing tissue damage, also activate mechanisms of cytoprotection. The ROS sensitive transcription factor Nrf2 induces tissue repair mechanisms through transcriptional activation of phase II antioxidant pathways. Here, for the first time, we correlate Nrf2 in human liver with histological, immunological and biochemical markers of injury and inflammation post liver transplantation (LT). Paired biopsies were acquired from donor livers (n = 17) prior to and following the IR phase of LT. Each biopsy was assessed using the Modified Suzuki Scoring system (histological grading of degree of IR injury) and the polymorphonuclear infiltrate quantified. Nrf2 protein levels were measured using Western blots. Oxidised and reduced glutathione levels were measured using the Glutathione assay kit (Cayman Chemical Co.) and IL-8 was measured by ELISA. The data was analyzed using the SPSS statistical package. All reperfusion biopsies showed evidence of IR injury with significantly increased Suzuki scores and polymorphonuclear infiltration. (P<0.05). GSH levels decreased following IR as did the GSH: GSSG (redox ratio). Median GSH in donor...
and reperfusion biopsies was 146.88 µM/g (25.52-307.94) and 75.80 µM/g (7.18-180.15) respectively. Nrf2 protein levels post-IR were decreased as compared to pre-IR (P<0.01). IL-8 levels in post-IR biopsies were decreased as compared to pre-IR (P<0.01). IL-8 levels increased in post-IR biopsies (median donor and 75.80 µM/g (25.52-307.94) respectively). Nrf2 protein levels in donor liver post-IR may be cytoprotective as they correlate with Nrf2 levels. Recipients of donor organs with high Nrf2 could induce the trans-differentiation of ADSC into hepatic lineage cells. ADSC could be a source of hepatic lineage cell transplantation.

Disclosures:
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MURINE ADIPOSE TISSUE DERIVED STROMAL CELLS TRANS-DIFFERENTIATE INTO HEPATIC LINEAGE CELLS BY BASIC FIBROBLAST GROWTH FACTOR

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Background and Aims: During the past few years, multiple studies have revealed that bone marrow-derived mesenchymal stromal cells (BMSC) can be trans-differentiated into various cell types under proper conditions. Recently, mesenchymal stromal cells (MSC) are also reported to exist in adipose tissue. Compared with BMSC, adipose tissue derived stromal cells (ADSC) can be easily and repeatedly harvested from patients by a simple, minimally invasive method. ADSC also have a potential to differentiate into various cell types, including hepatic lineage cells. However, the molecular mechanism underlying this phenomenon has remained unclear. To address this issue, we investigated effective differentiation method to differentiated ADSC into hepatic lineage cells. Methods: (1) ADSC was isolated from the subcutaneous adipose tissue of 12-week-old male C57Bl6/J mice. ADSC was cultured in 3 dimensional culture system (3D) of collagen gel with or without 20ng/ml of the growth factors (bFGF, HGF, OSM, HGF+OSM+0.1% of DMSO) or on plastic dishes (2 dimensional culture system; 2D) with or without bFGF (20ng/ml) for 2 or 4 weeks. To investigate the effect of these growth factors, the gene expressions of AFP, albumin, CK18, CK19, PEPC, G6Pase, Cytp2b9, and transthyretin (TTR) were examined by RTPCR. (2) Albumin secretion into the media by ADSC (2D) was evaluated by ELISA.

(3) Glycogen synthesis, urea production, and low-density lipoprotein (LDL) uptake, were confirmed by periodic acid-Schiff staining, a colorimetric assay, and an LDL uptake assay, respectively in ADSC (2D). (4) ADSC was cultured with bFGF (20ng/ml) (2D or 3D) for 4 weeks. We assessed the expressions of albumin and CK18 proteins of ADSC by immunohistochemistry. Results: (1) Albumin was induced in ADSC (3D) cultured with bFGF, OSM, and HGF+OSM+DMSO. ADSC cultured with bFGF for 4 weeks induced hepatocyte markers gene expression most effectively, and revealed the gene expressions of albumin, CK18, PEPC, G6Pase, Cytp2b9 and TTR. ADSC (2D) cultured with bFGF for 4 weeks expressed albumin, CK18, PEPC, G6Pase, and TTR. (2) Albumin secretion by ADSC (2D, 3D) cultured with bFGF was significantly higher than control. The bFGF treated ADSC showed the ability to uptake LDL. (4) ADSC (2D, 3D) was stained for both albumin and CK18. Conclusions: Basic fibroblast growth factor could induce the trans-differentiation of ADSC into hepatic lineage cells. ADSC could be a source of hepatic lineage cell transplantation.

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DO INJECTED AUTOLOGOUS BONE MARROW CELLS WORK IN PATIENTS WITH ADVANCED LIVER CIRRHOSIS?

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Background/Aims: Liver cirrhosis (LC) is the end stage of chronic liver disease and is extremely difficult to treat. Although recent studies examining the role of bone marrow cells (BMCs) in regeneration or fibrosis using liver fibrosis/cirrhosis animal models have given conflicting results, human clinical trials of BMC infusion for cirrhotic patients have had positive results. In this study, we evaluated the safety and effect of autologous BMC infusion (ABMI) on the liver in patients with advanced LC. Methods: Six patients (three males) aged between 18 and 75 years with a clinical diagnosis of advanced LC (Child-Pugh class B or C), with a total bilirubin less than 3.0 mg/dl, a platelet count exceeding 50,000/uL, and no viable hepatocellular carcinoma on magnetic resonance imaging (MRI) were enrolled. Autologous BMCs were harvested from the ilium under general anesthesia and infused into a peripheral vein after RBC depletion and mononuclear cell concentration. Serologic tests, MRI, and biopsy were performed before and 1, 3, and 6 months after the procedure. The quality of life of the patients was surveyed using a questionnaire. Results: The mean patient age was 57 years (range 47–64 years), and the mean number of infused mononuclear cells was 6.83 x 10^9. The serum albumin and cholesterol level increased and the prothrombin time was shortened. The ascites of the patients improved or disappeared after stopping oral diuretics or reducing their requirement. All patients showed improved symptoms and quality of life. The fibrosis index measured using MRI indicated reduction of fibrosis in some patients. The liver volume measured using MRI had increased in some patients. In serial biopsies, a significant increment in the progenitor cell compartment, including the ductular reaction and intermediate hepatocytes, was consistently noted. No serious adverse events occurred. Conclusions: Autologous BMC infusion for advanced LC resulted in improved liver function and subjective symptoms with progenitor cell compartment activation. ABMI can be used as a bridging modality in select patients with decompensated LC.

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N-ACETYLCYSTEINE IMPROVES THE METABOLIC AND SYNTHETIC FUNCTION OF HUMAN HEPATOCYTES ISOLATED FROM SEVERELY STEATOTIC DONOR LIVERS

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The availability of good quality hepatocytes has limited progress in the development of human hepatocyte transplantation. Marginal donors are the main source of tissue available for cell isolation, but the isolated hepatocytes obtained are of low viability and are usually not suitable for clinical use. The most common source is livers rejected on the grounds of steatosis. The aim of this study was to evaluate the effects of addition of the antioxidant N-acetylcysteine (NAC) during isolation of human hepatocytes from severely steatotic donor livers. Methods: Human hepatocytes were isolated from 10 donor livers (7M, 3F) rejected for transplantation on the grounds of being severely steatotic (>60%). The median donor age was 55y (range 44-76y) and cold ischaemia time 19h (range 11-29h). The left lateral segment of the liver was dissected into two pieces, which were cannulated and randomised to control and NAC. A standard collagenase liver digestion technique was used with and without NAC (5mM) added to the first perfusion buffer. The hepatocytes obtained were plated and cultured for 24h and then assays performed: SRB staining for hepatocyte attachment, MTT for cell metabolic activity, [14C]-leucine incorporation, albumin ELISA and urea production. Results: A significantly higher cell viability and cell yield were obtained from tissues perfused with NAC (median 82%; range 72-91 and 2.6x10^6 cells/g; 0.5-5.5) compared to control (71%; 38-82, p=0.005 and 1.1x10^6 cells/g; 0.3-3.6, p=0.005). Cell attachment (median 1.19 OD unit; range 0.08-2.46 vs. 0.59 OD unit; 0.07-2.06, p=0.017) and overall cell metabolic activity (median 0.22 OD unit; range 0.12-0.28 vs. 0.10 OD unit; 0.09-0.19, p=0.005) were both significantly higher in cells from tissues perfused with NAC. Protein synthesis from [14C]-leucine incorporation (median 857 cpm/well; range 275-1938 vs. 795 cpm/well; 238-1560, p=0.012) and albumin content (median 339 ng/mg protein/well; range 180-1227 vs. 197 ng/mg protein/well; 124-1144, p=0.012) in hepatocytes from tissues perfused with NAC were significantly higher than control. Urea production was not significantly affected by NAC. Preliminary experiments suggested that N-acetylcysteine decreased hepatocyte iNOS expression. Conclusions: This study demonstrates that addition of NAC during isolation of hepatocytes from steatotic donor liver tissue, significantly improves the metabolic and synthetic function of the isolated cells. Incorporation of NAC in the hepatocyte isolation protocol should make better use of rejected steatotic livers and thus increase the availability of hepatocytes for clinical cell transplantation.

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PURIFICATION OF A SPECIFIC CELL POPULATION CONTAINING ALL OF THE REPOPULATION POTENTIAL OF FETAL LIVER STEM CELLS FOR THE NORMAL ADULT RAT LIVER

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Cell transplantation is a promising alternative to liver transplantation. Previously, we showed a high level of long-term liver replacement after transplantation of unfractionated embryonic day (ED)14 fetal liver stem/progenitor cells (FLSPC). However, hematopoietic stem cells, a major component in unfractionated fetal liver cell preparations engraft in other organs, e.g. bone marrow, spleen and lung, and may cause undesirable side effects or complications. Therefore, for most clinical applications, cell isolates need to be highly enriched before cell transplantation. Dlk-1, a member of the delta-like family of transmembrane proteins, is highly expressed in human and rodent fetal livers. We therefore isolated Dlk-1+ cells from ED14 fetal liver and examined their hepatic gene expression profile and characteristics in vitro and proliferative and differentiation potential after transplantation into the normal adult rat liver in comparison to Dlk-1- fetal liver cells. Methods & Results: We have purified rat ED14 FLSPC to 95% homogeneity using immunomagnetic microbeads (MACS). Dlk-1-enriched fetal liver cells are AFP+/CK-19. Anti-Dlk-1 selected rat fetal liver cells exhibit all the cell culture and gene expression characteristics expected for hepatic stem/progenitor cells. Compared to Dlk-1- cells, Dlk-1+ cells preferentially express AFP, Alb, CK-19, G6-Pase, CPS-1, TAT, TRPO, OSMR, Hex, HNF-3β, HNF-4α, CYP3A1 and E-Cadherin, all of which are related to hepatocytic or hepatic progenitor cell functions. After transplantation of Dlk-1+ fetal liver cells (0.4 to 1.9x10^6 cells), these cells engrafted in the liver, proliferated and differentiated into both mature hepatocytes and bile duct structures. These cells express unique hepatocyte-specific proteins and are structurally and functionally indistinguishable from host hepatocytes throughout the entire hepatic lobules. The level of repopulation exceeded 15% at 6 months after cell transplantation. In selected areas, liver repopulation reached 50% and transplanted cell clusters became confluent and generated complete new liver lobules. In contrast, Dlk-1- fetal liver cells do not repopulate the normal adult rat liver, however, they engraft in other organs. Conclusions: This is the first study showing a method to separate a highly enriched hepatic cell fraction that contains all the hepatic fetal liver stem/progenitor cell types identified to date and all of the normal liver repopulation potential found in the fetal liver. This approach is a major step forward in the development of protocols that will be essential for future clinical applications.

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A CRITICAL ROLE OF FAS (CD95) IN ALLOREJECTION OF TRANSPLANTED HEPATOCYTES

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BACKGROUND: As with whole organ transplantation, allograft rejection is a major problem in hepatocyte transplantation,
requiring long-term immunosuppression. Death receptors such as Fas (CD95) are highly expressed at hepatocyte cell surface. Fas-mediated apoptosis has been implicated as an important mechanism of liver injury and liver transplant rejection. We showed previously that expression of genes of the E3 domain of the adenoviral genome (AdE3) in donor rat hepatocytes prevented their rejection after transplantation into allogeneic recipients. AIM and HYPOTHESIS: Our objective is to determine the mechanism of hepatocyte allorejection with the ultimate goal of modifying donor cells to prevent immune rejection. Since AdE3 gene expression did not downregulate cell surface MHC class I in the donor cells, but markedly reduced cell surface Fas, we hypothesized that abrogation of Fas-FasL signaling by host alloreactive CTLs was critical in preventing allorejection. METHODS: We tested this hypothesis in two steps. First, we injected C57Bl6 mice with 109 pfu of an adenovector Ad-RIDβ-expressing the receptor internalization and degradation complex (RIDβ) which degrades cell surface "death receptors" including Fas. Control mice received an adenovector expressing an irrelevant gene (Ad-UGT1A1). Two days later the mice were challenged with an agonistic anti-Fas mAb (Jo2). Liver injury and hepatocyte apoptosis were assessed by serum ALT measurement and TUNEL staining of liver sections, respectively. Second, we transplanted hepatocytes from Fas-deficient lpr mice or congenic wildtype C57Bl6 mice, genetically marked by transduction with LacZ into fully allogeneic BalbC recipients. RESULTS: Hepatic expression of AdE3 proteins resulted in marked reduction of Fas-mediated apoptosis and abrogation of liver injury as assessed by serum ALT levels. LacZ-marked hepatocytes from lpr mice survived in allogeneic BalbC mice, whereas hepatocytes from wildtype C57Bl6 mice were rejected within 10 days. CONCLUSION: Down-regulation of cell-surface Fas on donor hepatocytes prevented Fas-mediated apoptosis. Lack of Fas expression in hepatocytes is sufficient to prevent their rejection after transplantation into allogeneic recipients.

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MECHANISMS REGULATING SURVIVAL OF DONOR CELLS IN THE NON-HEART BEATING RAT LIVER

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The shortage of donor organs poses a major restriction for cell therapy programs. To determine whether additional sources of cells could be developed, we examined non-heart-beating (NHB) donors. F344 donor animals were killed with i.v. KCl and maintained at 4 degrees C with instillation of hepwarin in portal vein to avoid thrombosis for cell isolation. Tissue analysis from NHB donors during 15 min to 40 h period after death showed normal lobular architecture, morphological integrity and absence of significant hepatic apoptosis or necrosis. TUNEL indicated that despite onset of hepatic apoptosis in NHB donors after 30 h, its extent was limited and DNA ladder analysis verified the findings. To demonstrate regulation of global gene expression, we analyzed whole NHB liver and normal liver RNAs with Affymetrix 230 2.0 Arrays (30K genes). Data analysis showed that >2-fold differential gene expression was limited in NHB donors after 4 h to a single metabolic gene; after 16 h to 95 genes; and after 30 h to 372 genes. Ontological groupings showed representation of multiple biochemical processes, including those affected by hypoxia, although genes in apoptosis, death signaling, DNA damage, ATM or NF-kb pathways were not represented, indicating significant integrity at the genetic level. Next, we isolated hepatocytes from a series of NHB donors and found that capacity to exclude trypan blue declined within 15 min after death without change subsequently from donors after up to 4 h (50-55% viable). By contrast, viability of hepatocytes from donors beyond 6 h declined, although viability of cells from donors after 6, 16 or 24 h was similar (20-30%). To enhance viability of NHB donor cells, we studied whether donor pretreatment to perturb adrenergic (labetalol, phentolamine), calcium channel (verapamil) or KATP channel (diazoxide) will be helpful. However, these treatments did not improve cell viability from NHB donors 3, 6, 16 or 24 h after death. Finally, we examined the capacity of F344 NHB donor cells to engraft in DPP4-rats after intrasplenic transplantation. After 7 d, survival and engraftment of transplanted cells from NHB donors up to 4 h after death resembled that of cells from live donors with integration in the liver parenchyma. However, cells from NHB donors between 6-24 h after death engrafted rarely. Conclusions: The NHB donor liver remains intact for prolonged periods and can offer healthy hepatocytes capable of engrafting in the liver. As perturbations in the NHB donor liver were limited, this suggests that damage to hepatocytes likely emanated from reperfusion during cell isolation, which should be amenable to manipulations.

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HUMAN HEPATOCYTE GROWTH FACTOR EXPRESSION IN ENDOTHELIAL PROGENITOR CELLS ENHANCES REGENERATIVE PROPERTIES IN RAT CIRRHOTIC LIVER

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Background: Previously we reported that endothelial progenitor cell (EPC) transplantation could reduce established liver fibrosis and promote hepatic regeneration in a cirrhotic liver rat model. In the present study, we investigated the hypothesis that gene transfer can be used to achieve phenotypic modulation of EPCs. Especially, we investigated the effect on regenerative properties of human hepatocyte growth factor (hHGF)-transduced EPCs in rat cirrhotic liver. Methods: Rat EPCs were isolated from rat bone marrow cells. In vitro, rat EPCs cultured for 7 days were transduced an adenovirus encoding hHGF gene (AdhHGF) or bacterial beta-galactosidase gene (AdlacZ) as a control. In vivo, recipient rats were injected i.p. with carbon tetrachloride (CCl4) twice weekly for six weeks before initial administration of EPCs. CCl4 was then re-administered twice weekly for more four weeks, and LacZ-transduced EPCs (Td/L-EPCs) or hHGF-transduced EPC (Td/H-EPCs) transplantation were carried out for these same four weeks. Examination items were as follows. 1) the measurement of rat HGF concentration in plasma and in liver tissues by ELISA, 2) the morphometry of fibrotic areas by Azan-Mallory staining, 3) immunohistochemistry using anti-collagen-type I, fibronectin, TGF-β, α-SMA, and Ki67 antibodies, and 4) blood chemistry. Results: Rat HGF plasma levels of rats transplanted with Td/H-EPCs disclosed significantly higher than those of rats transplanted with Td/L-EPCs after CCl4 treatment.
for 10 weeks (48.0 ± 27.9 versus 34.5 ± 15.2 ng/mL). In the AdhHGF-transduced group, the degree of liver fibrosis was suppressed compared to those in the AdLacZ-transduced group (4.29 ± 1.4 versus 5.25 ± 1.0 %), and the expression of type-I collagen, fibronectin, TGF-β and α-SMA was diminished after CCl₄ treatment for 10 weeks although insignificantly. Ki67-positive hepatocytes in the AdhHGF-transduced group were significantly increased (13.3 ± 1.1 versus 10.5 ± 1.8 %; p<0.05). Moreover, serum total protein and albumin levels were significantly higher in the AdhHGF-transduced group than those in the AdLacZ-transduced group. Conclusions: This enhanced regenerative acceleration of transplanted EPCs by HGF transfer will provide novel therapeutic strategy for hepatic regeneration in patients with severe cirrhosis.

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684 INITIAL CLEARANCE OF TRANSPLANTED CELLS FROM THE LIVER IS REGULATED IN PART BY COX-1-MEDIATED INFLAMMATORY MECHANISMS AND COX-INHIBITION IMPROVES CELL ENGRAFTMENT

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To develop robust applications of hepatocyte transplantation, insights into cell engraftment mechanisms will be helpful. Cell transplantation leads to activation of both beneficial and deleterious events in the liver, including Kupffer cells, phagocytes, hepatic stellate cells, and endothelial cells, that affects cell engraftment. In particular, release of inflammatory mediators, e.g., cytokines, impairs transplanted cell engraftment. Here, to establish whether Cox-1 and Cox-2-dependent mechanisms serve roles in clearing transplanted cells, we studied groups of DPPIV-F344 rats that were subjected to intrasplenic transplantation of 10-20 million freshly isolated F344 rat hepatocytes. Analysis of cell transplantation-induced perturbations included hepatic expression of Cox-1 and Cox-2, Kupffer cell activation, onset of cytokine-dependent liver inflammation through hepatic biliary excretion of 99m-Tc-mebrofenin and changes in cell engraftment. RT-PCR and immunostaining showed that Cox-1 but not Cox-2 was expressed in the liver of DPPIV- rats even without cell transplantation. After cell transplantation, hepatic Cox-1 expression increased within 6 h and persisted throughout the 7 d period of the study, whereas Cox-2 was not expressed. Similarly, cell transplantation was associated with rapid activation of Kupffer cells as shown by increased carbon incorporation. Moreover, hepatobiliary excretion of 99m-Tc-mebrofenin, which is perturbed by the release of TNF-alpha and IL-6, was abolished after cell transplantation. To demonstrate whether perturbation of Cox-1 activation by prior treatment of animals with Naproxen, a non-specific Cox inhibitor, will benefit cell engraftment, we performed dose-ranging studies in DPPIV-rats. Administration of Naproxen 2 h before cell transplantation decreased systemic inflammation, as indicated by normalization of 99m-Tc-mebrofenin excretion. Furthermore, morphometric analysis of transplanted cell numbers in Naproxen-treated rats and untreated control rats showed significant increases in transplanted cell engraftment after Cox-inhibition. Conclusions: The inflammatory response activated by cell transplantation extends to Cox-1-mediated processes in the liver leading to impairment of transplanted cell engraftment. As interference with Cox activation improved cell engraftment, these findings establish further roles for intrinsic inflammatory mechanisms in clearance of transplanted cells, as well as the possibility of thwarting these deleterious consequences with pharmacological approaches. The findings will be helpful in optimizing clinical strategies for cell transplantation.

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685 DISTINCTIVE CHARACTERIZATION OF PERIPHERAL TOLERANCE IN PATIENTS WITH PRIMARY BILIARY CIRRHOSIS

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Although several groups have described autoreactive T cells in primary biliary cirrhosis (PBC), there remains a major intellectual gap in understanding loss of tolerance. This loss of tolerance is critical for developing the hallmark of PBC, the multi-lineage anti-mitochondrial response. To address this issue, we developed 8 unique T cell clones (TCC) specific for the major mitochondrial antigen, the E2 component of pyruvate dehydrogenase. The qualitative and quantitative characteristics of these TCCs were studied and mapped in detail with respect to their selective activation by co-stimulatory signals. Importantly, using readouts of not only cytokine production, but also stimulation with putative mimics of PDC-E2, we found that, in patients with PBC, silencing in the periphery rather than clonal deletion, maintains tolerance. In particular, we studied the ability of these TCC specific clones for PDC-E2 163-176 to proliferate or become anergic in the presence of co-stimulation signals. TCC were stimulated with either human PDC-E2 163-176, an E coli 2-oxoglutarate dehydrogenase mimic (OGDC-E2 34-47), or analogs with amino acid substitutions using HLA-matched allogeneic PBMC or mouse LDR53 fibroblasts as APC. Based on their differential responses to these peptides in the different APC systems, TCC were classified as co-stimulation-dependent or -independent. Only co-stimulation dependent TCC could become anergic. TCC with co-stimulation dependent responses to OGDC-E2 become anergic to PDC-E2 when preincubated with mimic, even if co-stimulation independent for PDC-E2 163-176. Anergic TCC produced IL-10. One selected TCC could not become anergic after preincubation with PDC-E2 163-176 pulsed LDR53 but became anergic using LDR53 pulsed with PDC-E2 peptide analogs with a substitution at a critical TCR binding site. TCC that only respond to peptide-pulsed PBMC, but not LDR53, proliferate with peptide-pulsed CD80/CD86 transfected LDR53; however, anergy was not induced with peptide-pulsed LDR53 transfected with only CD80 or CD86. These data highlight that co-stimulation plays a dominant role in maintaining peripheral tolerance to PBC-specific antigens. They further suggest that, under specific circumstances, molecular mimicry of an autoantigen may restore rather than break peripheral tolerance. Furthermore, based on
686 PRIMARY SCLEROSING CHOLANGITIS IS AN INDEPENDENT PREDICTOR OF EARLY HEPATIC ARTERY THROMBOSIS FOLLOWING PRIMARY LIVER TRANSPLANTATION: A COHORT MULTI-CENTRE STUDY

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BACKGROUND Primary sclerosing cholangitis (PSC) is an uncommon relentlessly progressive disease leading to liver failure for which liver transplantation is the only established long-term treatment. Controversy exists however as to whether PSC increases the risk of early hepatic artery thrombosis (HAT), a catastrophic complication of liver transplantation. The objective of this multi-centre cohort study is to examine this hypothesis in a large risk-adjusted analysis.

PATIENTS AND METHODS

We examined the impact of PSC on the risk of early (<3 months) HAT and thrombotic graft loss among 6,297 adult first single-organ liver transplant recipients in the UK and Ireland during the period 1 March 1994-31 March 2006, adjusting for recipient, donor and graft characteristics. Independent verification of our findings was sought by undertaking a risk-adjusted analysis of the impact of PSC on the time to thrombotic graft loss among 58,034 such recipients reported to the OPTN/UNOS database in the US between 1 October 1987-31 March 2006. Multiple logistic and Cox regression models were used in the analysis as appropriate.

RESULTS The incidence of early HAT among patients transplanted for PSC in the UK and Ireland (n=571) was significantly higher than that among those transplanted for other liver diseases (OR 1.63 95%CI 1.10-2.40). This association persisted even after adjustment for other risk factors (OR 1.86 95%CI 1.24-2.81). Among those who ultimately lost their graft in the first year (n=1,254), the incidence of early HAT was even higher among PSC recipients compared to those transplanted for other diseases (unadjusted OR 1.97 95%CI 1.24-3.12, adjusted OR 2.19 95%CI 1.33-3.61). Similarly, the risk of thrombotic graft loss within the first year among PSC recipients in the US (n=4,150) was significantly higher than those transplanted for other diseases, both with and without risk-adjustment (unadjusted HR 1.81 95%CI 1.53-2.13, adjusted HR 1.67 95%CI 1.41-1.97). In both cohorts, the incidence of thrombotic graft loss after the first post-transplant year was similar in PSC and non-PSC recipients. PSC recipients who had developed early HAT had a significantly higher risk-adjusted graft loss than those who had not (HR 12.73 95%CI 6.54-24.80).

CONCLUSIONS PSC is an independent predictor of early HAT following primary liver transplantation, leading to substantially greater recipient morbidity and futility of a scarce resource. Our results suggest the need for detailed pre-operative screening for underlying thrombophilia, increased postoperative surveillance for early HAT and consideration of appropriate prophylactic antithrombotic therapy in this patient population.

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687 IMPAIRMENT OF THE INDOLEAMINE 2,3-DIOXYGENASE PATHWAY IN PRIMARY BILIARY CIRRHOSIS

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The immunomodulatory effects of the tryptophan-catabolizing enzyme indoleamine-2,3-dioxygenase (IDO) have been elucidated at a cellular level and implicated in the pathogenesis of several complex diseases. Defects within the regulatory T cell compartment are one of the characteristics of primary biliary cirrhosis (PBC), an autoimmune chronic cholestatic liver disease, a phenotype that has also been shown in disease-mimicking animal models of this disease. In fact, human PBC manifests a quantitative decrease in the T regulatory lymphocyte compartment while three murine models mimicking several features of the disease are associated with specific dysfunctions of T cell proliferation. Since IDO constitutes an inducible control mechanism of T lymphocyte proliferation, we hypothesized that IDO dysregulation could lead to altered frequency and/or function of T cells at the level of antigen processing/presentation ultimately contributing to tolerance breakdown. We investigated (i) the IDO induction profile and function in peripheral blood monocytes in 30 women with PBC and 20 controls using RTFPCR and HPLC methods, (ii) the IDO expression in 5 PBC and 14 control liver tissues by immunohistochemistry and, (iii) the association of a TGF-beta gene promoter polymorphism in 120 PBC and 120 control DNAs. Results demonstrate that (i) both IDO expression and activation manifest an impaired IFN-γ response in peripheral monocytes. After stimulation, IDO transcription was significantly lower in monocytes from patients with PBC compared to healthy controls (0.1143 ± 0.019 vs 0.051 ± 0.013, P<0.007), patients also manifested lower rates of trp to kyn transformation when compared to healthy controls (0.4621 ± 0.222 vs 0.0867 ± 0.03, P<0.005). (ii) A peculiar IDO expression profile in intrahepatic bile duct cells characterized early stage PBC with a typical apical pattern observed if the enzyme was expressed in the cholangiocyte. Further, we observed significantly different gain-of-function -509 T allele frequencies within the TGF-beta promoter region in the two populations (0.600 in PBC vs. 0.504 in controls, P=0.035). In conclusion, we submit that an impaired IDO induction might represent a contributing factor in PBC pathogenesis in association with several specific defects in the target tissue and a susceptible genetic background.

Disclosures:
The following people have nothing to disclose: Sabine Oertelt-Prigione, Tin K. Mao, Carlo Selmi, Koichi Tsuneyama, Aftab A. Ansari, Ross L. Coppel, Pietro Invernizzi, Mauro Poddà, M. Eric Gershwin
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INDUCTION OF PRIMARY BILIARY CIRRHOSIS IN GUINEA PIGS USING CHEMICAL XENOBIOTIC IMMUNIZATION: IMPLICATIONS FOR TOLERANCE AND THE ETIOLOGY OF HUMAN PBC

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Although significant advances have been made in dissecting the effector mechanisms in autoimmunity, the major stumbling block remains defining the etiological events that precede disease. Primary biliary cirrhosis (PBC) illustrates this paradigm because of its high degree of heritability, its female predominance and its extraordinarily specific and defined immune response and target destruction. In PBC, the major autoantigens belong to E2 components of the 2-oxo-acid dehydrogenase family of mitochondrially-located enzymes which share a lipoylated peptide sequence that is the immunodominant target. Using rigorous combinatorial chemistry, our previous work demonstrated that synthetic mimics of the lipopeptide molecule such as 6-bromohexanoate (6-BH) react intensely with PBC sera but not controls. This observation prompted us to immunize guinea pigs with 6-BH conjugated to BSA and to follow them for 2 years. Herein, we provide serologic and immunohistochemical evidence that such immunized guinea pigs not only develop antimitochondrial autoantibody responses similar to human PBC, but also develop autoimmune cholangitis after 18 months. Firstly, 6BHBSA-immunized guinea pigs produced antibodies to PDC-E2, BCOADC-E2, and OGD-E2 as early as 4 wk after the initial immunization, and 100% of animals were AMA positive by 12 weeks. Most importantly, at 18 months post-immunization, guinea pigs demonstrated significant lymphoid cell infiltrates surrounding damaged bile ducts and mild to moderate infiltration of lympho-plasmacytes and vacuolated histiocytes in portal areas where interlobular bile ducts were surrounded by variably swollen lympho-plasmacytes with a vacuolated cytoplasm and an irregular luminal border, or showed an eosinophilic shrunken appearance with pyknotic nuclei. Portal inflammation was distributed heterogeneously within the liver and in some portal areas, interlobular bile ducts were not clearly observed as in bile duct loss. In contrast to the pathological changes of interlobular bile ducts, septal to large bile ducts did not show any significant pathological changes. Mild piecemeal necrosis (interface hepatitis) was observed in the affected portal tracts. In summary, the observed histology was similar to the chronic non-suppressive destrucitve cholangitis that characterizes PBC. In conclusion, these data reflect the likelihood that in PBC, the multi-lineage anti-mitochondrial response is a pathogenic mechanism and that loss of tolerance and subsequent development of biliary lesions depends on either modification of the host mitochondrial antigen or a similar breakdown due to molecular mimicry.

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RISK OF CARDIOVASCULAR AND CEREBROVASCULAR EVENTS IN PRIMARY BILIARY CIRRHOSIS: A POPULATION-BASED COHORT STUDY

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Background: Patients with primary biliary cirrhosis (PBC) have a 2.7 fold increase in death compared with general population the cause of which is unclear. Risk factors such as smoking and hyperlipidemia have been associated with PBC. Therefore, patients with PBC may have higher risk of major vascular events. Methods: We compared the risk of developing Myocardial Infarction (MI), Stroke and Transient Ischaemic Attack (TIA) in a cohort of PBC patients and a general population control cohort. Subjects were selected from General Practice Research Database (GPRD). For each case, 10 controls were chosen from the same general practice matched on age and sex. Hazard ratios and 95% confidence intervals were calculated. Results: There were 930 patients (88% female) in our PBC cohort that were compared with 9202 controls. There were similar proportions of smokers, and subjects with hypertension in PBC and control groups. However, PBC patients had lower Body Mass Index (BMI) [Chi-square=11.4, df=4, P=0.02] and higher frequency of diabetes [Chi-square=16.3, df=1, P<0.001]. Less than 5% of subjects in each group received lipid lowering agents. During a total of about 43000 person years of follow-up, 244 MI's, 591 Stroke's and 221 TIA's were identified. Incidence rates per 1000 person years for MI, stroke, and TIA during the study period were 6.0 (3.9-9.1), 13.9 (10.5-18.3) and 3.3 (2.0-6.0) for PBC cohorts and 5.6 (5.0-6.4), 14.1 (13.0-15.3), and 5.3 (4.6-6.0) for control cohorts respectively. Hazard ratios for vascular events in PBC cohort compared to control cohort were 1.04 (0.67-1.62), 0.98 (0.73-1.31), 0.66 (0.38-1.16) for MI, Stroke and TIA respectively. Hazard ratio for any vascular event was 0.99 (0.77-1.27). Adjustment for confounders did not change the results. Conclusions: PBC is not associated with an increased risk of myocardial infarction, stroke or TIA. Major vascular events are unlikely to account for the increased mortality in PBC patients. Nor is there excess morbidity from these conditions. Hypercholesterolemia related to PBC may not require treatment with lipid lowering agents.

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NOD2,2445 MICE, A SPONTANEOUS MODEL OF HUMAN PRIMARY BILIARY CIRRHOSIS, DEVELOP PDC-E2 SPECIFIC AUTOREACTIVE T CELLS

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Primary biliary cirrhosis (PBC) is a liver specific autoimmune disease characterized by antimitochondrial autoantibodies

Disclosures:

The following people have nothing to disclose: Patrick S. Leung, Ogyi Park, Koichi Tsuneyama, Mark J. Kurth, Kit S. Lam, Afzab A. Ansari, Ross L. Coppel, M. Eric Gershwin.
... be the key to successful immunotherapy. Response to PDC-E2 will be the effector mechanism for biliary antigenic driver of this disease. Finally, we submit that these imply that PDC-E2 is a major autoantigen and likely to be of NOD.2445 mice as a model of human PBC, but also further NOD.2445 mice. These data highlight not only the relevance splenic CD4 T cells specific to PDC-E2 (0.04 to 0.1%) in C57BL/6 mice when either BSA or PDC-E2 were used flow cytometry. Importantly, there was no detection of reactive stimulated T cells was examined by ELISpot and intracellular medium alone. Two days later, IFN-γ production of the antigen-stimulated T cells was examined by ELISpot and intracellular flow cytometry. Importantly, there was no detection of reactive T cells in C57BL/6 mice when either BSA or PDC-E2 were used as antigens. Furthermore, the NOD.2445 mice had a similar frequency of reactive T cells to BSA as control C57BL/6 mice. However, there was an extraordinarily high frequency of splenic CD4 T cells specific to PDC-E2 (0.04 to 0.1%) in NOD.2445 mice. These data highlight not only the relevance of NOD.2445 mice as a model of human PBC, but also further imply that PDC-E2 is a major autoantigen and likely to be the antigenic driver of this disease. Finally, we submit that these data provide further evidence that the multi-lineage, multi-cell response to PDC-E2 will be the effector mechanism for biliary damage in humans with PBC. Manipulation of this pathway will be the key to successful immunotherapy.

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691 GENETIC POLYMORPHISM OF HLA-DR AND CYTOKINE GENES IN JAPANESE PATIENTS WITH PRIMARY BILIARY CIRRHOSIS (PBC) – HLA-DRB1*0405 CONFFERS SUSCEPTIBILITY TO GP210-TYPE PROGRESSION OF PBC–

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Background/Aims: Anti-gp210 and anti-centromere antibodies are different risk factors for hepatic failure-type (gp210-type) and portal hypertension-type (centromere-type) progression, respectively, in primary biliary cirrhosis (PBC). To study the underlying immunogenetic mechanisms for each type of disease-progression, we examined the HLA-DR and cytokine gene polymorphism in 150 well-characterized PBC patients who have been registered to National Hospital Organization Study Group for Liver disease in Japan (NHOSLJ). Methods: Serum antibodies to gp210 and centromere were measured by EUISA over periods extending from 2-289 (median 59) months of observation. HLA-DRB1 genotype was determined by sequence-based typing (SBT) of group-specific PCR products. Genetic polymorphism in the cytokines TNF-α, IFN-γ, TGF-β1, IL-10, IL-6 and MDXR were analyzed by polymerase chain reaction-sequence specific primers (SSP). Results: HLA-DRB1*0803 confers susceptibility to PBC development in Japanese population [OR=1.93, 95% CI: 1.17, 3.18] and is a risk factor for positive anti-centromere antibodies [OR=2.24, 95% CI: 0.93, 5.33], HLA-DRB1*0405 is a significant risk factor for positive anti-gp210 antibodies [OR=2.10, 95% CI: 1.10, 4.45] and is a significant negative risk factor for positive anti-centromere antibodies [OR=0.27, 95% CI: 0.09, 0.71]. In addition, IFN-γ intron +874 (T/T + T/A) genotype was a risk factor for anti-gp210 antibodies: production [OR=2.38, 95%CI: 0.97, 5.96] with reference to IFN-γ (A/A) genotype. While HLA-DRB1*0803 nor HLA-DRB1*0405 per se were not risk factors for disease-progression, HLA-DRB1*0405 carriers who progressed to advanced stage were all positive for anti-gp210 antibodies, indicating that the development of anti-gp210 antibodies is an essential step for the progression of PBC in HLA-DRB1*0405 carriers. In addition, we found a novel haplotype of MDXR gene polymorphism (Hap*2) which determines the development of jaundice in gp210 positive PBC patients. Conclusions: While HLA-DRB1*0803-linked genetic factor confers a susceptibility to PBC development and anti-centromere antibodies-production, HLA-DRB1*0405-linked genetic factor confers a susceptibility to anti-gp210 antibodies-production and gp210-type progression of PBC in Japanese population. Furthermore, genetic polymorphism of MDXR gene (Hap*2) confers a susceptibility to the development of jaundice resulting in end-stage hepatic failure in gp210-positive PBC patients. These results indicate that multiple different genetic factors are involved at multiple steps of disease-progression in PBC.

Disclosures:
A CRITICAL ROLE OF INVARIANT NKT CELLS IN EXACERBATING THE BILIARY LESIONS IN A TGF-β RECEPTOR II DOMINANT-NEGATIVE MOUSE MODEL OF PRIMARY BILIARY CIRRHOSIS

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A key question in human PBC revolves around the mechanisms involved in destruction of small intrahepatic bile ducts. Autoreactive T cells are induced by CD83+ dendritic cells (DCs) leading to the generation of autoantibodies against the E2 subunit of the pyruvate dehydrogenase enzyme complex (PDC-E2). Innate immunity has also been postulated to play a key role in the exacerbation of liver injury. For example, natural killer (NK) cells are increased in number in the liver of PBC patients and recruited more efficiently, resulting in exacerbated hepatic damage which is hypothesized to be due to their higher cytotoxic ability. While CD1d expression and the frequency of invariant NKT (iNKT) cells are both increased in PBC liver, the involvement of such iNKT cells in the pathogenesis of PBC is not clear. It is important to note that whereas there is an increase in hepatic presence of iNKT cells in PBC patients as compared to controls, there is in fact a concomitant decrease in the hepatic presence of iNKT cells activated by α-galactosylceramide (α-GalCer) in young but not older δTGFβRII mice, suggested an age dependent role of iNKT cells. These data demonstrate that iNKT cells in δTGFβRII mice are a critical factor in liver injury.

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ment should ideally be targeted to patients with low bone mass because of limited availability of BMD technology in some communities and cost considerations. We aimed at comparing the performance of several risk score indices to identify PBC patients who are more likely to have low bone mass who should be referred for BMD measurements. METHODS: We studied 363 patients with PBC. BMD of lumbar spine and femoral neck was measured. BMD values were converted into T-score separating patients with normal BMD (T>-1), osteopenia (T-1 to -2.5), and osteoporosis (T<-2.5). We included a subcategory of "low-BMD" for patients with T<-2 of either lumbar spine or femoral neck to detect those pre-osteoporotic patients that should be referred for BMD measurements. Four risk score indices that were created and validated in postmenopausal women from the general population were calculated in our PBC patients. Those indices are the Osteoporosis Self-assessment Tool (OST) which is based only on age and weight, and three other risk tools including the Osteoporosis Risk Assessment Instrument (ORAI), the Simple Calculated Osteoporosis Risk Estimation (SCORE), and the Osteoporosis Index of Risk (OSIRIS) which are based on age and weight in combination with up to four additional variables such as race, and history of estrogens use, rheumatoid arthritis, and non-traumatic fractures. ROC analyzes and the AUC were computed for each index to evaluate their discriminatory performance and accuracy. Additionally, three risk categories (low, moderate, and high) were used for each score index according to the developer’s recommendation for DXA referral. RESULTS: The AUC was consistently high for the four indices indicating good test performance (Table). OST, SCORE, ORAI, and OSIRIS identified respectively 90%, 89%, 88%, and 94% of the patients at high risk for low-BMD (T<-2) who subsequently should have been recommended for densitometry; and 84%, 65%, 60%, and 84% of patients at low risk for low-BMD who subsequently should not have been recommended for densitometry. CONCLUSIONS: Simple risk score indices are effective and efficient tools to help target high-risk patients with BMD for measurements. As the OST index is the simplest and quickest to calculate, OST may be used as a first-line prescreening tool in PBC patients.

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<th>Risk Score Indices</th>
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**AUTONOMIC DYSFUNCTION IN PRIMARY BILIARY CIRRHOSIS IS ASSOCIATED WITH STRUCTURAL BRAIN ABNORMALITIES, PARTICULARLY IN THE GLOBUS PALIDUS**

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Autonomic dysfunction (AD) (assessed using symptom assessment tools, heart rate variability (HRV), baroreflex sensitivity (BRS) and dynamic testing in the context of the Valsalva manoeuvre and tilt testing) is a frequent finding in the autoimmune liver disease primary biliary cirrhosis (PBC). Recent studies from our group have confirmed that the degree of AD seen in individual patients is unrelated to the severity of their liver disease, with AD being a frequent finding even in patients with confirmed early stage disease. Although the consequences of AD, such as the symptom of fatigue, have been a focus of recent work, the underlying mechanisms that lead to AD in PBC have received less attention. The aim of this study was to examine structural brain lesions in early stage PBC and to determine whether their presence and position associated with AD. Par-
ticular attention was paid to deep white matter lesions (DWML) which have been linked with AD and associated cognitive impairment development in the dementia literature. 29 female subjects with early stage PBC were included. BRS was assessed using the sequence method and HRV using spectral analysis using the continuous beat-to-beat Taskforce system (CNSystems; Austria). High-resolution T2w images (TR/TE = 2000/60ms, 0.49x0.49mm, 3mm thick) were acquired with a Philips 3T Intera Achieva with a dedicated head coil. DWML load was assessed by two independent observers according to the Scheltens classification criteria. A consensus score regarding total lesion load (TLL) was reached. DWML were found to be present in all 29 PBC subjects suggesting that the presence of organic brain change is universal in PBC. BRS showed a significant correlation with Total Lesion Load (TLL). When individual brain areas were considered the lesion load in the Globus Pallidus (GP) inversely correlated (non-parametric) with impaired BRS (p=0.03; r=-0.4). In conclusion, AD in PBC is associated with the presence of structural brain abnormalities when assessed using MR techniques. These abnormalities are particularly in those brain areas that are associated with autonomic nervous system function. Dynamic and longitudinal studies are needed to determine the nature of the association between AD and structural brain lesions in PBC.

Scheltens, Brain 115, 753 (1992)

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RESULTS OF THE FRENCH STUDY OF RISK FACTORS FOR PRIMARY BILIARY CIRRHOSIS

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BACKGROUND AND AIM: Primary biliary cirrhosis (PBC) is a complex disease thought to result from combination of genetic and environmental factors. Elucidating these factors are critical for understanding the pathophysiology of the disease and developing new therapeutic approaches. A large case-control study recently performed in the US has identified several environmental risk factors associated with PBC, namely urinary tract infections (UTI), tobacco smoking, cosmetics use and hormone replacement therapy (HRT). Accordingly, our study was carried out in a large scale in France to determine whether these results could be confirmed and considered as paradigm of risk factors for PBC. METHODS: Patients with PBC and unrelated controls matched for sex, age and geographical location were subjected to a standardized questionnaire regarding demographic characteristics, lifestyle, personal and familial medical history and reproductive history. Comparisons between cases and controls were performed using the Wilcoxon test for continuous variables and the Fisher exact test for categorical variables. Multiple logistic regression models were used for multivariate analysis. RESULTS: 222 patients with PBC (89% female; mean age, 51 years) and 509 controls were enrolled in the study. Data analysis indicated that having a first-degree relative with PBC (adjusted odds ratio [AOR] 6.82, 95% confidence interval 1.16 – 32.93) or with autoimmune thyroid disease (AOR 5.30, 95%CI 1.38 – 28.07), a personal history of UTI (AOR 1.89, 95%CI 1.26 – 2.84), or a past history of active or passive smoking (AOR 3.12, 95%CI 1.95 – 5.00) were significantly associated with increased risk of PBC. The frequent use of hair dye was not shown to confer disease susceptibility. A history of use of HRT was not more frequent in women with PBC, however the past use of oral contraceptives was found to be significantly associated with a decreased risk of the disease (AOR 0.64, 95%CI 0.43 – 0.95). The age at first pregnancy was significantly lower in PBC than in control women (24.0 ± 4.8 years vs. 25.0 ± 4.5; p=0.0096) while the mean number of pregnancies in the two groups was similar. CONCLUSION: The present data: (a) confirm the major genetic predisposition to autoimmune disorders and PBC; (b) reinforce the strong association between UTI, tobacco smoking and risk of PBC development; (c) suggest that exogenous estrogens may confer protection against PBC.

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PRIMARY BILIARY CIRRHOSIS (PBC) WITH INITIAL NORMAL BILIRUBIN CONCENTRATION: TREATMENT WITH URSEDOXYCHOLIC ACID (UDCA) DOES NOT AFFECT LIVER-RELATED SURVIVAL

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Background and aims: Early PBC is considered the subgroup which can benefit from long-term UDCA therapy. We evaluated a cohort of early PBC patients with prolonged follow-up, treated or untreated with UDCA in order to confirm this assumption. Methods: 204 PBC patients with ≤17 µmol/L serum bilirubin concentration at referral (149 patients referred to Royal Free Hospital, London and 55 referred to University of Padua). 88 (84 females, median age 58 yrs) were treated with UDCA and 116 (110 females, 57 yrs) remained untreated. All but 2 patients continued UDCA treatment for the entire follow-up time. Survival free of death and liver transplantation (LT) and time to onset of complications were evaluated with Kaplan-Meier plots and log rank test. Results: At referral untreated group and UDCA group were not different for symptoms (48% vs 39% asymptomatic, respectively), albumin (median 43 vs 42 g/L), alkaline phosphatase (median 232 vs 251 IU/L), Mayo risk score (median 3.9 vs 4.0). Median bilirubin was 8 µmol/L (range 3-16) in non-UDCA group and 9 µmol/L (range 2-17) in UDCA group (p=0.04). Follow-up ranged from 1 to 22 yrs in both groups (median 6 yrs in untreated and 8 in treated). Median rate of bilirubin increase from referral was significantly higher in untreated (median 0.3 µmol/L/year, range 10.0–70.0) than in UDCA group (median 0.0 µmol/L/year, range 2.6–40.0). LT occurred in 4 (3%) vs 2 (2%) and death in 24 (21%); 16 non liver-related: 9 for cardio-respiratory disease, 2 for cerebrovascular accident and 5 for extrahepatic malignancy) vs 2 (2%, all liver-related) in non-UDCA and UDCA groups respectively. Kaplan-Meier analysis showed significant better survival (p=0.002) in UDCA group, but after censoring non liver-related deaths the difference disappeared (p=0.11). By dividing all patients according to low (≤3.6), intermediate (3.7-4.1) and high (≥4.2) Mayo risk score, overall survival was significantly better in UDCA group with respect to untreated, but only in those with high Mayo risk (p=0.008), and again this difference disappeared after censoring non liver-related deaths (p=0.06). There was no difference in the first onset of any liver
COMBINATION ANTIRETROVIRAL THERAPY WITH COMBIVIR ATTENUATES AUTOIMMUNE BILIARY DISEASE IN THE NOD.c3c4 MOUSE MODEL OF PRIMARY BILIARY CIRRHOSIS

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A human betaretrovirus resembling the mouse mammary tumor virus (MMTV) has been characterized in patients with primary biliary cirrhosis (Xu et al., PNAS, 2003). The NOD.c3c4 mouse develops AMA, autoimmune biliary disease and liver failure. We have shown that NOD.c3c4 mice have a significantly higher burden of MMTV infection in the liver compared to control mice without innate immune deficiency and that the virus is predominantly localized to bile ducts. Aim: Clinical trials suggest that PBC patients treated with combination antiviral therapy demonstrate significant histological and biochemical benefit (Mason et al., 2004, American Journal of Gastroenterology). The goal of this study was to investigate whether antiviral therapy can impact on the development of autoimmune biliary disease in the NOD.c3c4 mouse. Methods: Twenty 5 to 8 week old NOD.c3c4 (Taconic) were randomized to either placebo (n=10) or Combivir (n=10), dosed at Zidovudine 3mg and Lamivudine 1.5mg per day. Serial blood samples were taken for hepatic biochemistry studies and mice were sacrificed at 20 weeks of age. A liver pathologist ranked coded liver samples using the Ishak score and the percentage of liver demonstrating biliary cyst formation was measured in 5 magnification sections using MetaVue software analysis. Hepatic RNA was assessed for viral burden using real-time RT-PCR with MMTV gag and pol primers normalized to beta-actin. Results: Serial hepatic biochemistry studies showed diminished alkaline phosphatase in the Combivir treated mice within the first 10 weeks of therapy but this was not sustained. At sacrifice, there was no difference in hepatic weight for Combivir vs. placebo treated animals. Histological evaluation showed a significant decrease in the necro-inflammatory score (0.8 Combivir vs. 1.5 placebo, p=0.015) as well as the bile duct injury [0.3 Combivir vs. 1.0 placebo, p=0.015]. Therapy had little impact on bile duct cyst formation (8% Combivir vs. 10% placebo). When compared to mice receiving placebo, Combivir therapy reduced viral burden by 26% to 47% as measured by the pol and gag gene RT-PCR, respectively. Conclusions: Combivir treatment positively impacted on inflammation and bile duct damage in the NOD.c3c4 mouse, as previously reported for patients with PBC. Even though Combivir therapy lacked potency in inhibiting MMTV, the bile duct damage and inflammation was attenuated suggesting a central role for MMTV in the autoimmune biliary disease. As Combivir treatment is insufficient to halting disease in mice and in humans with PBC, the NOD.c3c4 mouse will provide a good model for testing highly active anti-retroviral therapy.

Disclosures:
The following people have nothing to disclose: Min Chen, Don Graham, Safwat Girgis, Guangzhi Zhang, Shawn Wasilenko, Mark Kneteman, Chelsea McDougall, Yun-yuan Li, Michael Sakalian

HLA DRB1 ALLELES IN FRENCH PATIENTS ACCORDING TO THE CLINICAL PRIMARY BILIARY CIRRHOSIS PHENOTYPE

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Background: Elucidating predisposing genetic associations is a crucial step in understanding the pathophysiology of complex disease such as primary biliary cirrhosis (PBC). Polymorphic variants of HLA are thought to be determinant not only of susceptibility but also of the clinical phenotype of several autoimmune disorders. This could be one of reasons for the apparent complex relationship between HLA polymorphisms and PBC. The only consistent reported association in PBC is the DRB1*08 allele. However at least, in Europeans, this association accounts for a minority of patients. A strong protective association with DRB1*11 and DRB1*13 has also been reported. Objective: Our study was aimed to investigate the contribution of HLA DRB1 alleles to susceptibility to PBC and to variants of the disease according to mode of presentation and severity. Methods: HLA DRB1 alleles of 146 well-characterized PBC patients (64 with liver transplantation) and 16 PBC-Auto-Immune Hepatitis (AIH) overlap syndrome were typed by reverse line blot assay of PCR-amplified DNA. We used as controls a study of 356 families residing in Paris (1). Results: The allele frequency of HLA DRB1*07 was higher in patients with pure PBC than in controls (19.9% vs 13.5%, p=0.009, odds ratio (OR)=1.6). There were also significant protective associations with DRB1*11 (6.2% vs 13.2%, p=0.001, OR=0.43) and DRB1*15 (5.5% vs 13%, p=0.0004, OR=0.39). Several relevant pronostic parameters and their relationship to HLA DRB1 alleles were analysed. Higher frequencies of HLA DRB1*07 and DRB1*03 were associated to age at diagnosis <45 yrs or positive antinuclear dots and anti-gp210 antibodies status. HLA DRB1*08 and DRB1*15 were less frequent in patients with an age at diagnosis <45yrs, in patients who had liver transplantation or with positive antibodies status. In patients with PBC-AIH overlap syndrome there was a significant association with HLA DRB1*03 compared to healthy controls (25% vs 10.1%, p=0.016, OR=2.97). Conclusions: Our data show that in France: (a) The common form of PBC is associated with HLA DRB1*07; (b) absence of association with DRB1*08, a finding as previously reported in Italy, suggests that PBC in France has a different genetic background than that reported in Northern Europe and United States; (c) HLA DRB1*03 is specifically associated with PBC-AIH overlap syndrome; (d) Taken together these results show that HLA polymorphisms contribute not only susceptibility but also to clinical expression and severity of PBC. (1) Pedron B et al, Human Immunology 2005,66,721-731

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CD8 T CELLS PLAY A CRITICAL ROLE IN PRIMARY BILIARY CIRRHOSIS OF DN TGF-βRII MICE

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Human primary biliary cirrhosis (PBC) is characterized serologically by antimitochondrial antibodies (AMA) and histologically by an intense T cell portal infiltrate that destroys bile ducts. The infiltrating T cells have been identified as both CD4 and CD8 cells with a dominance of CD8+ cells with disease progression. Furthermore, using tetramer technology, we have previously demonstrated the presence of a significantly higher autoreactive CD8 T cell precursor frequency in the liver compared to blood of patients with PBC and noted that such cells are higher in early disease. These data suggest that the biliary damage is mediated by CD8 cells. To address this issue, we have taken advantage of the PBC-like disease exhibited by dnTGF-βRII mice and focused our attention on the liver T cell infiltrate. Importantly, these mice have a directed expression of a dominant-negative form of TGF-βRII, under the direction of the CD4 promoter and exhibit nearly identical disease as humans with PBC. One major advantage to this animal model is the ability to perform cell transfer. Therefore to address the issue of T cell effector mechanisms, we isolated dnTGF-βRII splenic CD4+ or CD8+ T cells and transferred these populations into Rag1-/- mice. Importantly, these mice have a direct expression of a dominant-negative form of TGF-β receptor type II (dnTGF-βRII), under the direction of the CD4 promoter and exhibit nearly identical disease as humans with PBC. One major advantage to this animal model is the ability to perform cell transfer. Therefore to address the issue of T cell effector mechanisms, we isolated dnTGF-βRII splenic CD4+ or CD8+ T cells and transferred these populations into Rag1-/- mice. Importantly, the CD8+ T cells transfer group demonstrated a significant expansion of T cells and the presence of portal tract infiltrates in recipient mice. In contrast, although CD4+ T cells did expand in the recipient group, they did not home or focus within the portal tracts. Our results demonstrate that the impaired TGFβ signaling pathway in these mice leads to a CD8 cytotoxic T cell population that plays a critical role in biliary cell damage. These data have implications not only for understanding TGFβ signaling and autoimmunity in these mice, but also in developing appropriate focused immunotherapy to prevent biliary damage.

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ANTIBODIES TO SS-A/RO-52KD AND CENTROMERE IN PRIMARY BILIARY CIRRHOSIS

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Background: Recent data indicate that antinuclear reactivities are associated with an unfavourable clinical course in primary biliary cirrhosis (PBC). Aim: We investigated frequency and clinical significance of a wide spectrum of “rheumatological” antinuclear antibodies in the field of autoimmune chronic liver disease, with special regard to PBC. Methods: The study population included: 105 patients with PBC [89 anti-mitochondrial antibody (AMA) positive, 16 AMA negative], 80 with type 1 autoimmune hepatitis (AIH), 30 with type 2 AIH, 52 with primary sclerosing cholangitis (PSC), 30 with systemic lupus erythematosus (SLE) and 50 blood donors. Patients and controls were tested by immunoblot assay (IB) (RecomLine-Mikrogen, Germany) for the presence of antibodies to the following extractable nuclear antigens (ENA): RNP-68kD, RNP-A, RNP-C, Sm-B, Sm-D, SS-A/RO-52kD, SS-A/RO-60kD, SS-B/La, P0 (ribosomal phosphoproteins), PCNA, Scl-70 and Jo-1. Anti-centromere antibodies (ACA) were also searched by IB (RecomLine-Mikrogen, Germany) and indirect immunofluorescence on HEp-2 cells. Results: The overall prevalence of IB-detected anti-ENA in PBC (30%) was higher than in AIH-1 (2.5%, p<0.0001), AIH-2 (0%, p<0.0001) and PSC (11.5%, p=0.006) and lower than in SLE (53%, p=0.03). The most frequent anti-ENA reactivity in PBC was anti-SSA/RO-52kD (28%, 29/105), ACA were detected by IB in 21% PBC patients and never in the other subjects (p<0.0001). Anti-SSA/RO-52k and ACA occurred with similar prevalences in PBC cases with and without AMA. However, half of AMA negative PBC cases presented at least one of these antibodies. Anti-SSA/RO-52KD positive PBC patients had a more advanced histological stage (p=0.01), higher serum levels of bilirubin (p=0.01) and IgM (p=0.03) compared with negative ones at the time of diagnosis. Moreover, they were more frequently suffering from Sicca Syndrome, occurring in 27.5% (8/29) anti-SSA/RO-52KD positive, but only in 6.5% (5/76) anti-SSA/RO-52kD negative PBC cases (p=0.006). Conversely, the positivity for anti-CENP-B was not associated with distinct features. Positive cases, however, were more frequently affected by the CREST syndrome, occurring in 32% (7/22) of them, but only in 1% (1/83) of negative patients (p=0.0001). Conclusions: In the autoimmune liver disease setting, anti-SSA/RO-52KD and ACA behave as AMA-independent markers of PBC and can thus be of diagnostic relevance especially in AMA negative PBC. Anti-SSA/RO-52KD antibodies identify PBC patients with a more advanced and active disease.

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INTERCELLULAR TRANSPORT AND TRANSLOCATION OF IGA AMA IN PRIMARY BILIARY CIRRHOSIS

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Several laboratories, particularly at UC Davis, have attempted to define a role of mucosal anti-mitochondrial antibodies (AMA) of the IgA class in the pathogenesis of primary biliary cirrhosis (PBC) through a combination of cytology assays and/or immunofluorescence microscopy. Indeed, it has been suggested that the presence of the poly immunoglobulin receptor (pIgR) on biliary epithelial cells allows the basolateral transcytosis of IgA. Such studies have been helpful but also limited by availability of monoclonal dimeric IgA and have generally used canine MDCK cells as a surrogate for human biliary cells. We have now taken advantage of a unique patient with PBC who has a monoclonal gammopathy with extraordinary titers of dimeric IgA AMA. We purified such autoantibodies and labeled them with Alexa 488. The ability of these monoclonal antibodies to be...
transported and to co-localize was studied by confocal dual staining microscopy using a well characterized line of human biliary epithelial cells (BECs). The cells were grown on 0.4 μm transwell support membranes, IgA AMA- Alexa 647 was added to the basal compartment of the transwell and the cells were incubated for 15 min at 37 °C. In parallel, control antibodies were used throughout. Importantly, the monoclonal IgA AMA from our patient with PBC, but not control IgA, was transported and translocated to the mitochondria and co-localizes with PDC-E2. We now postulate herein that there is a direct effect of mucosal IgA on mitochondrial function and that a necessary component of this process is the unique pIgR. This extraordinary observation allows us to further postulate that the abnormal kinetics of BEC proliferation is due to the inability of apoptotic BECs to be cleared because of the binding of mucosal IgA.

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704 PROGRESSION IN PBC: NEW INSIGHTS FROM FOLLOW-UP LIVER BIOPSIES 10 YEARS AFTER DIAGNOSIS

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BACKGROUND: Primary Biliary Cirrhosis (PBC) runs a variable course. Biomarkers that predict likely disease progression are needed. AIM: To identify early characteristics that may serve as markers of disease progression in PBC. METHODS: A chart review of PBC patients with a repeat liver biopsy after initial histological diagnosis. Retrievable dual biopsies were reevaluated blinded to date for degree of fibrosis (Ludwig classification), portal inflammation, eosinophilic infiltration, interface hepatitis, lobular hepatitis, lobular necrosis, granulomas, feathery degeneration, ductopenia, duct injury by lymphocytes, florid duct lesions and ductular reaction. Definition of response to ursodeoxycholic acid (UDCA) was: complete responder (CR) normalization of alkaline phosphatase (ALP) within 2 years of initiating therapy, non-responder (NR) less than 50% decline of ALP, partial responder (PR) between CR and NR. Statistical analyses used either paired t tests, Mann-Whitney U test, χ² test, Fisher’s exact test, Kruskal Wallis test or ANOVAs with Bonferroni-adjusted pair wise comparisons. RESULTS: Mean age (56 patients) at baseline was 46 ± 9 (range 30-61), 52 (92.9%) female and 45 (80.4%) AMA positive. All but one received UDCA (13-15mg/kg/d) with CR or PR response in 38 (69.1%), and 17 (30.9%) were NR. On the initial liver biopsy report, 6 (10.7%), 26 (46.4%), 13 (23.2%), 9 (16.1%) and 2 (3.6%) patients had stage 0, 1, 2, 3 and 4 of fibrosis, respectively. Repeat biopsies performed 9.2 ± 2.5 years after their initial biopsy showed NRs to UDCA therapy had more fibrosis progression (14 out of 17, p=0.006). Baseline ALT values in those with fibrosis progression were higher than in those without (114 ± 91 IU/L vs. 73 ± 52 IU/L, p=0.045). Baseline serum gp210 titers were higher with fibrosis progression than without (21.3 ± 35.1 U vs. 8.3 ± 13.9 U, p=0.046) and correlated with response to UDCA (CR: 3.9 ± 0.3 U, PR: 8.6 ± 14.2 U, NR: 36.1 ± 43.8 U, p=0.004). Re-review of 22 retrievable dual biopsies indicated that both interface hepatitis (p=0.037) and ductopenia (p=0.003) on initial biopsy correlated with NR to UDCA but not with fibrosis progression. SUMMARY: Higher ALT and gp210 titer at baseline and failure to achieve a biochemical response with UDCA are associated with fibrosis progression in PBC. Both interface hepatitis and ductopenia may predict responsiveness to UDCA but not fibrosis progression, this latter finding may due to the small sample size.

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705 RITUXIMAB FOR PRIMARY BILIARY CIRRHOSIS (PBC) REFRACTORY TO URSEDIOXYCHOLIC ACID (UDCA)

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Background: Rituximab, an anti-CD20 monoclonal antibody that selectively depletes B-cells, has shown promise in autoimmune-body-associated, immune-mediated disorders including rheumatoid arthritis. Since UDCA leads to biochemical normalization in only a minority of PBC patients, additional treatment options are necessary. The objective of this study was to assess the safety and efficacy of rituximab in patients with PBC refractory to UDCA. METHODS: 14 PBC patients refractory to UDCA (≥13 mg/kg/day for ≥6 months) received two rituximab infusions (1 g) at days 1 and 15 and were followed for up to 12 months. The primary outcome was the proportion of patients with normalization and/or ≥25% improvement in alkaline phosphatase (ALP) at 6 months. Quality of life was assessed by the Fatigue Severity Scale (FSS) and pruritus by an ordinal scale (0=none; 1=mild; 2=severe; 3=excruciating; and substantial sleep disturbance). All data are medians (ranges). Comparisons were made using the signed-ranks test for matched pairs. Results: The median age was 53 years (range 35-71); 92% were female and AMA+. The median PBC duration and UDCA dosage were 3.6 years (0.9-10.8) and 15.3 mg/kg/d (13.6-27.5), respectively. Twelve and 8 patients have completed 6 and 12 months of follow-up, respectively. Although rituximab was well-tolerated, one patient withdrew due to an asthma exacerbation during the first infusion and is excluded from analyses. Effective B-cell depletion was observed in all patients (baseline vs. 6 mo. CD19+ cells: 239 vs. 1/L; P=0.003). ALP normalization and/or ≥25% improvement was observed in 25% of patients at 6 (n=3/12) and 12 months (n=2/8) of follow-up. Significant reductions in median ALP, serum IgM levels, and AMA titers were observed at 6 months. The AMA titer fell by at least one dilution in 75% (9/12); 2 patients became AMA-negative. Pruritus and fatigue also improved (Table). Three patients developed human anti-chimeric antibodies (HACA); all had effective B-cell depletion. Conclusions: Selective B-cell depletion with rituximab shows promise as a therapy for PBC. This phase I study demonstrated no safety concerns with rituximab, but larger controlled studies are necessary to confirm its efficacy.

Biochemical, Serologic and Clinical Response to Rituximab

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Baseline (m=13)</th>
<th>M6 (m=12)</th>
<th>M12 (m=8)</th>
<th>P-value (M6 vs baseline)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALT (U/L)</td>
<td>65 (9-306)</td>
<td>45 (25-206)</td>
<td>41 (21-242)</td>
<td>0.10</td>
</tr>
<tr>
<td>ALP (µmol/L)</td>
<td>259 (142-1152)</td>
<td>205 (108-955)</td>
<td>211 (152-1478)</td>
<td>0.05</td>
</tr>
<tr>
<td>AMA</td>
<td>1/120 (9-1/640)</td>
<td>1/80 (1-1/640)</td>
<td>1/80 (1-1/640)</td>
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<tr>
<td>IgM (g/l)</td>
<td>3.64 (2.65-8.87)</td>
<td>2.00 (1-8.497)</td>
<td>2.25 (0.81-7.16)</td>
<td>0.002</td>
</tr>
<tr>
<td>Pruritus</td>
<td>1 (0-3)</td>
<td>0 (0-3)</td>
<td>0 (0-3)</td>
<td>0.03</td>
</tr>
<tr>
<td>FSS</td>
<td>36 (11-59)</td>
<td>29 (12-55)</td>
<td>33 (21-55)</td>
<td>0.06</td>
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</table>

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The following people have nothing to disclose: Abdel Aziz Shaheen, Mark Gordon Swain, Samuel S. Lee, Shirley Cole, Shauna Coffey-Williamson
BILIARY INFECTION WITH MOUSE MAMMARY TUMOR VIRUS IN THE NOD.C3C4 MOUSE AND OTHER MOUSE MODELS OF PRIMARY BILIARY CIRRHOSIS

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Three mouse models with innate immune defects have recently been reported that develop spontaneous AMA and biliary lesions. Endogenous mouse mammary tumor virus (MMTV) is found in the genome of most mouse strains that can be expressed to form infectious viral particles in mice. A human betaretrovirus resembling MMTV has been linked with PBC (Xu et al. PNAS, 2003). Aim: To assess whether endogenous MMTV is associated with autoimmune biliary disease in the PBC mouse models. Methods: Livers were isolated from female 12 week-old mice. The innate immune deficient models, NOD.c3c4, CD4 directed dominant negative TGF-β receptor II (dnTGF-βRII) and IL-2 receptor α knockout (IL-2Rα/-) were compared with control mice, BALB/c, C57/bl, NOD.1tj, SJL and the Pera/Eij mouse lacking endogenous MMTV. Anti-gp52Su and anti-p27Ca were used to localize MMTV by confocal microscopy and anti-CK7 to identify biliary epithelium. Hepatic RNA was assessed by real-time RT-PCR to quantify MMTV using gag and pol primers normalized to beta-actin. Proviral MMTV was assessed by PCR of hepatic DNA. Results: MMTV gag and pol gene expression was detected in all mice except for the Pera/Eij that lacks endogenous MMTV; this was especially marked in the NOD.c3c4 mice with biliary cyst formations. Conclusions: In mouse liver, MMTV predominantly replicates in the biliary epithelium. The viral burden of MMTV was much higher in mice with innate immune deficiency and autoimmune biliary disease, providing a model to investigate the viral and autoimmune pathogenesis of cholangitis and AMA production. These findings also provide additional support for the association of a human betaretrovirus with PBC.

Quantification of MMTV in mouse liver

<table>
<thead>
<tr>
<th>Strain</th>
<th>MMTV gag</th>
<th>MMTV pol</th>
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<tbody>
<tr>
<td>NOD.c3c4 (n=5)</td>
<td>50</td>
<td>10</td>
</tr>
<tr>
<td>IL-2R α/- (n=3)</td>
<td>35</td>
<td>7.0</td>
</tr>
<tr>
<td>dnTGF-βRII (n=3)</td>
<td>116</td>
<td>8.1</td>
</tr>
<tr>
<td>BALB/c (n=4)</td>
<td>4.1</td>
<td>2.7</td>
</tr>
<tr>
<td>C57Bl (n=3)</td>
<td>9.3</td>
<td>3.1</td>
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<tr>
<td>NOD.1tj (n=3)</td>
<td>5.8</td>
<td>2.1</td>
</tr>
<tr>
<td>SJL (n=3)</td>
<td>2.0</td>
<td>0.7</td>
</tr>
<tr>
<td>Pera/Eij (n=5)</td>
<td>0</td>
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</table>

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INCIDENCE AND MORTALITY OF PRIMARY SCLEROSING CHOLANGITIS IN THE UK: A POPULATION-BASED COHORT STUDY

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INTRODUCTION: There is little information about the occurrence in the general population of Primary Sclerosing Cholangitis (PSC) in the UK and the risk of associated mortality and malignancy compared with the general population. The few population based studies available from the world literature give incidence figures ranging from 0.07 to 1.3 per 105 per year [1]. Results of the risk of cholangiocarcinoma have been as high as 1.5% per annum [1]. AIM & METHODS: We identified 223 people with PSC and 2,217 control subjects from the General Practice Research Database in the UK. We calculated incidence rates (1991-2001) and mortality rates and used Poisson and Cox regression to make comparisons between populations. RESULTS: Overall there were 149 incident cases corresponding to an incidence rate per 100,000 person years of 0.41 (95% CI 0.34-0.48) and a prevalence in 2001 of 3.85 per 100,000 (95% CI 3.04 to 4.80). PSC was almost twice as common in men as in women, and was associated with inflammatory bowel disease in almost half of cases. There was a 3-fold mortality rate increase (Hazard ratio 2.92 (95% CI 2.16-3.94)) in people with PSC compared to the general population, a 2-fold increase in risk of any malignancy, a similar increase in GI malignancy and a 40-fold increase in the risk of primary liver cancer (HR 2.23 and 37.44 respectively) Conclusions: This study provides we believe the most reliable estimates of the occurrence of PSC in the UK and of its risk in terms of death and malignancy yet available.

<table>
<thead>
<tr>
<th>Death</th>
<th>Rate (per 10,000)</th>
<th>Hazard Ratio</th>
<th>95% CI</th>
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<tbody>
<tr>
<td>Control</td>
<td>3.4</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>PSC</td>
<td>123</td>
<td>3.02</td>
<td>2.16-3.94</td>
</tr>
<tr>
<td>All Malignancy</td>
<td>1.5</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Liver cancer</td>
<td>2.23</td>
<td>4.72</td>
<td>3.04-4.80</td>
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The following people have nothing to disclose: Timothy R. Card, Masoud Solaymani-Dodaran, Joe West
708 STIMULATION OF THE INNATE IMMUNE SYSTEM IS NECESSARY FOR LIVER-SPECIFIC CD8+ T LYMPHOCYTES TO INDUCE AN AUTOIMMUNE HEPATITIS

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INTRODUCTION. Autoimmune hepatitis (AIH) is a chronic disease characterized by a progressive destruction of the hepatic parenchyma. It is currently hypothesised that a viral infection could trigger an AIH in a genetically predisposed individual. The aim of this study is to determine, using a murine model of AIH, if an activation of the innate immune system by pathogen-like specific ligands for toll-like receptors (TLR) in presence of autoreactive T-cells could induce a break of immunological liver tolerance. METHODOLOGY. NP-specific T-cell clones directed against the main epitope of the LCMV nucleoprotein (NP), were adoptively transferred to TLR-NP transgenic mice expressing NP specifically in the liver. CpG (20ug) and Poly(I:C) (500ug) were injected intraperitoneally to stimulate the TLR-9 and TLR-3 receptors. ALT levels were monitored throughout the experiment. Mice were sacrificed 8 days following cell transfer and liver were excised for histology and immunohistochemistry. The expression level of adhesion molecules, chemokine and chemokine receptors were quantified. Hepatocyte apoptosis was evaluated by TUNEL assay. RESULTS. Mice co-injected with CpG and Poly(I:C) following IV transfer of NP-specific T cells showed elevated serum levels of ALT and liver inflammation. In absence of Toll-like receptor ligands (CpG and Poly(I:C)), mice transferred with liver-specific T cells maintained normal levels of ALT and liver histology (p<0.05). Liver ICAM-1 expression levels increased in response to co-injection of CpG, Poly(I:C) and liver-specific T-cells, while CpG and Poly(I:C) alone were sufficient to induce upregulation of VCAM-1 expression levels (p<0.05). CpG and Poly(I:C) coinjection with or without IV transfer of liver specific T cells did not modify liver Vap-1 expression levels. In mice who received CpG/Poly(I:C) and liver-specific T-cells, CXC3 liver expression was down-regulated. CONCLUSIONS. CD8+ liver antigen-specific cells need the activation of the innate immune system through Toll-like receptors (TLR-9 and TLR-3) by pathogen mimics (CpG and Poly(I:C)) to cause liver inflammation and hepatocyte apoptosis. Stimulation of the innate immune system induces an up-regulation of ICAM-1 and VCAM-1, thus, increasing T cell homing to the liver. Therefore, in genetically predisposed individuals, a viral infection could induce an up-regulation of specific adhesion molecules in the liver resulting in autoreactive T-cell recruitment and development of an autoimmune hepatitis.

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709 CLINICAL RELEVANCE OF GENETIC AND SEROLOGIC MARKERS IN AUTOIMMUNE HEPATITIS: A SINGLE CENTER EXPERIENCE WITH 296 PATIENTS

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Autoimmune hepatitis (AIH) is a chronic necroinflammatory liver disease associated with hypergammaglobulinemia, serum autoantibodies and hepatitis with lymphoplasmacytic infiltration, which is responsive to immunosuppressive therapy. The aim of our study was to determine genetic and serologic factors in a cohort of patients with AIH and to correlate them with clinical features. 296 patients diagnosed as having AIH were enrolled. Clinical data were retrospectively reviewed. Genomic DNA was available from 210 patients and the frequency of HLA alleles A1, B8, DRB1*03, DRB3*01, DQA1*05, DQA1*03 and DQB1*02 were analysed and compared with 100 healthy controls. 170 patients were diagnosed as type 1, 44 as type 2 and 82 as type 3. 73% of the patients were female and 27% male. 88% of the patients were younger and 12% older than 65 years. Median duration of follow-up was 11.3 years. Based on the serologic profile, there were no significant differences between the different groups with respect to extrahepatic manifestations, response to therapy, progression to cirrhosis, liver transplantation and liver-related deaths. However, patients with type 2 were significantly younger at diagnosis than patients with type 1 or 3. Similar to previous studies, the above mentioned HLA alleles were more frequently detected in patients with AIH in comparison to controls. Regarding the AIH subgroups, there were no significant differences in the distribution of alleles between type 1 and 2 patients. Patients with AIH type 3 displayed more often the haplotype A1-B8-DRB1*03-DRB3*01 than type 2 patients, and DRB1*03 when compared to AIH type 1 and type 2. Patients with DR3, DR52 and DR3-DR52 were more often male, showed an advanced fibrosis, a worse clinical outcome and a higher frequency of SLA/LP, but less immune mediated syndromes than subjects with other HLA antigens. Overall, there were no significant differences in the clinical course of male and female patients, but DRB1*04 and DRB1*04-DRB4*01 were more often detected in women; in contrast, men had a higher frequency of B8, DRB1*03, DRB3*01 and A1-B8-DRB1*03-DRB3*01. Patients older than 65 years had a more moderate clinical course than younger patients. Frequency of DRB4*01 and DRB1*04-DRB4*01 was increased in patients over 65 and the frequency of DRB1*03 and DRB1*03-DRB3*01 in patients under 65 years. Taken together, our data suggest that clinical features of adult AIH patients are not influenced by the antibody status. In contrast, HLA alleles appear to not only determine susceptibility to AIH, but also the clinical expression of the disease.

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710 PERIPHERAL, BUT NOT CENTRAL, TOLERANCE NOR LEVEL OF AUTOANTIGENIC EXPRESSION IS RESPONSIBLE FOR THE GENDER BIAS IN SUSCEPTIBILITY TO EXPERIMENTAL TYPE 2 AUTOIMMUNE HEPATITIS

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INTRODUCTION. Autoimmune hepatitis (AIH) is a disease of unknown etiology characterized by a progressive destruction of the hepatic parenchyma. In type 2 AIH, 90% of cases are 80% diagnosed before the age of 18. Recently, we have developed an experimental model of type 2 AIH based on DNA vaccination with human autoantigens (CYP2D6 and FTCD) in C57BL/6 mice. Through molecular mimicry, this xenoinmunisation breaks the immune tolerance to murine hepatic autoantigens and induces an AIH. This model shows clinical, laboratory and histological characteristics of
711 PREDICTIVE FACTORS FOR HEPATOCELLULAR CARCINOMA IN TYPE 1 AUTOIMMUNE HEPATITIS: IMPACT OF GENDER, STAGE, HLA PHENOTYPE AND TREATMENT

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Background/Aim: Hepatocellular carcinoma (HCC) is an uncommon but serious occurrence in autoimmune hepatitis, and factors predictive of this risk are needed to optimize early detection. Our aim was to determine the predictors of HCC in autoimmune hepatitis that could focus and improve screening strategies. Methods: 227 patients with definite type 1 autoimmune hepatitis underwent ultrasonography and serum alpha fetoprotein determinations at 6-12 month intervals during 114±6 months (range, 6-409 months; median, 93 months). All patients were negative for viral infection, and they had been screened at least once for HCC (screening episodes, 1212; range, 1-30). Results: Nine patients developed HCC (4%), and each had cirrhosis for at least 73 months prior to the neoplasm (mean duration of cirrhosis, 132±14 months; range, 73-200 months; median, 132 months). Patients developing HCC were more commonly male (56% vs 17%, P=0.01) than patients without HCC, and they had cirrhosis at accession or during follow-up (100% vs 40%, P=0.0003) and features of portal hypertension (esophageal varices, ascites, or edema) more frequently (67% vs 12%, P=0.0004). Patients with HCC also had worsening laboratory tests during corticosteroid therapy (treatment failure) [33% vs 16%, P=0.03] and progression to cirrhosis (56% vs 18%, P=0.002) more often than patients without HCC as well as longer periods of immunosuppressive treatment (57±27 months vs 29±3 months, P=0.04). Male gender (OR, 6.8; 95% CI, 1.6-23.9, P=0.009), cirrhosis for ≥10 years (OR, 14.7; 95% CI, 2.9-73.2, P=0.001), features of portal hypertension (OR, 14.1; 95% CI, 3.3-59.9, P=0.0003), immunosuppressive treatment for ≥3 years (OR, 4.4; 95% CI, 1.2-17.1, P=0.03), and treatment failure (OR, 6.3; 95% CI, 1.4-27.6, P=0.01) were associated with increased risk of HCC by logistic regression analysis. The incidence of HCC in men with cirrhosis was 4-fold greater than in women with cirrhosis (13 vs 3 cases per 1000 patient-years), and the men were distinguished from the women by younger age (42±3 years vs 47±1 years, P=0.03) and lower frequency of HLA DRB1*04 (28% vs 48%, P=0.03). Women with cirrhosis and HLA DRB1*04 had a lower frequency of HCC than men with cirrhosis (0% vs. 21%, P=0.01), especially men without HLA DRB1*04 (0% vs 19%, P=0.04). Conclusions: Male gender, cirrhosis of ≥10 years duration, features of portal hypertension, immunosuppressive treatment for ≥3 years, and worsening laboratory tests during corticosteroid therapy identify patients at risk for HCC, and these risk factors should focus the screening strategy. Women with cirrhosis and HLA DRB1*04 have a low risk of HCC. Disclosures: The following people have nothing to disclose: Aldo J. Montano-Loza, Herschel A. Carpenter, Albert J. Czaja

712 GLUCOCORTICOID RESISTANCE IN AUTOIMMUNE HEPATITIS ASSOCIATES TO INCREASED EXPRESSION AND ACTIVITY OF P-GLYCOPEPTIDE IN T CELL

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Background and Aim: Though autoimmune hepatitis (AIH) patients respond to glucocorticoid (GC) therapy comparatively well, resistant to GC and relapse are often observed clinically. Recently, it has been reported that ABC transporter such as MRPl and P-glycoprotein (Pgp), which excrete intracellular GC, were expressed on T cell and Pgp relates to GC resistance in autoimmune diseases. We studied how these molecules relate to GC resistance in AIH by analyzing the expression and activity of Pgp and MRPl in T cell of AIH patients. Patients and Methods: Clinically and pathologically diagnosed 43 AIH patients and 36 healthy volunteers were enrolled. CD4 and CD8 cells were separated from peripheral blood. Surface expression of Pgp and MRPl were analyzed by FACS using Pgp specific MRK-1.6 antibody and MRPl specific MRPm5 antibody. Pgp activity was evaluated by measuring intracellular rhodamine123, which was added cell culture in advance, in the presence of Pgp blocker PSC833. AIH patients were divided to GC therapy resistant group (n=17: daily maintenance dosage of GU was more than 10mg) and responded group (n=26: less than 10mg). They were also divided into relapsed group (n=27: patient who had relapse during maintenance therapy) and non-relapsed group (n=16). Results: Pgp expression of AIH were significantly higher than control
GCD: 16.4 vs 3.7, CD8: 16.5 vs 3.1, p < 0.001. Pgp expression in resistant group or relapsed group were significantly higher than responded group (CD4: 20.1 vs 10.5, p < 0.01, CD8: 17.6 vs 12.7, p < 0.05) or non-relapsed group (CD4: 20.6 vs 12.6, p < 0.01, CD8: 19.5 vs 13.2, p < 0.05). Pgp activity of AIH was higher than control, however no significant difference was observed. Pgp activity of resistant group was significantly higher than responded group (CD4: 35.4 vs 28.2, p < 0.05, CD8: 34.5 vs 22.9, p < 0.05). Though Pgp activities of relapsed group were not significantly higher compared with non-relapsed group, they were significantly higher compared with control (CD4: 35.5 vs 25.9, p < 0.05, CD8: 33.1 vs 23.8, p < 0.05). There was no significant correlation between the level of Pgp activity and serum levels of ALT, IgG. MRP1 expression was not different between AIH and control. MRP1 expression was different between resistant group and responded group, relapsed group and non-relapsed group; either. The expression of Pgp and MRP1 were not correlated. Conclusion: Our analysis revealed that increased expression and activity of Pgp in T cell participate in the mechanisms of GC resistance in AIH. These results indicated that the expression and activity of Pgp in T cell could be used as the marker to predict GC resistance in AIH therapy.

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713 POTENTIATION OF THE INHIBITORY EFFECT OF INTRAHEPATIC NKT LYMPHOCYTES BY A NOVEL NON-DEGRADABLE Beta-Glucosylceramide ANALOG IS ASSOCIATED WITH DECREASED STAT 1 PHOSPHORYLATION

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Glycolipid presentation by the CD1 receptor to NKT regulatory lymphocytes is important for antigen recognition. The therapeutically potential for harnessing NKT cell responses has been demonstrated in several immune-mediated disorders. Betaglycolipids were recently suggested to exert an immune-modulatory effect on NKT lymphocytes, alleviating immune-mediated disorders. The addition of a thio-group has been shown to turn beta-glycolipids, into a non-degradable molecule. Purpose: To determine the effect of the ligand structure on intrahepatic NKT lymphocyte function in NKT-mediated immune hepatitis. Methods: ConA hepatitis, an NKT-mediated disorder, was induced in C57BL/6 mice (n=8). Mice were injected with non-degradable beta-glucosylceramide containing the thio-group (GCT), beta-glycolipid (GC), or PBS. FACS analysis of intrahepatic NKT lymphocytes and measurement of the serum IFN-g levels by ELISA were performed. Phosphorylation of STAT1 and 6 were determined by western blot. Liver damage was assessed by determination of serum transaminases and degree of apoptosis, using TUNEL assay. Results: Administration of GCT led to a more pronounced inhibitory effect on intrahepatic NKT lymphocytes than GC (a decrease of 84% versus 53%, compared with PBS, respectively, p < 0.05). This effect was associated with a significant increase in STAT 1 expression observed in GCT versus GC-treated mice (130% versus 68%, respectively, compared with PBS). Both GCT and GC led to a decrease of STAT1 phosphorylation (43% and 29%, respectively) along with a decrease in IFN-g serum levels (16% and 26%, for GCT and GC, p < 0.05). Serum IL-4 levels did not change significantly. These effects were associated with alleviation of ConA immune-mediated hepatitis, as determined by a similar substantial decrease in serum transaminases (AST: 58.1% and 55.3%; and ALT: 70.4% and 63.4% for GCT and GC, respectively, p < 0.05), and a profound decrease in apoptosis found in TUNEL assay (66% and 55% for GCT and GC, respectively). Conclusion: Alteration of the chemical structure of the beta-glucosylceramide by replacing the O-glycosidic bond with a S-glycosidic bond resulting in a non-degradable molecule, significantly suppresses intrahepatic NKT lymphocytes. These results suggest that cellular events that alter the enzymatic pathways of glycosylceramidases, serve as an initial stimulus for NKT-cell activation in vivo. This novel ligand may serve as an immune-modulatory therapy in patients with immune-mediated liver injury.

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714 ASSOCIATION OF CYTOTOXIC T-LYMPHOCYTE ANTIGEN 4 GENE POLYMORPHISMS WITH TYPE 1 AUTOIMMUNE HEPATITIS IN JAPANESE

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Background & Aims: Autoimmune hepatitis (AIH) is an organ-specific autoimmune disease characterized by chronic inflammation of the liver, elevated transaminase levels, hypergammaglobulinemia, serum autoantibodies, histologic evidence of interface hepatitis, and a favorable response to immunosuppressive treatment. Although the HLA DRB1*0405 allele in Japanese has been identified as independent determinants of susceptibility to AIH, the role of other genetic factors is largely unknown. The cytotoxic T lymphocyte antigen 4 (CTLA4; CD152) is an inhibitory receptor expressed on the cell surface of activated memory T cells and on CD4+CD25+ regulatory T cells, and acts largely as a negative regulator of T cell responses. As CTLA4 polymorphisms have recently been linked with several autoimmune diseases including AIH in Caucasians, we sought to clarify if CTLA4 polymorphisms are associated with disease susceptibility in Japanese patients with type 1 AIH. Methods: We genotyped 76 patients with AIH (median 56 years: female 82%) and 100 ethically matched controls for allelic determinants using TaqMan genotyping assays at four polymorphism sites: -1722 and -318 in the promoter, +49 in exon 1, and +6230 in the 3' untranslated region. Results: Positivity for the SNP -1722C was significantly increased in patients with AIH (OR = 2.13, 95% CI, 1.14–3.98; P = 0.026) than in healthy subjects, indicating a significant association of the -1722T/T genotype with AIH, which was significantly decreased (OR = 0.47, 95% CI, 0.25–0.88; P = 0.026) in comparison with healthy controls. Compared with -1722 C/C patients, these patients were younger (56 vs. 63 yrs; P = 0.01) and had significantly lower serum levels of aspartate aminotransferase (313 vs. 765 IU/L; P = 0.031) and bilirubin (1.1 vs. 8.6 mg/dL; P = 0.027). Analysis of allelic frequencies revealed no significant difference between patients with and without the
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OUTCOME AND SURVIVAL IN CHILDHOOD ONSET AUTOIMMUNE SCLerosing CHOLANGITIS AND AUTOIMMUNE HEPATITIS: A 13 YEARS FOLLOW-UP STUDY

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Background: As part of a long-term prospective study (Hepatology 2001;33:544-553), we have previously reported that autoimmune hepatitis (AIH) and autoimmune sclerosing cholangitis (ASC) in childhood are distinct entities, with similar modes of presentation, histological features and response to treatment. The prevalence of ASC is similar to AIH but ASC affects boys and girls equally. Diagnosis of ASC relies on the demonstration of cholangiopathy on radiological imaging at presentation. Aim: To assess long-term outcome of patients with AIH and ASC recruited in our original study. Patients and Methods: 55 consecutive paediatric patients with clinical and/or biochemical evidence of liver disease and a serological profile compatible with AIH were recruited from 1984 to 1997 and prospectively followed-up, to date. At the time of diagnosis, all underwent liver biopsy and direct cholangiography. Twenty-eight patients were diagnosed with AIH (F:M ratio 22:6) and 27 with ASC (F:M ratio 15:13) based on the absence or presence of radiological features of cholangiopathy. Most patients were treated with prednisolone and azathioprine (Az). Ursodeoxycholic acid (UDCA) was added in those with ASC. Results: At last follow-up (median 13 yrs, range 8-29 yrs), 53/54 patients are alive, one patient with ASC having been lost to follow-up. Of the 28 patients with AIH, 5 (18%) have successfully stopped all immunosuppression, 3 are on Az alone, 1 on prednisolone alone, 13 on prednisolone and Az, 2 on prednisolone, Az and UDCA, 1 on prednisolone, MMF and UDCA, one on CyA and prednisolone. Two patients (7%) underwent liver transplantation for end-stage liver disease. Of the 26 patients with ASC, 1 (4%) are off medication, 5 are on UDCA alone, 3 on UDCA and Az, 3 on prednisolone and UDCA, 1 on Az alone and 2 on prednisolone and Az, no treatment information being available for 3. Six patients with ASC (22%) have required liver transplantation, 3 of whom (50%) had recurrence of ASC 1, 3, 4 years after surgery, and requiring re-transplantation in two. One of these two patients had further ASC recurrence in the graft requiring re-transplantation 12 year later. One cirrhotic girl with ASC died of intra-abdominal haemorrhage after delivering a healthy infant. Conclusion: Although AIH and ASC have similar features at presentation, and initial response to immunosuppressive treatment is satisfactory, long-term follow-up shows that patients with ASC are significantly more likely to die or need transplantation than those with AIH (p < 0.009, Log Rank), and have a high rate of disease recurrence after liver transplantation.

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GENETICALLY DETERMINED ABNORMALITY OF THE PD-1/PD-LS PATHWAY MAY PREDISPOSE TO AUTOIMMUNE LIVER DISEASE

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Background: Programmed death-1 (PD-1) and its ligands - members of the CD28/B7 super-family - are central to the maintenance of peripheral tolerance, their experimental impairment resulting in autoimmune disease. Aims: To evaluate the expression of PD-1 on peripheral blood mononuclear cells (PBMCs) and T cells, and its ligand PD-L1 (B7-H1) on PBMCs and dendritic cells (DCs) in autoimmune liver disease (AILD). Methods: PD-1 and PD-L1 expression on PBMCs and DCs was evaluated by FACS using anti-Cd4, -CD8, -blood DC antigen-1 (BDCA-1), -PD-1 and -PD-L1 monoclonal antibodies in 32 AILD patients (PtS, 22 autoimmune hepatitis, 10 autoimmune sclerosing cholangitis, median age 10 years, range 3-19; 18 females). Fourteen patients’ first-degree relatives (FDR, median age 45, range 5 to 49; 7 females) and 9 healthy subjects (HS, median age 31 years, range 21-48; all females) were also studied. Up-regulation of PD-1 and PD-L1 was induced by 500 ng/ml lipopolysaccharide (LPS) 4-hour stimulation. Results: At baseline the frequencies of PD-1 expressing PBMCs (Pts 3.71 ±0.37, FDR 3.68±0.5 vs HS 6.31±0.78, P<0.01) and CD4 T cells (Pts 1.45±0.17, FDR 2.06±0.77 vs HS 2.67±0.28, P=0.001 and P=0.23) were lower in patients and relatives than in controls. PD-L1 expressing PBMCs (Pts 1.31±0.26, FDR 0.58±0.13 vs HS 1.35±0.43) and DCs (Pts 0.35±0.07, FDR 0.19±0.05 vs HS 0.48±0.09) were similar in Pts and HS but lower in FDR than in HS (P<0.05 for both). After LPS stimulation, PD-1 expressing PBMCs (Pts 4.39±0.63, FDR 5.23±0.93 vs HS 7.37±1.32, P=0.017 and P=0.14) and CD4 T cells (Pts 1.95±0.26, FDR 2.61±0.71 vs HS 2.27±0.23, P=0.05 and P=NS) increased marginally in all groups but remained lower in Pts and FDR than HS, while PD-L1 expressing PBMCs increased marginally in Pts (from 1.31±0.26 to 1.48±0.21) and HS (from 1.35±0.43 to 1.89±0.43) but significantly in FDR (from 0.58±0.13 to 1.38±0.41, P=0.05). PD-L1 expressing DCs increased significantly in Pts (from 0.35±0.07 to 0.88±0.15, P=0.002), FDR (from 0.19±0.05 to 0.49±0.06, P=0.003) and HS (from 0.48±0.09 to 1.17±0.22, P=0.07), remaining lower in FDR than HS (P<0.02). Summary and conclusion: Our data show that PD-1 expressing PBMCs and CD4 T cells are numerically impaired in AILD patients and their first degree relatives, and that AILD patients have an imbalance between PD-1 and PD-L1 expression. The abnormality of the PD-1/PD-L1 pathway appears to be genetically determined and may predispose to the development of AILD.

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THE USE OF MYCOPHENOLATE MOFETIL IN THE TREATMENT OF AUTOIMMUNE HEPATITIS IN PATIENTS REFRACTORY TO OR INTOLERANT OF CONVENTIONAL TREATMENT

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Background: Autoimmune hepatitis (AIH) is characterized by progressive hepatocellular inflammation often progressing to
cirdrosis. Standard treatment consists of corticosteroids alone or in combination with azathioprine (AZA). Remission will be achieved in over 80% of cases and long-term treatment with azathioprine with or without steroids has been shown to be effective at preventing relapse and maintaining remission. Alternative therapies are warranted for individuals who are refractory to or intolerant to standard therapy. There is no standard treatment for steroid refractory AIH or for those intolerant to azathioprine. Mycophenolate mofetil (MMF), a potent inhibitor of guanosine nucleotide synthesis, is a promising alternative treatment of autoimmune hepatitis. Aim: To assess the outcome of therapy with MMF in patients with refractory AIH or intolerance to azathioprine. Methods: Patients diagnosed and treated for AIH from 1994 to 2007 at our center were identified. Response to medication was recorded. Patients converted to MMF were evaluated for tolerance and response. Results: 90 patients were included in the study. 48% had complete clinical and laboratory resolution of their disease, 30% had relapsing disease and 22% had partial or no response on prednisone and AZA. MMF was initiated in 21 patients (24%) after an average of 42 months of treatment with conventional therapy. The reason for MMF conversion was failure of conventional treatment (group 1) in 57% and intolerance to steroids or azathioprine (group 2) in 43%. 17/21 patients had a follow up greater than 6 months. 8/17 patients were converted to MMF for refractory disease. 0/8 patients had biochemical resolution of disease following conversion. 9/17 patients were converted to MMF for intolerance to conventional treatment. 8/9 patients (88%) maintained complete remission following conversion (p=0.002). In patients converted to MMF, a mean decrease in steroids from 17.5 mg to 6.5 mg was seen (p<0.001). Conclusion: In patients with AIH who are intolerant to conventional therapy, mycophenolate mofetil is well tolerated. The majority of patients switched to MMF secondary to azathioprine intolerance remain in clinical and laboratory remission. Mycophenolate mofetil did not induce remission in those refractory to conventional therapy, but use of mycophenolate mofetil did allow a significant mean decrease in steroid use in those patients. Larger, prospective studies are needed to better assess the role of MMF as a frontline and second line agent in the treatment of AIH.

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LYMPHOTOXIN ALPHA GENE POLYMORPHISMS IN PEDIATRIC AUTOIMMUNE HEPATITIS TYPE 1
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of serum transaminases compared with a decrease in GC and combined treatment groups (an increase of 231% and 136%, vs. a decrease of 72%, 66% and 81%, 76%, for ALT and AST, respectively, p< 0.05). aGalCer led to a 10% decrease in TUNEL-positive hepatocytes, while treatment with GC or the combination led to a significant decrease of 82% and 61%, respectively (p< 0.05). Conclusion: GC, a natural betaglycolipid, decreases the immune-mediated liver damage of aGalCer, associated with a decrease in intrahepatic NKT cells and a Th2 shift. These data suggest that optimization of the NKT ligand structure can determine its immune modulatory effect, with a role for CD1d, and that different ligands may ameliorate immune mediated damage.

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ANTIBODIES TO DOUBLE-STRANDED DNA (ANTI-DSDNA) IN LIVER DISEASE: HIGH SPECIFICITY FOR TYPE 1 AUTOIMMUNE HEPATITIS (AIH-1) AND USEFUL MARKER FOR THE DIAGNOSIS OF PRIMARY BILARY CIRRHOSIS (PBC) - AIH OVERLAP SYNDROME  
Georgios Pappas, Alessandro Granito, Paolo Moraturi, Silvana Maccariello, Luigi Muratori, Rodolfo Ferrari, Fabio Cassani, Silvia Ferri, Valentina Cipriani, Chiara Quarneti, Francesco B. Bianchi; Department of Internal Medicine, Hepatology, Cardioangiology, University of Bologna S.Orsola-Malpighi Hospital, Bologna, Italy  
BACKGROUND AND AIMS Antibodies to ds-DNA play a well established role in the diagnosis, pathogenesis and prognosis of systemic lupus erythematosus. Anti-dsDNA have been detected also in AIH-1 and sporadically in other chronic liver diseases. Conclusive data are still lacking about their prevalence in other autoimmune liver disorders and their specificity for AIH-1, especially in European patients. To determine the prevalence of anti ds-DNA in the whole spectrum of autoimmune liver disease: type I and type II AIH, PBC and PSC. To evaluate whether anti-dsDNA are specific for type I AIH and whether in this setting they are of clinical and prognostic relevance.  
PATIENTS AND METHODS: 310 consecutive patients with autoimmune liver diseases and 114 matched controls with other chronic liver diseases were studied (table 1). Anti-dsDNA were searched for by IIF on Crithidia luciliae a test considered to be high specific. RESULTS: The frequency of anti-dsDNA in the studied population is reported on table 1. Their overall specificity for type-1 AIH was 96%. Anti-dsDNA seropositivity in AIH-1 was associated with older age (p = 0.006), higher γ-globulin, IgG (p = 0.01) and bilirubine serum levels (p = 0.01) and lower albumine (p = 0.03). Seropositive patients tend to have a higher prevalence of severe fibrosis and cirrhosis (p = 0.07) and had more frequently the allele DR4 (p = 0.06). Six out of the 10 anti ds-DNA positive PBC patients had an IAIHG score corresponding to “probable” AIH. In these patients an overlap PBC-AIH syndrome could thus be suspected. These overlap cases had a higher titer of anti-dsDNA. None of the seronegative PBC patients reached a score sufficient for “probable” AIH. CONCLUSIONS: In the spectrum of liver disease anti-dsDNA represent a diagnostic marker of AIH-1. Their occurrence at a high titer in PBC should suggest the diagnosis of overlap PBC-AIH syndrome. In AIH-1 anti-dsDNA correlate with older age, DR4, higher bilirubin and lower albumin serum levels and histological evidence of severe fibrosis and cirrhosis. Their presence thus suggests a more advanced stage of liver disease.

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THE IMPACT OF ETHNICITY ON THE NATURAL HISTORY OF AUTOIMMUNE HEPATITIS  
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Background and aims: The impact of ethnicity on the natural history of autoimmune hepatitis [AIH] has not been well characterized. The aim of this study was to assess the natural history of AIH in Blacks compared to others (non-Blacks). Patients and methods: This was a 10-year (June 1996 to June 2006) retrospective analysis of patients with AIH from a single tertiary care center. The diagnosis of AIH was defined by the criteria established by the International AIH Club. Poor outcome was defined as liver failure at presentation, failure to achieve remission, need for liver transplantation and or death. Results: 101 patients with AIH were found eligible for the study. Black patients were more likely to have cirrhosis (56.7% vs. 37.5%, p=0.061), have liver failure at initial presentation (37.8% vs. 9.3%, p=0.001) and be referred for liver transplantation (51.3% vs. 23.4%, p=0.004). The overall mortality was also significantly higher in Black patients (24.3% vs. 6.2%, p=0.009). Compared to non-Blacks, Blacks had more advanced hepatic fibrosis (3.6 ± 2.7 vs. 2.1 ± 2.4, p=0.013). Kaplan Meier analysis showed that probability of developing a poor outcome was significantly higher in Blacks (p=0.003). Independent predictors of poor outcome were Black ethnicity, presence of cirrhosis, and fibrosis stage at presentation. Black males were the group most likely to have a poor outcome (85.7%). Conclusions: Blacks, especially Black men with AIH, have more aggressive disease at initial presentation, are less likely to respond to conventional immunosuppression and have a worse outcome compared to non-Blacks.  
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POPULATION-BASED STUDY OF ALASKA NATIVE PERSONS WITH AUTOIMMUNE HEPATITIS: CLINICAL PRESENTATION, RESPONSE TO TREATMENT AND RELAPSES

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Background: Studies have demonstrated a high prevalence of autoimmune hepatitis (AIH) in Alaska Native persons (AN). Clinicians have felt these patients showed a dramatic response to treatment with rapid drops in transaminase levels after initiating therapy. The conventional wisdom is that achievement of remission in adults averages 6 months or more. We conducted a review of AN diagnosed with AIH to determine clinical presentation, how rapidly patients responded to treatment and the frequency of relapse after treatment was initiated. Methods: A review of patient records was performed from a statewide (Alaska) registry of AN diagnosed with AIH since 1981. All persons included met the International Autoimmune Hepatitis Group criteria for definite or probable AIH and excluded those with overlap syndrome. Unlike the AASLD guidelines, which define remission as ALT and AST less than twice the upper limit of normal, we defined remission as ALT and AST less than 40. Standard treatment was initiated with methylprednisolone 32 mg/day or prednisone 40 mg/day and azathioprine 50-150 mg/day, with a slow tapering of the corticosteroid based on

200 patients with autoimmune hepatitis between 1999 and 2004. 84 of 200 patients met the diagnosis of AIH according to revised scoring system of the International Autoimmune Hepatitis Group. 29 patients were excluded secondary to insufficient clinical information or absence of liver biopsy specimens. The charts of the remaining 55 patients were analyzed for age, gender, serum aminotransferase levels, serum autoantibody levels and treatment regimens. Seropositivity was defined as ANA >1:40 and ASMA >1:40. Liver biopsy specimens were reviewed by two pathologists who were blinded to clinical and serological data. Degree of liver injury on histology was defined by four individual categories including portal inflammation, lobular inflammatory activity, piecemeal necrosis and cirrhotic nodules. Each category was graded on a scale of 0 to 4. Mann-Whitney test was used to evaluate the difference between means. Results: The 55 patients with AIH had a mean age of 52 years ± 15 years, with 41 (75%) being female. Positive ANA titers were found in 36 patients (66%), 36 patients (66%) were ASMA positive, 27 (49%) were both ANA/ASMA positive and 10 (18%) were both ANA/ASMA negative. The patients with autoantibodies were similar to the patients without autoantibodies with respect to age, gender and treatment regimen. There was no statistically significant difference in the ALT and/or AST at diagnosis and at 1, 6 and 12 months post treatment between the seropositive and the seronegative patients. Degree of lobular inflammatory activity was slightly higher in the seropositive group vs. the seronegative group (mean score, 1.4 vs. 1) but results were not statistically significant (p=0.25). Both groups had similar degree of portal inflammation (1.4 vs. 1.6, p=0.71) and piecemeal necrosis (0.93 vs. 1, p=0.69). Centrilobular necrosis was found in liver biopsy specimens of 2 (4%) seropositive patients and 0 seronegative patients. Conclusions: The presence or absence of autoantibodies does not appear to correlate with histological severity of disease and does not predict response to immunosuppressive therapy in these patients. Based on our study, autoantibodies should not be used to predict AIH activity or outcome and patients with clinical features consistent with AIH should be treated regardless of serologic status.

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SEROLOGICAL MARKERS DO NOT PREDICT LIVER HISTOLOGY IN AUTOIMMUNE HEPATITIS

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Objective: The present study is to determine if the presence of serum autoantibodies in patients diagnosed with autoimmune hepatitis (AIH) correlates with histological and biochemical severity of disease and response to treatment. Methods: Electronic billing records at our university hepatology practice revealed 200 patients with autoimmune hepatitis between 1999 and 2004. 84 of 200 patients met the diagnosis of AIH according to revised scoring system of the International Autoimmune Hepatitis group. 29 patients were excluded secondary to insufficient clinical information or absence of liver

after stimulation by their natural ligand lypopolysaccharide (LPS) through Toll-like-receptor-4 (TLR4), trigger adaptive immune responses. Monocytes abound in the portal/pericellular infiltrate in autoimmune hepatitis (AIH). Aim: To investigate the effect of T-regs on monocyte TLR4 expression in the presence or absence of LPS in AIH. Patients and methods: 16 patients with ANA/ASMA-ve AIH (median age: 14.7 years; 7 females) and 7 normal controls (median age 28 years; 7 females) were studied. Monocytes, T-cells and CD4+CD25- T-cells were isolated from peripheral-blood-mononuclear-cells using immunomagnetic beads. Monocyte TLR4 expression (assessed as mean fluorescence intensity) was evaluated by flow cytometry before and after 24-hour co-culture with T-regs (or with CD4+CD25- T-cells as control) in the absence or presence of LPS. To assess whether T-reg effect on monocytes is due to cell-to-cell contact and whether is mediated by IL-10, transwell experiments and neutralization assays using anti-IL-10 antibodies were performed. Results: Before LPS stimulation and in the absence of T-regs the expression of TLR4 on monocytes was similar in controls and patients. Following T-reg addition it increased by 29% (from 34.6±4.9 to 44.5±5.8; P=0.03) in controls and by 39.8% (from 41.9±3.4 to 58.7±6.7; P=0.025) in AIH. Following LPS stimulation in the absence of T-regs, monocyte TLR4 expression increased by 21% (P=0.1) in normal and by 15.9% (P=0.1) in AIH. After T-reg addition in the presence of LPS, TLR4 increased by 23% (from 42.5±4.2 to 52.3±7.7; P=0.046) in controls and by 33% (from 48.7±3.5 to 64.6±5.1; P=0.016) in AIH. Following T-reg addition, the expression of TLR4 on monocytes was higher in patients than in controls both in the absence (P=0.12) and presence (P=0.059) of LPS. No effect on monocyte TLR4 expression was observed when CD4+CD25- T-cells were added instead of T-regs. T-reg effect on monocyte was mainly mediated by a cell-to-cell mechanism, the increased TLR4 expression being lower (17% in normal and 16% in AIH) when monocytes were kept separated from T-regs than when they were in direct contact (29% and 39.8% respectively). Increased TLR4 expression was inhibited by addition of anti-IL-10 by 86% in controls and 91% in patients. Conclusions: Unexpectedly, T-regs increase TLR4 on monocytes through cell-to-cell contact in the presence or absence of IL-10, the effect on monocyte was mainly mediated by a cell-to-cell mechanism. The following people have nothing to disclose: Maria Serena Longhi, Giorgina Mieli-Vergani, Yun Ma, Diego Vergani

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treatment response. Results: Eighty patients (76 women, 4 men) met the criteria for definite (68) or probable (12) AIH. The average age at diagnosis was 51.2 years (range 15-78). Twenty-one (26%) presented with symptoms of acute hepatitis; 4 were diagnosed incidentally with normal liver function tests; 4 had mild symptoms (fatigue) with transaminase elevation, and 35 had no reported symptoms but elevated transaminases. Ten patients were not treated and 7 treated had inadequate follow-up. Of the remaining 63, one (1.5%) has not achieved remission; time to achieve normal transaminase levels was 3-6 months in 7 (11%), 1-2 months in 8 (13%), and less than one month in 47 (76%). In 70 treated patients, relapses were documented in 20 (29%): 4 were noncompliant in taking their medications, 7 had bone marrow suppression and had to stop azathioprine, and 9 were unable to tolerate azathioprine or the dose could not be raised above 50 mg/day due to neutropenia. Conclusions: Alaska Native persons with AIH appeared to respond more rapidly to treatment than other reported populations, as the majority normalized their liver enzymes within one month or less of starting therapy. The majority of those who relapsed received inadequate dosing of azathioprine due to intolerance, noncompliance or bone marrow suppression.

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BROAD CLINICAL VARIABILITY OF A CHOLESTATIC LIVER DISEASE IN ELEVEN SIBLINGS WITH ONE SINGLE MUTATION IN THE ABCB4 GENE

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Background: Cholestatic liver disease is one of the major reasons for progressive liver damage and liver failure. A family of eleven siblings from a small village in Transylvania allowed us to study the broad clinical variability of cholestatic liver diseases with one single gene defect. Methods: Clinical and histopathological features of the family members were analysed to assign them to the different groups of: affected with elevated liver parameters and/or definite histological changes, mildly affected with intrahepatic cholestasis of pregnancy or subtle histological changes and healthy. Assuming a monogenetic inheritance linkage analysis was performed using Affymetrix 50K Gene Chips. Genes that cause cholestatic disease and candidates from the linkage analysis were analyzed by immunofluorescence, expression level and sequencing. Results: Three patients died at the age of 5, 7 and 43 due to biliary cirrhosis. Three patients developed elevated liver parameters and histologic fibrosis/cirrhosis during adulthood. These six were considered strongly affected. Five others had normal liver values but pruritus during pregnancy or very subtle histological changes. These were considered mildly affected. None of the above were considered healthy. Extensive pedigree studies revealed distant consanguinity. Under the autosomal-recessive model taking into account the relationship, a maximal LOD-score of 3.88 could be obtained on chromosome 7q21.1-7q22. Detailed characterization of the 149 genes at the locus revealed that homozygosity for the single variant Arg788Trp (c.2364c>t) in the ABCB4 gene co-segregated with severity of liver disease, whereas heterozygosity was found in all mildly affected patients. The variant is located in a transmembrane domain of the ABCB4 protein which is involved in phospholipid-export into the bile. Expression level and localization were normal compared to controls. The overall histological changes were unspecific showing ductopenia and subtle periductal fibrosis in some samples. Conclusion: Both, linkage analysis and sequencing led to the recognition of one single genetic defect of the ABCB4 gene as the cause for the variety of cholestatic liver disease from intrahepatic cholestasis of pregnancy to fibrosis, and cirrhosis and death in childhood and adulthood.

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IDENTIFYING LEARNING NEEDS OF ADULT LIVER TRANSPLANT RECIPIENTS

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Purpose: The purpose of this study was to determine how informed patients felt after liver transplant in terms of various aspects of follow-up care and to identify factors that influence knowledge deficits. Methods: A survey was sent to 129 patients who received a liver transplant in 2005 and 2006. It consisted of 102 questions and included a modified version of the Patient Learning Needs Scale (PLNS), which was used to measure patients' perceptions of how informed they felt about managing their health care after transplant. This scale consisted of multiple items divided into four categories: (1) Medications; (2) Health Problems; (3) Follow Up; and (4) Quality of Life / Psychosocial Issues. Patients rated how informed they felt about items in each category. Other survey questions addressed factors likely to impact perceptions of learning. Results: Sixty-eight (53%) surveys were returned. The majority of respondents indicated not having an accurate idea about life after transplant. PLNS items relating to quality of life and psychosocial issues scored significantly lower (indicating patients felt less informed) when compared to the three other categories (p < .001). Also, items relating to health problems, particularly those that dealt with symptom management, scored lower when compared to medications (p = .008) and follow-up categories (p = .048). Factors that may have influenced PLNS scores were analyzed by t-test or one-way ANOVA. Seven patients had a previous transplant, but showed no difference in learning outcomes. Time on the waiting list and length of stay did not have a significant impact. Scores did show a trend towards greater levels of knowledge reported in patients who waited 13 to 24 months and toward more informational needs in patients who were hospitalized for longer periods of time. Factors having the greatest impact on information deficits included pain, fatigue, feeling overwhelmed, and side effects of medications while hospitalized. Time away from the transplant unit, inability to attend inpatient classes, and absence of a support person were identified as additional obstacles in patients with longer lengths of stay. Conclusions: Patients felt inadequately informed about psychosocial and quality of life issues after liver transplant. Overall, patients felt informed about medications and follow-up cares required
after liver transplant, but felt less informed in terms of management of the symptoms and health problems they may have. We are currently developing innovative patient education methods, including a DVD and online tutorial, to address these concerns.

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RESULTS OF A UNIQUE HEPATITIS C OUTREACH PROGRAM IN PROVIDING SCREENING, EDUCATION, AND REFERRAL IN A MIDWEST STATE

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Introduction: Hepatitis C virus (HCV) has been found to be an unrecognized viral infection in many individuals who may be at risk. However, education and screening of the general public by federal or state health agencies is not mandated or funded. The Missouri Hepatitis C Alliance (MOHCA) is a non-profit, primarily volunteer organization. MOHCA was started in 1998 to provide support for those diagnosed and undergoing treatment for HCV. MOHCA provided public educational presentations, local awareness advertising, newspaper articles, support/education group announcements, fund raising events, radio interviews, television talk shows. MOHCA also worked with 44 different County Health Departments and Clinics. MOHCA provided public educational presentations, local awareness advertising, newspaper articles, support/education group announcements, fund raising events, radio interviews, television talk shows. MOHCA also worked with 44 different County Health Departments and Clinics. Aim: The purpose of this retrospective study was to evaluate and report the number of individuals who have received education and screening by MOHCA through various efforts. It was presumed by bringing this service to the public higher rate of HCV virus exposure would be found than has been previously reported by health agencies. Methods: The MOHCA database was searched from January 2005 through May 2007 to identify HCV positive individuals. HCV antibody screenings were done using the IgM Elisa-3 and 2005 individuals were tested in Mid-Missouri. Results: During the period from Jan 2005 through May 2007 MOHCA provided education to 1846 individuals, businesses, and various settings. The eight various monthly support/education groups had a total of 704 in attendance. Of those tested, 593 (29.6%) were found to be HCV antibody positive. When Positive amplification was done by PCR, 84 (4.2%) were found not to be viremic (PCR < 5 IU/ml). Of those tested 1062 (53%) were male and 942 (47%) were female. However, HCV antibody positivity was found to be lower in both males 261 (13%) than females 332 (21.8%) of those tested. Conclusion: In rural Mid-Missouri when education and screening are offered to those at risk, screening finds more individuals who are HCV antibody positive. The time and effort of this organization is an example of how those who are potentially at risk can be sought out through educational and support efforts, resulting in higher rates of previously reported infection rates through general screening methods used.

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HEALTH-RELATED QUALITY OF LIFE IN PATIENTS WITH CHRONIC LIVER DISEASE WITH OR WITHOUT HEPATOCELLULAR CARCINOMA (HCC) AWAITING ORTHOPHIC LIVER TRANSPLANTATION (OLT)

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Background: There have been relatively few studies of health-related quality of life (HRQOL) in patients with HCC (hepato-cellular carcinoma) awaiting an OLT. These patients are usually asymptomatic and the tumours have been found in routine follow-up. Objective: To compare the HRQOL of patients with HCC who are candidates for OLT with that of patients with other indications for OLT and without HCC. Methods: Observational study performed in the Liver Transplant Unit of the University Hospital of Bellvitge, Spain (May 2002- June 2006). Patients included were candidates for OLT with or without HCC. HRQOL was measured using the disease-specific Liver Disease Quality of Life (LDQOL) questionnaire. Three groups were compared: patients with HCC, patients with cirrhosis (CH) and VHC but no HCC, and patients with CH due to alcohol (CH ENOL), but no HCC. Sex, age, HCV, HBV, Child A, B, C, and MELD scores were also recorded. Results: A total of 157 patients were included for analysis (68 patients with HCC, 46 patients with CH ENOL, and 43 patients with CH VHC). Mean age (SD) of the overall sample was 52.6 (9.8) years and 73% were male. Distribution by age and sex was similar in the three groups studied. Patients with HCC had statistically significant higher scores (better HRQOL) on eight of the twelve disease-specific scales of the LDQOL than the group with CH and VHC, including symptoms of liver disease (p=0.000), effects of liver disease (p=0.000), concentration (p=0.002), memory (p=0.015), quality of social interaction (p=0.030), sleep (p=0.000), loneliness (p=0.043) and stigma (p=0.028). Statistically significant differences were found between HCC patients and patients with alcoholic cirrhosis on only two dimensions, symptoms of liver disease (p=0.014) and effects of liver disease (p=0.035). Conclusions: Patients with HCC who are candidates for OLT had better scores on several of the disease-specific scales of the LDQOL compared with candidates without tumours. This may have important consequences for measuring change over time and for between group comparisons where case mix varies between groups.

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POST TRANSPLANT TREATMENT OF HEPATITIS C: IS THERE AN IDEAL MONITORING PARAMETER?

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Background: The treatment of recurrent Hepatitis C (HCV) in the post transplant population has advanced with the use of pegylated interferons and ribavirin. The successes have been fraught with viral recurrence following the cessation of therapy. In the past, presence of HCV by liver tissue polymerase chain reaction (PCR) has been evaluated as a predictor of virologic relapse post therapy. With the introduction of the super sensitive transcription-mediated amplification (TMA) testing, results to <5 IU/ml, into clinical practice, the utility of tissue PCR in our treat-
ment algorithm is unknown. The aim of this study was to evaluate the need for tissue PCR in the present setting of super sensitive serum PCR testing. Methods: This retrospective study was conducted for patients transplanted and subsequently treated for HCV. Data collected included demographics, time from transplant, viral load at start of treatment, genotype, tissue PCR analysis by the National Genetics Institute, Los Angeles, CA, length of treatment, and 6 month post treatment viral load. Results: Fourteen patients met inclusion criteria. They were all male, with a mean age of 51.8 (range 43-60 years) and ethnicity including Caucasian (n=12), African American (n=1), and Asian (n=1). Average time from liver transplant to treatment was 30 months (range 3-92 months). Viral load average at start of treatment was 3,920,000IU/ml (range 400,000 - 11,600,000IU/ml) and there was a predominance of Genotype 1 (86%). The end of treatment serum PCR and liver tissue quantitative assay were negative (<1 copy/μl) in all patients and six month post treatment for serum PCR using TMA was negative in 13(93%). Conclusion: The above results indicate that successful termination in the treatment of recurrent HCV can be monitored using only serum TMA. In comparison to previous work using PCR values with higher limit of detection numbers, these results suggest that TMA serum PCR sensitivity is currently high enough to obviate the need for tissue PCR testing. These findings will impact the treatment algorithm for the post transplant recurrent HCV patient by allowing for the development of standard of care treatment timeline and testing parameters.

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TITLE: MULTIDISCIPLINARY COLLABORATION IMPROVES HEPATITIS C MANAGEMENT AND CARE FOR HIGH-RISK PATIENTS IN A METHADONE MAINTENANCE CLINIC

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Background: Patients accessing care through the ORT (Opiate Replacement Therapy) are at high risk for chronic blood borne illnesses. Frequently veterans in the ORT clinic and other substance abuse programs have missed appointments to assess their hepatitis and other liver-related risks, obtain education/counseling, vaccinations, and follow-up care. In order to improve access to care, and improve compliance; formal communication was initiated between the liver and substance abuse/mental health teams. Aim: To describe the interdisciplinary collaborations and outcomes of a liver well-being initiative in a methadone clinic. Results: Over 105 patients accessed the ORT clinic at the San Francisco Veterans Administration for methadone in 2006. Prior to program implementation in 2006, 60% are HCV antibody positive and 75% of these patients did not return for follow-up care (including labs, vaccinations for hepatitis A and B, and appointments with a provider for ‘liver’ evaluation). However, 10 months after the program was initiated the following was completed: a formal joint team meeting and protocol established between clinic chiefs/nurses, a protocol was established for HAV and HBV vaccinations, staff (100%) within the ORT clinic have become familiar with HAV/HBV/HCV screening, a survey to all patients to assess their needs (90% participation), and space was dedicated to liver and non-ORT care. To date, all patients have received HCV education and counseling, 80% have received the flu vaccine, and all (100%) ORT patients now have a primary medical provider. As well, two patients have agreed to and successfully initiated HCV treatment through weekly on-site injections at the ORT clinic site. Conclusion: Collaboration between the ORT and liver nurses is critical in providing optimal care to veterans infected or at risk for hepatitis C. Interdisciplinary collaboration can result in improved liver health and treatment opportunities for methadone patients. Program participant feedback, including responses from a HCV Awareness Day, patient surveys and other providers suggest that this may be a model for other liver and other health services to improve HCV management and care in high risk patients.

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IMPROVEMENT OF QUALITY AND EFFICIENCY IN HEPATOLOGY CLINIC AT A VA MEDICAL CENTER

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1. Background: The high prevalence of chronic hepatitis C (HCV) among veterans has a significant impact on current and future health care resources. Unpredicted shortage of providers led to a mismatch between available clinic slots and number of requested consultation. 2. Method: Retrospective data abstraction from work load reports between 2004 and 2006 was performed. Several interventions were initiated in 2004 and 2005. a) Establishing of a dedicated HCV care team (physician, nurse practitioner, nurse, pharmacist) b) Optimizing the scheduling process to reduce no-show rates and clinic down time c) Streamline the visit with delegating non-physician work d) Implementation of pre-set order menus for blood work and medication e) Introduction of a HCV group class for new patients 3. Result: MD and NP provided Hepatology service for 44 weeks each in 2004, and for 44 and 20 weeks in 2006, respectively. In 2004 and 2006, the number of available appointment slots were 880 and 735 with 677 and 700 slots used, respectively leading to an increase in used clinic capacity from 77% to 95%. The total number of visits increased by 121. On average, 7.7 patients per week were seen in clinic in 2004 and 17.4 patients in 2006 demonstrating an increase in efficiency by a factor of 2.25. Forty-four more patients can be seen in clinic in 2005. a) Establishing of a dedicated HCV care team (physician, nurse practitioner, nurse, pharmacist) b) Optimizing the scheduling process to reduce no-show rates and clinic down time c) Streamline the visit with delegating non-physician work d) Implementation of pre-set order menus for blood work and medication e) Introduction of a HCV group class for new patients 4. Conclusion: Clinic management and patient care in Hepatology clinic at our VA medical center could be improved with simple but effective measures, considerably leading to an increase of scheduled appointments, patient visits, and reduction of no-show rate. A dedicated team combined with a trusting patient/provider-relationship is the keystone for continuity of care in patients with chronic diseases. The HCV group class ensured a standardized education for new patients. This project may be applied to other specialty clinics to improve clinic management and patient care.

Disclosures:
732 SURVIVAL OUTCOMES IN LIVER TRANSPLANT RECIPIENTS SUFFERING FROM NON-ALCOHOLIC STEATOHEPATITIS

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Background: Recent reports from the Center for Disease Control suggest that the obesity trends in the United States will continue to worsen in the coming decades. As a result, liver transplant centers can expect a higher number of non-alcoholic steatohepatitis (NASH) patients to present for liver transplantation (LTx). Information pertaining to NASH recidivism is available but long-term survival and lifestyle change data is limited. The aim of this study is to explore long-term outcomes and recidivism in LTx recipients suffering from NASH. Methods: A retrospective review of LTx patients with NASH from the pre-transplant evaluation up to five years posttransplant was completed. All patients underwent transplantation between 1997 and 2006, inclusive. Data collected included demographics, morbidity issues, presence of coronary artery disease (CAD), presence of diabetes mellitus, serum hemoglobin A1C values, body mass index (BMI), lifestyle changes, and survival information. Nutritional consultation by a registered dietitian was available to all pre and post liver transplant patients. Kaplan Meier survival analysis was employed. Results: Twenty-eight patients with NASH received LTx during the study period. There were 15 female (54%) and 13 male (46%) with 27 Caucasians and 1 African American. Mean age was 57.6 (range 44 to 76 years of age). All patients had diabetes mellitus. The mean BMI was 32.3 (range 25.8 to 43.9). Post-LTx, there was no significant change in BMI (32.3 vs 32.8) or mean Hgb A1C (7.1% vs 5.7%) (p>0.5). The one year survival was 77% and the three year survival was also 77%. There were no predictors for graft or patient survival. Conclusion: This cohort of NASH related patient demonstrates a poor short- and long-term survival, hampered ability to lose BMI points following liver transplantation. In light of the recent CDC data demonstrating a marked increase in obesity for our nation, the above patient cohort reveals a health care concern for liver transplant patients in terms of long-term survival and multiple post-transplant morbidities.

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733 OCTREOTIDE BOLUS INJECTION AND AZYGOS BLOOD FLOW (AZBF) IN PATIENTS WITH CIRRHOSIS: SUSTAINED DECLINE AND VISIBLE EFFECT AT READMINISTRATION

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Octreotide (OCT) improves the initial control of variceal bleeding, but hemodynamic changes are usually transient and attributable to the effect of bolus administration. Whether a repeat bolus infusion may induce hemodynamic changes in the collateral is not fully elucidated. Aim: To study the acute effects of octreotide on AzBF in patients with cirrhosis and portal hypertension, following, 1) a 50 mcg bolus infusion, and 2) a repeat 50 mcg bolus after 60 minutes. Patients and Methods: We performed invasive measurements of AzBF (azygous vein cannulation and continuous thermal dilution technique) in 12 consecutive patients at baseline and at 10 minutes interval following a 50 mcg OCT bolus infusion, for a total of 60 minutes. Then, a 50 mcg OCT bolus was readministered and AzBF measured for another 20 minutes. Patients (mean age 51.4 yrs [30-69]) had biopsy-proven cirrhosis (alcoholic in 9 patients; Pugh’s score 8.8 + 0.3) and clinically significant portal hypertension (HVPG 19 + 1 mmHg). Four patients were on beta-blockers. Cardiac output was 6.7 + 0.4 L/min, and mean arterial pressure (MAP) was 84 + 1.8 mmHg. Results: (expressed as mean + SEM). Protocol 1: Mean AzBF value at baseline was 660 + 138 ml/min. The 50 mcg OCT bolus was followed at 10 minutes by a 34% decrease in AzBF as compared to baseline value. This AzBF decline was maintained over the 60-minute study period (36% + 1.4%). All values were statistically significant from baseline AzBF measurement (p<0.01). MAP values remained stable. Protocol 2: At 60 minutes, the repeat 50 mcg bolus infusion caused a further significant (p<0.01) decline in AzBF, although the response was blunted (-18% + 1.2%). Conclusion: in patients with cirrhosis and portal hypertension, the hemodynamic effect of a 50 mcg OCT bolus on mean AzBF is sustained and amenable to a further (but blunted) response at readministration. These findings suggest the clinical benefit of OCT is mostly related to OCT given as a bolus.

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734 SLEEP-WAKE ABNORMALITIES DO NOT CORRELATE WITH NEUROPSYCHIATRIC PERFORMANCE IN PATIENTS WITH CIRRHOSIS

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Sleep-wake inversion is a feature of overt hepatic encephalopathy (HE; Sherlock et al, 1954); while milder sleep disturbances are common in patients with cirrhosis, even in the absence of significant neuropsychiatric impairment (Córdoba et al, 1998). The aim of this study was to determine the relationship between sleep-wake abnormalities and the presence and the degree of HE in patients with cirrhosis. Ninety-three patients with biopsy-proven cirrhosis (56 males, Child A 62, B 20, C 11; mean age 57 yrs, range [34-80]), classified as neuropsychatically unimpaired (n=61) or as having minimal (n=12) or overt HE (n=20) using clinical (Conn et al, 1977), psychometric (Weissenborn et al, 2001) and EEG (Amadio et al, 1999) criteria, underwent evaluation of their sleep-wake patterns. A structured interview (subjective sleep evaluation - SSE) and a set of validated questionnaires were used to assess diurnal preference (Horne & Östberg, 1976), night sleep quality (Pittsburgh Sleep Quality Index - PSQI; Buysse et al, 1989) and day-time sleepiness (Epworth Sleepiness Scale - ESS; Johns et al, 1991). Twenty-six healthy volunteers (16 males, 47 [21-84] yrs) served as controls. Differences in sleep-wake indices between groups were examined by one-way ANOVA/ANCOVA (age adjustment); post-hoc analysis was performed using the Scheffé test; values are expressed as weighted averages. Categorized indices (normal/abnormal) were compared by the Pearson $\chi^2$. Patients
with cirrhosis slept significantly less well than healthy volunteers both subjectively (SSE: 1.87 vs. 1.35, p = 0.004) and objectively (PSQI: 8.39 vs. 4.22, p = 0.0001; PSQI abnormal 66% vs. 24%, χ2 = 13.7, p = 0.0002) and had more pronounced day-time sleepiness (ESS: 7.2 vs. 4.9, p = 0.069; ESS abnormal 32% vs. 0%, χ2 = 6.2, p = 0.012); a significantly higher number of awakenings (2.2 vs. 1.0, p = 0.001) and a longer time-to-fall-asleep (32 vs. 12 min, p = 0.043); there were no differences in diurnal preference, mean bed-time and mean wake-up time. There was no significant relationship between the presence or degree of HE and any of the sleep-wake indices. Similarly there was no relationship between the degree of hepatic dysfunction and sleep-wake variables, apart from the fact that patients with decompensated cirrhosis had more pronounced day-time sleepiness (p = 0.03). These results confirm the high prevalence and the severity of sleep-wake disturbances in patients with cirrhosis, which are independent of the presence and degree of HE. This sheds doubt on the appropriateness of including sleep parameters in scales for the diagnosis and grading of HE (Ferenci et al, 2001; Hourmand-Ollivier, 2006; Spahr et al, 2007).

735 MIDODRINE VERSUS ALBUMIN IN THE PREVENTION OF PARACENTESIS – INDUCED CIRCULATORY DYSFUNCTION IN CIRRHOTICS: A RANDOMIZED PILOT STUDY

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Intraavenous albumin has been used to combat paracentesis – induced circulatory dysfunction (PICD) in patients with cirrhosis with tense ascites. However, the use of albumin is costly and controversial. Splanchnic arterial vasodilatation is primarily responsible for circulatory dysfunction in these patients. Vasodilator therapy has been used to prevent PICD in cirrhotics. However, there are various problems with their use. There are no reports of use of midodrine, an oral α-adrenergic agonist, on the prevention of PICD in cirrhotics. In this randomized pilot study, we evaluated the effects of midodrine and albumin in the prevention of PICD in cirrhotics with tense ascites. Forty patients with cirrhosis and tense ascites underwent therapeutic paracentesis with midodrine or albumin in a randomized controlled trial at a tertiary centre. Effective arterial blood volume was assessed by measuring plasma renin activity at baseline and 6 days after paracentesis. Effective arterial blood volume as assessed by plasma renin activity before and 6 days after paracentesis did not differ in two groups (43.18 ± 10.73 to 45.90 ± 8.59 ng/ml/hr; p = 0.273 in the albumin group and 44.44 ± 8.44 to 41.39 ± 10.21 ng/ml/hr; p = 0.115 in the midodrine group). A significant increase in 24 hours urine volume and urine sodium excretion was also noted in midodrine group (p < 0.01 and p < 0.001, respectively). The cost of midodrine therapy was significantly lower when compared with that of albumin (p < 0.001). Conclusion: Midodrine is as effective as albumin in preventing PICD in cirrhotics, but at a fraction of the cost. Midodrine also resulted in increase in 24 hours urine volume and sodium excretion.

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736 MATERNAL AND FETAL OUTCOME IN 27 WOMEN WITH BUDD-CHIARI SYNDROME (BCS) AND 41 PREGNANCIES

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Although BCS mainly affects young women, data on BCS and pregnancy are sparse. Prognosis, when revealed by pregnancy, seems poor (1-year survival <50%). Pregnancy is often contraindicated in women with BCS. AIMS: (1) to assess characteristics and outcome of BCS revealed by pregnancy; (2) to determine if pregnancy should be contraindicated in women with known BCS. PATIENTS AND METHODS: Maternal and fetal features were assessed in 2 groups: Group 1, 7 patients who had BCS revealed by pregnancy; Group 2, a multicenter cohort of 20 women with BCS who became pregnant during follow-up; Compared with Group 3: 37 women with BCS without pregnancy at diagnosis or during follow-up. RESULTS: (1) In Group 1 (mean age 31 ± 5 yrs), diagnosis of BCS occurred in 3 patients during pregnancy and in 4 during postpartum. There were 3 miscarriages, 1 early preterm delivery and 3 healthy newborns (2 preeclampsia). Within 2 months of delivery, 2 underwent TIPS and 1 emergency liver transplantation (LT). All patients were alive at 38 ± 6 months. (2) In Group 2, 20 patients (mean age 29 ± 7 yrs), all asymptomatic for at least 1 year, had 34 pregnancies within 73 ± 56 months after BCS diagnosis. Anticoagulation was used in 24 pregnancies, antiplatelet therapy in 4: 14 decompressive therapies were performed in 10 patients (angioplasties in 4, TIPS in 2, surgical shunt in 6, and LT in 2) 59 ± 40 months before pregnancy. Child-Pugh score was lower (6.6 ± 1.7 vs 8 ± 1.9, p = 0.03) in group 2 vs 3. Until 20 weeks’ gestation, 15/34 abortions or miscarriages occurred. The 19 other deliveries occurred before 32 weeks in 2 patients, between 32-36 weeks in 10 and after 37 weeks in 6. There was 1 intrauterine fetal death, but 18 infants had a favorable outcome. Obstetrical complications included intrauterine haematoma (n=3), preeclampsia (n=1) and placententa praevia (n=1). Absence of venous collaterals (7/8 vs 3/9; p = 0.05) and presence of factor II mutation (9/15 vs 3/9; p = 0.05) were significantly associated with poor outcome pregnancies. No patient had died 41 ± 38 months after last delivery. Ascites occurred in 2 patients: 1 had portal vein thrombosis with heparin-induced thrombocytopenia and 1, without anticoagulation, had TIPS obstruction. CONCLUSION: (1) 15% of BCS in women are revealed by pregnancy. Maternal outcome is excellent using current therapies, but infant’s prognosis is severely impaired. (2) When pregnancies occur in women with known BCS, half give birth to healthy infants. In presence of factor II mutation, and when venous collaterals...
are not identified, pregnancies seem particularly at risk. Anticoagulation should be maintained. Liver dysfunction is rare and due to new thrombosis.

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737 PROGNOSTIC FACTORS FOR HEPATORENAL SYNDROME (HRS) REVERSAL IN PATIENTS WITH TYPE 1 HRS ENROLLED IN A RANDOMIZED, DOUBLE-BLIND, PLACEBO-CONTROLLED TRIAL

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Background: Type 1 HRS is characterized by rapid onset of functional renal failure (i.e., doubling of serum creatinine (Scr) within 2 weeks in cirrhotic patients, and is associated with a poor prognosis (median survival of 2-4 weeks). Preliminary results from 2 recently reported randomized, placebo controlled clinical trials have demonstrated that terlipressin + albumin has superior HRS reversal rates compared to albumin alone. Objective: To identify factors predictive of outcome in patients with Type 1 HRS. Methods: This analysis investigated the following 8 baseline patient characteristics collected from 112 Type 1 HRS patients enrolled in the randomized, double-blind, placebo-controlled HRS trial known as OT-0412: treatment (terlipressin, placebo), age (>65, ≤65 years), gender (male, female), race (non-White, White), alcoholic hepatitis (absent, present), MELD score (per 1 point increase), Child-Pugh score, and Scr concentration. Wald Chi-Square tests from individual logistic regressions were conducted to determine those baseline patient characteristics that are predictive of HRS Reversal. An individual log-binomial regression model was used to assess probability of HRS Reversal. Results: Baseline Scr concentration (p = 0.010), baseline MELD score (p = 0.024) and treatment with terlipressin (p = 0.009) were found to be significant predictors of HRS Reversal for the ITT population; with lower baseline Scr levels, lower baseline MELD scores and initiation of terlipressin therapy being more predictive of HRS Reversal. The probability for HRS Reversal decreases by 39% and 5.5%, respectively, for each 1 mg/dL increase in Scr or 1 point increase in MELD score; while the probability for HRS Reversal improves by 271% with terlipressin treatment. Finally, further analysis showed that only patients with baseline Scr < 5.6 mg/dL and receiving ≥ 3 days therapy achieved HRS Reversal. Summary: Less severe disease (i.e., lower Scr and MELD scores) in Type 1 HRS patients provides the best chance for HRS reversal. Additionally, treatment with terlipressin + albumin provides significantly improved chance for HRS reversal compared to albumin alone. However, patients appear to need ≥ 3 days therapy in order for HRS therapy to be effective. Conclusion: These data suggest that there may be a benefit in earlier HRS diagnosis and initiation of terlipressin + albumin treatment in order to maximize the chance for HRS Reversal.

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738 PAROXYSMAL NOCTURNAL HEMOGLOBINURIA IN BUDD-CHIARI SYNDROME – RESULTS OF THE EN-VIE STUDY

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Background and aims. Approximately 5-10% of cases of Budd-Chiari Syndrome (BCS) is caused by paroxysmal nocturnal hemoglobinuria (PNH). PNH is an acquired disorder of hematopoietic stem cells, characterized by intravascular hemolysis and venous thrombosis. We have studied the relationship between BCS and PNH with respect to clinical characteristics, treatment outcome and prognosis. Methods. Data on baseline characteristics, treatment and survival was obtained from the EN-Vie Study, a prospective international multi-center cohort study of 163 patients with BCS. Study guidelines recommended testing for PNH by flow cytometry or Ham’s test and this was done in 77 patients. Results. From the group of 77 patients tested for PNH, 15 tests were positive (19%). These 15 patients with PNH were compared to the group of BCS-patients without PNH (n=62). Median follow-up for the total tested group was 19 months (range 0-31 months). Of the 15 patients with BCS and PNH, 10 had already been diagnosed with PNH at an earlier time but only 2 of these patients were on anticoagulant treatment when the diagnosis of BCS was made, both because of previous thrombosis elsewhere. When comparing the PNH-patients to the group of non-PNH-patients, sex ratio, age at diagnosis of BCS, clinical presentation and liver function tests did not differ significantly between the groups. Comparison of radiological data however, revealed that BCS-patients with underlying PNH presented with a significantly higher percentage of additional splanchnic vein thrombosis (SVT; i.e. portal vein, mesenteric vein or splenic vein thrombosis) (47% vs. 10%, p=0.002) at diagnosis. Despite the higher frequency of SVT in the PNH-group, the number of patients treated with Transjugular Intrahepatic Portosystemic Shunt (TIPS) during follow-up was similar between both groups (6/15 in PNH group vs. 22/62 in non-PNH-group). Of the 15 PNH-patients, 4 successfully underwent bone marrow or stem cell transplantation after the diagnosis of BCS. There was no significant difference in survival between patients with and without PNH. Conclusions. The EN-Vie Study shows that PNH appears to be a more frequent cause of BCS than previously thought. Despite the high risk of (fatal) thrombosis in PNH, none of the patients known to have PNH before diagnosis of BCS had anticoagulant treatment as primary prophylaxis. Our data shows that, despite a higher frequency of additional SVT, short-term prognosis of patients with BCS caused by PNH does not differ from BCS-patients without PNH. Bone marrow/stem cell transplantation and TIPS appear to be safe treatment options for patients with PNH and BCS.

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UROTENSIN II INCREASES PORTAL PRESSURE AND INDUCES HEPATIC FIBROSIS

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BACKGROUND: Serum urotensin II (UII), a potent vasoactive mediator, is upregulated in chronic liver disease and correlates with the degree of portal hypertension. However, the relevance of these findings in the pathogenesis of cirrhotic portal hypertension is unknown. AIM: To determine whether UII executes a pathogenetic role in the development of chronic liver disease and portal hypertension. METHODS: UII was administered by continuous infusion over 4 weeks using osmotic mini pumps in 20 Sprague-Dawley rats divided into three treatment groups: Controls (saline, n=7), low dose UII (1 nmol/kg/hr, n=8) and high dose UII (3 nmol/kg/hr, n=5). Systemic and portal haemodynamics were assessed and tissues harvested for RT-PCR analysis of transcripts of profibrotic markers, and morphometric quantification of fibrosis. RESULTS: Infusion of UII induced a significant dose-dependent increase in portal venous pressure (5.8 ± 0.4, 6.4 ± 0.3 and 7.6 ± 0.7 respectively, p = 0.03) and splenic weights (p < 0.01). Concurrently, high dose UII infusion was associated with an increase in hepatic transcript for TGF-β (p < 0.05) while collagen 1 transcript expression was increased (p < 0.05) following the low dose infusion. Liver tissue hydroxyproline content was elevated in rats receiving high dose UII infusion (p < 0.05). By computer morphometric analysis there was a dose response relationship between UII and portal tract fibrosis (p < 0.05). No systemic haemodynamic alterations were noted. CONCLUSION: Sustained infusion of UII over 4 weeks in rats is associated with an elevation in portal venous pressure and induction of hepatic fibrosis as evidenced by an elevation in hepatic hydroxyproline content and increased portal tract fibrosis. This response may be mediated in part via an induction of TGF-β. These findings have pathophysiological implications in human liver disease where increased UII levels have been observed.

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PROTON PUMP INHIBITOR USE IS ASSOCIATED WITH A HIGH RISK OF SPONTANEOUS BACTERIAL PERITONITIS

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Spontaneous bacterial peritonitis (SBP) is associated with a poor prognosis and predicts early mortality. It is hypothesized that bacterial translocation causes SBP. Proton pump inhibitors (PPIs) promote gastric bacterial overgrowth and have been associated with infectious complications. The aim of this study was to determine the association of PPI use with SBP occurrence in a case-control study. Methods: 985 inpatient charts of cirrhotics were reviewed for SBP (ascitic fluid WBC >500 ± bacteriologic confirmation without prior GI bleed). PPI use was defined as outpatient PPI intake before SBP admission. Appropriate PPI use was defined as Gerd, Barrett’s, non-variceal GI bleed & peptic ulcer; rest were inappropriate. Charts without SBP confirmation or clear medication lists and SBP with GI bleed were excluded. Demographic information, Child (CTP) score, outpatient SBP prophylaxis and PPI use were collected. Each SBP patient was matched to 2 cirrhotics without SBP during the same time period per chart review according to age. Each SBP patient was also matched to one age and CTP-score matched non-SBP patient to evaluate the effect of PPI separately. Results: 130 patients had suspected SBP but only 44 had ascitic fluid confirmation. These 44 were matched to 88 inpatient non-SBP cirrhotics by age (Table) and 44 by age and CTP score; all non-SBP patients had been admitted for conditions other than GI bleeding or SBP. Bacteriologic confirmation (n=12) showed 7 gram positive (5 on PPI) & 5 negative (3 on PPI p=NS) bacteria. CTP score and SBP prophylaxis rate was significantly higher in the SBP group (Table). On univariate logistic regression with SBP as the outcome, only CTP score (p=0.0001 OR: 9.9 CI: 3.4-28.8) and PPI use (p=0.0001 OR: 7.8 CI: 3.4-18) were significant. This significant association with SBP continued on multivariate logistic regression; CTP (p=0.0001, OR: 9.3 CI: 4.3-30) and PPI (p=0.0001, OR: 6.4, CI: 2.5-16). When only CTP C SBP and non-SBP cirrhotics were studied, PPI use was still significantly associated with SBP (p=0.0001 OR: 5.7 CI: 2.1-15). Conclusion: PPI therapy and as expected, high CTP scores are independently associated with SBP despite a higher antibiotic prophylaxis rate in SBP patients.

TABLE: SBP and non-SBP patient characteristics

<table>
<thead>
<tr>
<th></th>
<th>SBP (n=44)</th>
<th>Non SBP (n=88)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>54 ± 12</td>
<td>54 ± 12</td>
<td>0.9</td>
</tr>
<tr>
<td>% Cirrhosis</td>
<td>73%</td>
<td>68%</td>
<td>0.7</td>
</tr>
<tr>
<td>CTP C (%)</td>
<td>96%</td>
<td>75%</td>
<td>0.001</td>
</tr>
<tr>
<td>SBP Prophylaxis</td>
<td>36%</td>
<td>22%</td>
<td>0.02</td>
</tr>
<tr>
<td>on PPI</td>
<td>30(68%)</td>
<td>22(25%)</td>
<td>0.0001</td>
</tr>
<tr>
<td>Inappropriate PPI use</td>
<td>16(35%)</td>
<td>7(32%)</td>
<td>0.2</td>
</tr>
</tbody>
</table>

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PREDICTORS OF INTERMEDIATE-TERM RISK IN CIRRHOSIS: IMPLICATIONS FOR MINIMAL LISTING CRITERIA IN LIVER TRANSPLANTATION (LT)

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Background: MELD score, jaundice, coagulopathy, renal failure, hyponatremia and uncontrolled ascites identify patients in the terminal stages of cirrhosis whose short term prognosis (< 180 days) is poor, and who therefore merit “sickest first” priority for LT. However the role of these and other indicators for predicting survival or need for LT in the intermediate term (180 days to 2 years) has not been determined. Methods: We prospectively collected clinical data from 774 cirrhotic veterans referred for consideration of LT between 1/1/98 and 4/1/05; of these, 108 patients with hepatocellular carcinoma at time of referral were excluded. Outcomes (death, transplantation) through 4/1/07 were measured from date of referral and were confirmed through patient electronic records, VA central office transplant database and the social security death index. Findings: Of 666 cirrhotic patients, 174 died or were transplanted within 180 days. Of the remaining 492 patients, an additional 117 died and 98 underwent LT within 2 years (intermediate term). Compared to survivors, patients who died or underwent LT in the intermediate term were more likely to have history at referral of ascites, uncontrolled ascites, past SBP or encephalopathy, but did not differ with regard to history of variceal bleeding. Laboratory values that differed significantly
TERULIPRESSIN IMPROVES RENAL FUNCTION AND INDUCES NATRIURESIS IN PATIENTS WITH CIRRHOSIS AND ASCITES WITHOUT HEPATORENAL SYNDROME

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Background & Aims: Patients with advanced cirrhosis and ascites are characterised by circulatory dysfunction with splanchnic vasodilatation and renal vasoconstriction. Treatment with the potent vasoconstrictor terlipressin has a positive effect on renal function in patients with hepatorenal syndrome (HRS), which is often the end-stage of patients with decompensated cirrhosis and ascites. In this pathophysiological study we aimed to evaluate, if the acute administration of terlipressin also improves renal function in patients with ascites without HRS. Methods: 23 cirrhotic patients participated; 15 with non-refractory ascites were randomised to either terlipressin (n=11) or placebo (n=4); 8 had refractory ascites and all received terlipressin. Glomerular filtration rate (GFR) and clearances of sodium (CNa), lithium (CLi) and osmoles (Cosm) and urine sodium concentration (UNa) were assessed before and after injection of 2 mg terlipressin/placebo. Results: In the non-refractory ascites group (n=11), terlipressin induced an increase in: GFR (68.8 ± 5.8 vs. 91.6 ± 7.4 mL/min, p<0.005), CNa (0.89 ± 0.06 vs. 1.52 ± 0.44 mM/min, p<0.05), CLi (17.3 ± 2.7 vs. 21.5 ± 3.5 mM/min, p<0.05), Cosm (2.10 ± 0.24 vs. 3.06 ± 0.60 mL/min, p<0.05) and UNa (66 ± 12 vs. 87 ± 11, p=0.005) [Mean ±SEM]. In the refractory ascites group (n=8), terlipressin induced an increase in: GFR (31.3 ± 6.7 vs. 41.1 ± 11.0 mL/min, p<0.05), CNa (0.11 ± 0.06 vs. 0.35 ± 0.14 mM/min, p<0.05), CLi (5.5 ± 1.5 vs. 9.5 ± 3.0 mM/min, p<0.05) and UNa (12 ± 6 vs. 27 ± 8, p<0.005). In both treatment groups plasma norepinephrine (p<0.05) and renin (p=0.05) decreased and proANP increased (p<0.01) after terlipressin. All parameters remained unchanged after placebo.

Conclusion: Terlipressin improves renal function and induces natriuresis in cirrhotic patients with ascites without HRS. Vasoconstrictors may represent a novel future treatment modality in these patients.

Disclosures:
The following people have nothing to disclose: Aleksander Krag, Søren Møller, Jens H. Henriksen, Niels-Henrik Holstein-Rathlou, Fin S. Larsen

THE DEGREE OF DISTURBANCE OF RENAL BLOOD FLOW AUTOREGULATION IN CIRRHOSIS DETERMINES THE SEVERITY OF RENAL DYSFUNCTION

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Background and Aims: The mechanisms leading to altered renal blood flow (RBF) and renal function in hepatorenal syndrome are unclear. It has been proposed that activation of the sympathetic nervous system causes a rightward shift in the renal autoregulatory curve, such that RBF becomes critically dependent on renal perfusion pressure (RPP), which then contributes to the development of the hepatorenal syndrome. The aims of the study were to determine the relationship of RBF and RPP in patients with decompensated liver disease, and the effect on renal hemodynamics following insertion of a transjugular intrahepatic portosystemic shunt (TIPSS). Methods: Fifty-six patients were recruited into groups with 1) no ascites (n=13), 2) diuretic-responsive ascites (n=13), 3) intractable ascites (n=12), 4) type II hepatorenal syndrome (n=10) and 5) requiring a TIPSS for refractory ascites (n=8). We measured cardiac hemodynamics, RBF, RPP, portal pressure and norepinephrine levels, and mathematically modeled the renal autoregulatory curve using heuristic methods. Results: RBF correlated with RPP (r²=0.78, p<0.001) and inversely with the hepatic venous pressure gradient (r²=0.61, p<0.0001) and plasma norepinephrine levels (r²=0.78, p<0.0001). Norepinephrine increased with increasing disease severity, and this was associated with a rightward and downward shift of the RBF/RPP autoregulatory curve. (Figure 1A) TIPSS insertion reduced the hepatic venous pressure gradient and plasma norepinephrine levels (<0.001), and the RBF/RPP curve was shifted upwards. (Figure 1B) Conclusion: The relationship between RBF and RPP involves interplay between the sympathetic nervous system and the kidney. Disturbance in this relationship determines the severity of renal dysfunction in cirrhosis. An important mechanism through which TIPSS insertion improves renal function is through positive effects on RBF autoregulation.
HYponatremia: A MAJOR DETERMINANT OF IMPAIRED HEALTH-RELATED QUALITY OF LIFE IN CIRRHOSIS WITH ASCITES


Background: Patients with liver cirrhosis and ascites are prone to develop spontaneous bacterial peritonitis (SBP), leading to a substantial increase of morbidity and mortality in cirrhotic patients. SBP is commonly attributed to bacterial translocation from the intestine due to impaired mucosal barrier function (Wiest & Garcia-Tsao, Hepatology 2005;41:422-33). Recently, mutations in the NOD2 (nucleotide-binding oligomerization domain containing 2) gene have been associated with altered permeability of the intestinal wall in Crohn’s disease. Thus, we hypothesized that SBP in patients with liver cirrhosis is also associated with common NOD2 gene variants.

Patients and methods: Overall, we prospectively included 84 patients with histology proven liver cirrhosis or clinical signs of cirrhosis and ascites and monitored these patients for the development of SBP. SBP was defined as presence of PMN count > 250 cells/μL or positive bacterial culture from ascitic fluid. NOD2 gene variants (rs2066844 [R702W], rs2066845 [G908R], rs2066847 [3020insC]) were genotyped in all patients using S' exonuclease Taqman assays with fluorescent dye labeled probes. Power calculations were performed using P&S; the study was designed to detect a significantly increased OR of 5 with a power of >80%, based on risk allele frequency of 0.04. All genotype distributions are consistent with Hardy-Weinberg-equilibrium.

Results: Employing PMN count and bacterial growth in culture as diagnostic criteria, 20 patients (24%) and 12 patients (14%) were diagnosed with SBP during follow-up. Overall, the occurrence of SBP is associated significantly with carriage of the risk allele of the NOD2 G908R variant (PMN count: odds ratio (OR) = 7.63; p < 0.01). In addition, carriers of the minor allele of the NOD2 R702W variant display a higher risk for SBP indicated by bacterial growth only (OR = 7.00; P = 0.04). All NOD2 genotype distributions are consistent with Hardy-Weinberg-equilibrium.

Conclusions: Here we report a significant association of SBP with common coding variants of...
the NOD2 gene. NOD2 variants linked previously to impaired mucosal barrier function confer an increased SBP risk. We speculate that our findings, when validated in independent cohorts, serve to identify patients with cirrhotic ascites eligible for preemptive antibiotic treatment.

Disclosures:
The following people have nothing to disclose: Beate Appenrodt, Frank Grünhage, Martin Gentemann, Lydia Thyssen, Stephanie Schwartz, Tilman Sauerbruch, Frank Lammer

746 PLATELET COUNT/SPLEEN DIAMETER RATIO AND AASLD CRITERION FOR SCREENING ESOPHAGEAL VARICES IN PATIENTS WITH HEPATITIS C VIRUS-RELATED COMPENSATED CIRRHOSIS
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Background: Screening for esophageal varices (EV) is a mandatory yet expensive part of the diagnostic work-up of cirrhotic patients. Recently, attention was put on non-invasive, accurate, and cost-effective assessment of EV with the aim of improving the management of cirrhotic patients from both the medical and the financial point of view. Aims: To evaluate the diagnostic efficiency of the platelet count/spleen diameter in comparison with the criterion suggested by the AASLD (portal vein diameter >13mm and/or platelet count <140,000/mm3) for screening EV in patients with HCV-related compensated cirrhosis. Methods: Among a total of 311 cirrhotic undergoing EV screening, 114 had no hepatic encephalopathy and no ascites detected by abdominal ultrasound. Sensitivity, specificity, positive and negative predictive values, and efficiency for EV diagnosis of the platelet count/spleen diameter ratio (cut-off value=909) and of the AASLD criterion were evaluated in patients with compensated cirrhosis. Results: Prevalence of EV was 26.3% (6% with large EV). According to the AASLD criterion, endoscopy would have been avoided in 75 of these patients (none with EV) with a sensitivity of 100% (88.8-99.9%, 95% confidence interval) and a negative predictive value of 100% (95.3-100%), while 39 patients would have undergone endoscopy (9 without EV) with 89.3% (80.1-94.2%) specificity, 76.9% (61.5-87.3%) positive predictive value, and 92.1% (85.7-95.8%) diagnostic efficiency. Noteworthy, all the "false positive diagnoses" made by using the AASLD criterion were due to thrombocytopenia (i.e., platelet count <140,000). A platelet count/spleen diameter ratio value of 909 showed 100% sensitivity (88.8-99.9%), 97.6% specificity (91.8-99.3%), 100% negative predictive value (97.5-100%), 93.8% positive predictive value (79.8-98.1%), 2 patients without EV undergoing unnecessary endoscopy, and 98.3% efficiency (93.9-99.5%) for the diagnosis of EV. Conclusions: In patients with HCV-related compensated cirrhosis, the platelet count/spleen diameter ratio is a useful non-invasive tool for evaluating which patients should undergo endoscopy for EV screening, and its diagnostic efficiency is even better than the one obtained by applying the AASLD criterion. This study also provides the first practical evidence supporting the utility of the AASLD criterion.

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The following people have nothing to disclose: Eedoardo G. Giannini, Eram Anwar, Kaukab Bashir, Vincenzo Savarino, Adnan Agha

747 INTRANASAL DESMOPRESSIN IS EFFECTIVE IN PREVENTING BLEEDING AFTER DENTAL EXTRACTION IN CIRRHOTIC PATIENTS HAVING MODERATE DEGREES OF COAGULOPATHY
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BACKGROUND. Many patients with advanced liver disease have extremely poor dentition and require dental extractions. Cirrhotic patients waiting for liver transplantation who need dental extractions are typically given fresh frozen plasma (FFP) and/or platelets to correct coagulopathy. This is costly and may be associated with transfusion reactions and fluid overload. AIM. We evaluated the efficacy of intranasal desmopressin as an alternative to prevent bleeding in cirrhotic patients undergoing dental extraction. PATIENTS AND METHODS. Cirrhotic patients with platelet counts of 30,000-50,000/µL and/or INR 2.0-3.0 were randomized to receive blood transfusion (FFP 10ml/kg and/or 1 unit of single donor platelets, respectively) or intranasal desmopressin (300µg) before dental extraction. Patients with other bleeding disorders, receipt of blood transfusion within 2 weeks prior to study, renal insufficiency, allergy to desmopressin, or treatment with anti-platelet medications were excluded. All patients received prophylactic antibiotics 1 hour prior to procedure. A standard oromaxillofacial surgical treatment protocol was performed by the same surgeon (AM). Patients were followed up for post-extraction bleeding and side-effects over the next 24-48 hours. Study medication was provided by CSL Behring. RESULTS. Forty-one patients (21 in desmopressin group and 20 in blood transfusion group) were enrolled into the study. No significant differences were noted between the two groups in terms of gender (% males 67 vs. 70, respectively), age (median 51 vs. 50), INR (2.0 vs. 2.2), platelet levels (46,000 vs. 45,000/µL), creatinine (0.9 vs. 0.8 mg/dL), total bilirubin (2.8 vs. 2.8 mg/dL), ALT (30 vs. 42 U/L), albumin (2.7 vs. 2.5 g/dL), MELD score (16 vs. 17), or median number of teeth removed (3 vs. 4) (all p values > 0.28). The number of teeth removed ranged between 1-31 in the desmopressin group and 1-22 in the blood transfusion group. No patients in the desmopressin group required rescue blood transfusion after extraction. Four patients reused desmopressin at home. One patient in the transfusion group had bleeding after the procedure and required an additional transfusion. Another patient in the transfusion group experienced an allergic reaction at the end of transfusion, which was effectively treated with diphenhydramine. Treatment associated costs were lower for desmopressin ($700/patient) than for blood transfusion ($1500-3000/patient). CONCLUSION. Intranasal desmopressin is as effective as blood transfusion in achieving hemostasis in coagulopathic cirrhotic patients who undergo dental extractions, is much more convenient, less expensive and well tolerated.

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The following people have nothing to disclose: Carmen M. Stanca, Andre H. Montazem, Jin X. Zhang, Adeyemi Lawal, Thomas D. Schiano
Background and Aims. The study of refill kinetics after microbubbles destruction at contrast-enhanced ultrasound (CEUS) has proven useful to assess myocardial and renal perfusion. Preliminary data in healthy subjects suggested that this method may be used also for hepatic perfusion study. This study was aimed at assessing the use of regional hepatic perfusion (RHP) by CEUS in cirrhosis. Methods. 52 patients with liver cirrhosis undergoing hepatic vein catheterisation were included in the study. During a continuous iv infusion of microbubbles (SonoVue®, bolus of 0.5 mL followed by 3 mL/min), RHP was quantified using CEUS by destroying microbubbles at 95 seconds and measuring their tissue refill with intermittent harmonic imaging. A 25 seconds film of the refill curve, expressed by the exponential function $y = A(1-e^{-t})$ was electronically analysed; $A$ expresses blood volume fraction and $e$ microbubbles velocity. RHP is calculated as $A \times e$. Hepatic blood flow (HBF) was measured during ICG infusion at hepatic vein catheterisation. Hepatic venous pressure gradient (HVPG) was determined in the main hepatic vein (balloon-catheter), and cardiac index by thermal dilution (Swan-Ganz catheter).

Results. 26 patients had compensated cirrhosis, and 26 were decompensated. Overall, microbubble velocity was significantly higher than in a group of 10 healthy subjects ($r = 0.38 \pm 0.20$ vs. $0.22 \pm 0.05, p = 0.015$). RHP correlated with Child-Pugh score ($r = 0.333, p = 0.016$), and its objective components (albumin $r = 0.351, p = 0.011$; bilirubin $r = 0.291, p = 0.037$; prothrombin time $r = 0.341, p = 0.029$). RHP showed a direct correlation with HBF ($r = 0.515, p = 0.003$), and inverse correlations with indices of hepatic functional reserve: ICG extraction $r = 0.543, p = 0.001$; ICG plasma clearance $r = 0.391, p = 0.027$; ICG intrinsic clearance $r = 0.338, p = 0.050$. In addition, RHP correlated with the hyperdynamic state (cardiac index $r = 0.364, p = 0.019$; mean arterial pressure $r = 0.429, p = 0.003$; systemic vascular resistance $r = 0.459, p = 0.003$), and showed a weak direct correlation with HVPG ($r = 0.282, p = 0.050$).

Conclusions. Regional hepatic perfusion is increased in patients with cirrhosis, and correlates with the degree of liver failure and hyperdynamic state. Moreover, RHP correlates directly with HBF and with the reduction of liver functional reserve expressed by ICG clearance and extraction, suggesting that increased liver regional perfusion is mainly through anatomical or functional shunts. The determination of RHP by CEUS is a novel, objective, quantitative, non-invasive tool to assess disease severity in patients with liver cirrhosis.

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749 SILDENAFIL HAS NO EFFECT ON PORTAL PRESSURE BUT LOWERS ARTERIAL PRESSURE

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Background: In cirrhosis, portal hypertension is partially due to intrahepatic vasocstriction attributed to local underproduction of nitric oxide (NO). Phosphodiesterase V (PDE-V) inhibitors increase NO availability and can decrease portal pressure by decreasing intrahepatic resistance, however this vasodilatory effect may also occur in the systemic circulation. Unlike experimental studies, a study in 5 patients with cirrhosis showed that vardenafil, a PDE-V inhibitor, lowers portal pressure (Aliment Pharmacol Ther 2006;23:121). Aim: To re-evaluate the effect of sildenafil, a short-acting PDE-V inhibitor more commonly used for erectile dysfunction (ED) by patients with cirrhosis, on systemic and portal hemodynamics. Methods: Open label study of the effect of single oral doses of sildenafil on mean arterial and portal pressures in patients with compensated cirrhosis and portal hypertension as defined by a hepatic venous pressure gradient (HVPG) > 6 mmHg. HVPG was obtained by subtracting the free hepatic venous pressure (FHVP) from the wedged hepatic venous pressure (WHVP). After obtaining baseline measurements, patients received an oral dose of 25 mg of sildenafil, with repeat measurements at 30 and 60 minutes. In a subset of patients, an additional 25 mg was administered if no significant drop in blood pressure was observed at 60 minutes and measurements repeated 30 minutes later (i.e. 90 minutes from baseline). WHVP and FHVP tracings were read blindly and in random sequence by three independent observers. Results: Twelve patients were enrolled in the study. All were male; 10 had HCV cirrhosis (7 with alcohol) and 1 each had HBV and cryptogenic cirrhosis. Median age was 54 years (range 51-58). All were Child A. As shown in the table there was a significant decrease in systolic, diastolic and mean arterial pressure without any effect on WHVP or HVPG. Conclusion: As previously shown with other vasodilators, sildenafil at therapeutic doses for ED has a potentially deleterious systemic effect without a portal pressure-reducing effect. Therefore, its daily use is not recommendable. The search should continue for vasodilators that are specifically targeted to the intrahepatic circulation and that lack a systemic effect.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Baseline (n=12, median [IQR])</th>
<th>Median % change at 30 min (n=11)</th>
<th>Median % change at 60 min (n=12)</th>
<th>Median % change at 90 min (n=9)</th>
<th>$p$ (Friedman’s test)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systolic blood pressure</td>
<td>133 (123-148)</td>
<td>-0% *</td>
<td>-0% *</td>
<td>-0% *</td>
<td>0.008</td>
</tr>
<tr>
<td>Diastolic blood pressure</td>
<td>69 (60-76)</td>
<td>-5% *</td>
<td>-8% *</td>
<td>-8% *</td>
<td>0.040</td>
</tr>
<tr>
<td>Mean arterial pressure</td>
<td>92 (83-94)</td>
<td>-8% *</td>
<td>-7% *</td>
<td>-9% *</td>
<td>0.003</td>
</tr>
<tr>
<td>Heart rate (bpm)</td>
<td>67 (59-75)</td>
<td>0</td>
<td>+2%</td>
<td>+3%</td>
<td>0.056</td>
</tr>
<tr>
<td>WHVP (mmHg)</td>
<td>21.4 (18.0-27.0)</td>
<td>0</td>
<td>+2%</td>
<td>-1%</td>
<td>0.162</td>
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<tr>
<td>FHVP (mmHg)</td>
<td>11.2 (8.5-13.3)</td>
<td>+1% mind</td>
<td>+4%</td>
<td>+1%</td>
<td>0.007</td>
</tr>
<tr>
<td>HVPG (mmHg)</td>
<td>10.4 (6.6-13.0)</td>
<td>+1% mind</td>
<td>-4%</td>
<td>-5%</td>
<td>0.506</td>
</tr>
</tbody>
</table>

*significantly different from baseline
750 OXYGEN DESATURATION DURING SLEEP IN HEPATOPULMONARY SYNDROME

David T. Palma1, Miguel R. Arguedas1, Susan M. Harding2, Michael B. Fallon1, 1Medicine, University of Alabama at Birmingham, Birmingham, AL; 2Sleep-Wake Disorders Center, University of Alabama at Birmingham, Birmingham, AL

Background & Aim: Sleep alters respiratory mechanics and gas exchange that can adversely impact arterial oxygenation. Whether sleep affects oxygenation in hepatopulmonary syndrome is unknown. The aim of this study was to evaluate subjects with hepatopulmonary syndrome for the presence of oxygen desaturation during sleep. Methods: Twenty cirrhotic adults including 10 controls and 10 hepatopulmonary syndrome were recruited from the UAB cirrhosis clinic and underwent home pulse oximetry during sleep. Subjects at high risk for obstructive sleep apnea were excluded through the Berlin questionnaire. Subjects who spent > 10% of total sleep time with arterial oxygen saturation < 90% were classified as sleeptime oxygen desaturators. Sleeptime desaturation was correlated with clinical variables. Results: The mean age of the entire cohort was 54 years and 9 participants were males. The most common causes of liver disease were hepatitis C infection (45%) and alcoholic liver disease (15%). There was no statistically significant difference in age, gender, race, cause of cirrhosis, Model for End-Stage Liver Disease score or Body Mass Index between hepatopulmonary syndrome and control subjects. 7 of 10 hepatopulmonary syndrome subjects and none of 10 controls had sleeptime oxygen desaturation. The median percentage of total sleep time with arterial oxygen saturation < 90% was significantly higher in hepatopulmonary syndrome subjects than in controls (medians 25% vs. 0%, p = 0.005). Hepatopulmonary syndrome subjects had significantly lower wake time arterial oxygen saturation (medians 97% vs. 95%, p = 0.003) and mean sleeptime arterial oxygen saturation levels (medians 96% vs. 91%, p = 0.0008) than controls. Sleeptime desaturation directly correlated with alveolar-arterial oxygen gradient (p = 0.0007) and inversely with waketime arterial oxygen tension (p = 0.0007) and oxygen saturation (p < 0.0001). Conclusions: Oxygen desaturation occurred during sleep in 70% of hepatopulmonary syndrome subjects, the degree of which correlated with the severity of hepatopulmonary syndrome. Marked hypoxemia during sleep may occur in hepatopulmonary syndrome patients who by waketime oxygen values have only mild to moderate hypoxemia. We speculate that nocturnal oxygen desaturation could contribute to the observation that increased mortality in patients with hepatopulmonary syndrome is not confined solely to those with severe daytime hypoxemia.

Disclosures: The following people have nothing to disclose: David T. Palma, Miguel R. Arguedas, Susan M. Harding, Michael B. Fallon

751 A MULTI-CENTER CASE-CONTROL STUDY OF CLINICAL PREDICTORS AND FUNCTIONAL STATUS IN HEPATOPULMONARY SYNDROME (HPS)

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Introduction: The hepatopulmonary syndrome (HPS) occurs in 10-30% of cirrhotic patients and adversely affects survival with or without liver transplantation (OLT). Whether specific clinical features are associated with the presence of HPS and if HPS influences functional status are poorly defined. We evaluated clinical risk factors and functional status in patients undergoing OLT evaluation. Methods: We performed a case-control study at six US academic medical centers between 2004-2006. HPS cases had an abnormal alveolar-arterial oxygen gradient (A-aPaO2) and intrapulmonary vascular shunting on contrast enhanced transthoracic echocardiography (CE) in the absence of significant restrictive or obstructive lung disease. Controls had negative CEs or positive CEs with normal A-aPaO2 in the absence of lung disease. Health related quality of life was assessed using an SF-36 based disease-targeted quality of life instrument (LDQOL 1.0). Results: 71 patients with HPS were compared to 178 controls. The groups were similar in terms of age, gender, and etiology of cirrhosis. Clubbing and cyanosis were uncommon but more frequent in HPS (p<0.05), while spider angiomas and ascites were similar between the groups. HPS patients were less likely to have a smoking history than controls (48% vs 62%, respectively, p <0.05), while the frequency of TIPS, abnormal CXR findings, beta-blocker or antibiotic use were similar between the groups. Mean (±SD) MELD scores were identical between cases and controls (13 ± 4 vs 13 ± 6). HPS patients had significantly higher median [IQR] A-aPaO2 than controls [23 [19-35] vs 9 [3-12], p<0.0001] and lower PaO2 (75±13 mmHg vs 93±13 mmHg, p<0.0001). Patients with HPS reported more dyspnea than controls (46% vs 25%, p< 0.001) and had worse NYHA functional class (p<0.005). Cases also had lower mean (±SD) SF-36 global mental component scores (40±10 vs 45±11, p=0.02) and worse general health perceptions (26±14 vs 37±22, p<0.015), physical (28±24 vs 42±32, p<0.033) and emotional (50±34 vs 66±32, p<0.01) role limitations and emotional well being (56±21 vs 66±22, p<0.01) relative to controls. Symptoms of liver disease were also greater in HPS patients relative to controls (51±19 vs 59±22, p<0.03). Conclusions: Commonly assessed clinical variables do not reliably predict the presence of HPS and therapies targeted towards modulation of portal pressure or bacterial translocation do not appear to affect its presence. HPS is associated with pulmonary symptoms, impaired functional class and reduced quality of life. These findings highlight the importance of developing HPS screening algorithms and developing effective medical therapies.

Disclosures: The following people have nothing to disclose: Michael B. Fallon, Michael J. Krowka, Miguel R. Arguedas, James F. Trotter, Lisa Forman, Vijay Shah, R. S. Brown, Steven Zacks, Steven M. Kawut
Background: The gold standard test for the diagnosis of SBP is based on manual count of ascitic fluid polymorphonuclear (PMN) cells. However, the procedure is operator dependent and lysis of PMN cells during transport to the laboratory may lead to false negative results. Furthermore, ascites fluid culture is insensitive and leads to delays in diagnosis. Lactoferrin is a major component of PMN cell granules and has been shown to be a sensitive and specific marker of leukocyte activation. Aims: 1) To assess the sensitivity and specificity of ascitic fluid lactoferrin for the diagnosis of SBP and 2) To identify a clinically useful cutoff level that can be used for future development of a rapid bedside test. Methods: 218 consecutive ascites fluid samples from 148 patients (1-8 samples per patient) with liver cirrhosis at two tertiary care medical centers were examined for PMN counts, bedside culture, Gram stain and lactoferrin concentration. Quantitative measurements of ascitic fluid lactoferrin concentration were determined using a polyclonal antibody-based enzyme-linked immunosorbent assay (ELISA) specific for human lactoferrin by a laboratory blinded to the patients' clinical information and other laboratory results. Ascites fluid PMN cell count > 250/mL and/or a positive gram stain/culture were used for the diagnosis of SBP. Results: 22 (10%) samples fulfilled diagnostic criteria for SBP. Samples from patients with SBP had significantly higher lactoferrin concentration (median 3744 ng/mL, interquartile range (IQR) 788-9617) compared to non-infected samples (median 31 ng/mL, IQR 12-67); P<0.001. Using a cutoff level of 242 ng/mL, the assay had 96% sensitivity and 97% specificity to identify those with SBP.

Sensitivity and specificity of ascitic fluid lactoferrin for diagnosis of SBP at different cutoff levels

<table>
<thead>
<tr>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>Ascitic fluid lactoferrin levels (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>76</td>
<td>68</td>
</tr>
<tr>
<td>96</td>
<td>97</td>
<td>242</td>
</tr>
<tr>
<td>91</td>
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<td>458</td>
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<td>82</td>
<td>99</td>
<td>724</td>
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Disclosures:
James Boone - Employee: Other

The following people have nothing to disclose: Mansour A. Parsi, Sherif N. Saadeh, Nizar N. Zein, Gary L. Davis, Rocio Lopez, Maria R. Lepe, Linsheng Guo, Mohammad Ashfaq, Goran Klintmalm, Arthur J. McCullough

AQUAPORIN-1 DEMONSTRATES ARTERIAL CAPILLARY PROLIFERATION AND HEPATIC SINUSOIDAL TRANSFORMATION IN HUMAN CIRRHOTIC LIVER-RELEVANCE TO PORTAL HYPERTENSION

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Background: Defenestration of hepatic sinusoidal endothelial cells concomitant with collagen deposition in the space of Disse causes an increase in peripheral portal microvascular resistance in liver cirrhosis. Moreover, the development of scars in the cirrhotic liver is invariably accompanied by an intense microvascular proliferation, and is involved in portal hypertension. Aquaporins (AQP)s are key regulators in water channels across the cell cytoplasm. Although AQP's in the normal hepatobiliary system have been studied in mammals, little is known about the AQP localizations and changes in the hepatic microvascular system including sinusoids in cirrhotic liver, which may contribute to portal hypertension. The aim of this study is to clarify the localization of AQP-1 in the microvessels in normal and cirrhotic human liver. Methods: As human control liver samples, wedge biopsy specimens from the non-cirrhotic liver were obtained from patients who underwent surgical resection for metastatic liver carcinoma. Cirrhotic liver specimens were obtained from macroscopically cirrhotic portions surgically resected from patients who had hepatocellular carcinoma combined with hepatitis C-related cirrhosis. Immunostaining was performed on serial sections. Four-micrometer sections were cut from paraffin blocks of formalin-fixed tissue, liver specimens were reacted with anti-AQP-1 polyclonal antibody. Western blotting was conducted using fresh control and cirrhotic liver tissues. For in situ hybridization(ISH), Human AQP-1 peptide nucleic acid probes were used with a catalyzed signal amplification system. Results: In control liver tissue, AQP-1 was mainly localized in the portal venules, hepatic arterioles, and bile ducts in the portal tract, while AQP-1 was hardly detected in the sinusoids. In human cirrhotic liver tissue, AQP-1 expressions were observed mainly on periportal SECs, proliferative arterial capillaries opening into the sinusoid in the generating hepatic nodule, and proliferated bile ductules at the peripheral edge of nodules and fibrotic septa. In cirrhotic liver tissue, overexpressions of AQP-1 at protein and mRNA levels were demonstrated by Western blot and ISH, respectively. Conclusion: AQP-1 immunoreactivities were aberrantly expressed in response to the transformation hepatic sinusoidal endothelial cells as well as the proliferated arterial capillaries in cirrhotic liver, indicating angiogenetic responses induced AQP-1, leading to the pouring of arterial blood into the sinusoids, increasing sinusoidal microvascular resistance, and contributing to exaggerating portal hypertension in liver cirrhosis.

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LONG-TERM SURVIVAL IN PATIENTS WITH REGRESSION OF VIRAL-RELATED CIRRHOSIS

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BACKGROUND Historically thought as irreversible, there is mounting evidence that cirrhosis can regress, given that the underlying liver disease is controlled. Whether regression of cirrhosis has a beneficial clinical effect is uncertain and highly debated. METHODS We have prospectively followed 10 years (range 3 to 17 years) 100 patients with compensated biopsy-proven cirrhosis related to chronic viral hepatitis B or C. All received specific therapy. Patients were stratified on the basis of the decrease of their histopathological fibrosis score on a post-therapeutic liver biopsy. Regression of cirrhosis was defined as a decrease in the fibrosis score from F4 to less than or equal to F2 from the METAVIR classification. Observed liver-related complications and survival rates were compared between patients with or without regression of cirrhosis. RESULTS Regression of cirrhosis was observed in 24 patients (22 hepatitis C virus-infected patients, 2 hepatitis B virus-infected patients), among which 21 were long-term responders to therapy and 3 biochemical responders. The platelet count, the prothrombin time and the response to therapy were independent predictors of regression of cirrhosis. The incidence of liver-related complications, including hepatocellular carcinoma, ascitis, hepatic encephalopathy, varical bleiding was null in the group with regression of cirrhosis compared to 19 percent without regression (P<0.05 — log-rank test). The incidence of cirrhosis-related complications was also lower in patients with a sustained virological response, although 3 patients with chronic hepatitis C, without regression of cirrhosis, developed hepatocellular carcinoma despite complete response to therapy (P<0.01 — log-rank test). Finally, 15 patients without regression of cirrhosis died or underwent liver transplantation while none of the patients with regression of cirrhosis died or underwent liver transplantation (P<0.05 — log-rank test). CONCLUSION In this prospective follow-up of patients with viral hepatitis-related cirrhosis who received specific therapy, the absence of liver-related morbidity and mortality in patients with histologically-proven regression of cirrhosis establishes that regression of viral cirrhosis has a clear impact on morbidity and mortality. It is therefore important to check the fibrotic status of cirrhotic patients after treatment since both the prognosis and the follow-up can be completely different in case of cirrhosis regression.

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PROGNOSTIC VALUE OF LIVER STIFFNESS MEASUREMENT AND HEPATIC VENOUS PRESSURE GRADIENT IN PATIENTS WITH CHRONIC LIVER DISEASE: A PROSPECTIVE STUDY

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Hepatic venous pressure gradient (HVPG) has been proved to have a prognostic value in patients with chronic liver disease, on the one hand. On the other hand, liver stiffness (LS) as measured by transient elastography has been found to be correlated with the amount of fibrosis within the liver and to HVPG. The present study aimed to assess the prognostic value of LS and to compare it with HVPG performances. METHODS: LS and HVPG were measured on the same day in consecutive patients presenting with chronic liver disease and an indication for a transjugular liver biopsy. 78 of these patients were followed for one year, or until liver transplantation or the occurrence of a complication of their liver disease, namely: variceal bleeding, ascites, sepsis, encephalopathy, hepatocarcinoma or death. RESULTS: 27 (35%) of the 78 patients experienced at least one complication: bleeding in 6, ascites in 4, sepsis in 10, encephalopathy in 2, hepatocarcinoma in 1, or died (16). The performances of LS in predicting the occurrence of a complication were: AUROC = 0.83 [0.74-0.92], with a cut-off of 21.1 kPa, sensitivity was 83%, specificity 64%, PPV 53% and NPV 89%. Using HVPG, AUROC was 0.85 [0.76-0.94], and with a cut-off of 10 mmHg, sensitivity was 85%, specificity 63%, PPV 56% and NPV 89%. According to whether LS was below or above 21.1 kPa, the actuarial rates of the occurrence of a complication at one year were 10% and 50% respectively (p<0.05). These rates were 10% and 53% (p<0.05) in patients with a HVPG below or above 10 mmHg respectively. Results were similar when only patients with cirrhosis were considered. CONCLUSION: LS as assessed by transient elastography and HVPG have similar performances in predicting complications at one year in patients with chronic liver disease. Disclosures: The following people have nothing to disclose: Christophe Bureau, Marie-Angèle Robic, Sophie Métivier, Jean-Marie Pérón, Olivier Rouquet, Emmanuel Dupuis, Laurent Alric, Jean-Pierre Vinel

PROGNOSTIC INDICATORS OF REBLEEDING IN POOR HEMODYNAMIC RESPONDERS TO TREATMENT OF PORTAL HYPERTENSION

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Hemodynamic response to treatment of portal hypertension is considered adequate when the hepatic venous pressure gradient (HVPG) decrease to <12 mmHg or by >20% of baseline. It is strongly established that responders have a lower risk of variceal bleeding and better survival. Non-responders may benefit of a rescue therapy. However, up to 40%-50% of non-responders do not have rebleeding suggesting that further therapy is not required in this subgroup. The aim of this study was to investigate which factors may predict rebleeding among poor hemodynamic responders. METHODS: During a 10-years period 400 patients were included after an acute variceal
bleeding episode and received chronic treatment with drugs (β-blockers ± nitrates) or endoscopic ligation or both, to prevent recurrent bleeding. A baseline hemodynamic study was performed in 344 patients and a second study was performed in 302 patients 1-3 months later to assess response that was defined as a decrease in HVPG to <12 mmHg or >20%.

RESULTS: Mean age of included was 59±12 years, 65% were male, 48% had alcoholic cirrhosis, 81% were Child-Pugh class B/C at baseline and 49% at third month of follow-up. 114 patients (38%) were hemodynamic responders and 188 (62%) were non-responders. During the follow-up of 43±40 months 148 patients (37%) rebleed and 207 (52%) died. Rebleeding occurred in 16% of responders and 48% of non-responders (P<0.001) and death in 32% vs 52% respectively (P=0.001). Among non-responders, those who rebleed had as compared with those without rebleeding, a higher baseline bilirubin (P=0.01) and platelets (P=0.05) at 1-3rd month of follow-up, a greater rate of failure to control the acute bleeding episode (P<0.001) and were older (P=0.01). The multivariate analysis performed including these parameters identified the Child-Pugh score at 3rd month (OR=2.4, 95% Cl=1.5-3.7), failure to control the index bleeding (OR=1.7, 95% Cl=1.1-2.8) and HVPG at 1-3rd month (OR=1.1, 95% Cl=1.01-1.2) as independent predictors of rebleeding in non-responders. Rebleeding occurred in 70% of non-responders with Child-Pugh >7 at 3rd month, failure to control bleeding or HVPG >20 mmHg at 1-3rd month, and in 39% of those without any of these factors (P<0.001). CONCLUSIONS: Child-Pugh score at 3rd month, failure to control the index bleeding and HVPG at 1-3rd month of follow-up are independent prognostic indicators of rebleeding in poor hemodynamic responders to treatment of portal hypertension. These factors may improve the selection of non-responders that may benefit of rescue therapies.

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757 ADRENAI IMPAIRMENT IS FREQUENT FINDING IN STABLE CIRRHOSIS AND IS RELATED TO DISEASE SEVERITY

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Introduction Patients with cirrhosis and sepsis have recently been reported to have impaired responses to adrenal stimulation. This adrenal insufficiency has been correlated with liver disease severity and outcome. It is currently unknown whether this adrenal insufficiency is related to the critical illness or is an exacerbation of pre-existing adrenal insufficiency. We hypothesised that adrenal insufficiency may be prevalent in stable patients with cirrhosis. Methods: We evaluated patients with stable cirrhosis admitted to our unit for TIPS or transplant assessment. Cirrhosis was defined histologically or on imaging, clinical or laboratory findings. Adrenal function was tested using the 1µg or 250µg short synacthen tests (SST). Results 47 patients were studied. Median age 53 yrs (IQR 45-60). Disease severity was evaluated using the MELD score (median 17 IQR 13-21), MELD-Na (median 20 IQR 15-26), and Child Pugh score (CPS) (median 10, IQR 8-12). 57% of patients were Child-Pugh grade C, 32% grade B and 10% grade A. Baseline cortisol was <250µmol/L in 62% of patients. 60% showed an increment in cortisol less than 250 µmol/L and in 55% the peak cortisol level was less than 300 µmol/L after adrenal stimulation. Adrenal failure (AF) was defined as baseline cortisol <250µmol/L and increment <250µmol/L. AF was observed in 36 % of patients. There was no difference in the incidence of AF between patients who received the 1µg or 250µg synacthen tests, (p= 0.480) therefore the group was analysed as a whole. AF was observed more frequently in Childs-Pugh C patients versus Childs-Pugh B and A patients (p=0.047). Post stimulation cortisol levels were lower at all time points in Childs C compared to Childs A/B patients (p < 0.03). In univariate analysis albumin, INR and Bilirubin were significantly associated with AF. In multivariate logistic regression only INR was a significant predictor of AF (p=0.005). Conclusion Patients with stable cirrhosis show a high incidence of adrenal insufficiency when tested with SST. Abnormal SST responses are observed more frequently in patients with more severe liver disease. These data suggest that the high prevalence of adrenal insufficiency in septic cirrhotics may be an exacerbation of an underlying condition. Further work is needed to clarify the pathogenetic mechanisms involved and the prognostic significance of this finding.

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758 HEPATIC VENOUS PRESSURE GRADIENT PREDICTS THE FIRST CLINICAL DECOMPENSATION IN PATIENTS WITH CHRONIC HEPATITIS C-RELATED CIRRHOSIS

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BACKGROUND: Hepatic venous pressure gradient (HVPG) has a marked prognostic value in compensated patients with chronic hepatitis C (HCV)-related cirrhosis. However, it has not been tested in the compensated setting. AIM: To study the capacity of HVPG to predict the first clinical decompensation (CD) in patients with compensated HCV-related cirrhosis.

METHODS: All patients with compensated HCV-related cirrhosis who underwent a hepatic hemodynamic study (HHS) between 1/1997-12/2005 in our center were included. Clinical decompensation was defined as the appearance of ascites, variceal bleeding or encephalopathy. ROC curves were performed to select the best predictive value for CD of several predictors. Then, a multivariate Cox regression analysis was used to identify independent predictors of decompensation.

RESULTS: In the study period, 798 patients with HCV underwent HHS. Six hundred-twenty five patients were excluded due to: decompensated disease (259), previous liver transplantation (200), inadequate follow-up (59), chronic hepatitis without cirrhosis (55), previous TIPS (28) and other reasons (24). The remaining 173 patients with compensated disease (76% male; median age 55 [29-78] years; 74% genotype 1) were included in the study. They received HHS because of: transjugular liver biopsy (54), hepatocellular carcinoma (HCC, 50), evaluation for liver transplantation (23), before antiviral (20) or propranolol (15) therapy, and other reasons (11). Median follow-up was 25 [1-123] months. Fifty-seven (33%) patients developed CD during follow-up: 43 (75.5%) ascites, 10 (17.5%) variceal bleeding, and 4 (7%) hepatic encephalopathy. The probability of CD was 13%, 30% and 52% at 1, 3 and 5 years respectively. In univariate analysis age> 60 years, HVPG> 13 mmHg, MELD score> 9, albumin< 3.8 g/dL, previous antiviral therapy and existence of varices or HCC were significantly related to
CD. In multivariate analysis only age > 60 [HR 4.09 [2.29-7.32]; p=0.0001] and HVPG > 13 [HR 2.76 [1.50-5.12]; p=0.001] were independently associated to CD. HVPG value > 13 discriminated two populations with a different risk of CD: 19%, 31% and 65% Vs. 6%, 20% and 25% at 1, 3 and 5 years [HR 2.96 [1.65-5.31] p=0.0001]. A sensitivity analysis excluding those patients with HCC was performed. HVPG > 13 [HR 2.41 [1.02-5.67]; p=0.045], besides MELD score > 9 [HR 3.28 [1.35-8.01]; p=0.009] and previous antiviral therapy [HR 0.47 [0.24-0.93]; p=0.03], was retained in the model. CONCLUSION: HVPG independently predicts CD in patients with compensated HCV-related cirrhosis. Patients with a HVPG > 13 mmHg have a significantly higher risk of developing CD.

759 COMPARISON BETWEEN ENDOGENOUS THROMBIN POTENTIAL (ETP) AND INR TO ASSESS LIVER FUNCTION DECREASE IN PATIENTS WITH LIVER CIRRHOSIS

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Introduction: Advanced liver cirrhosis is characterized by a reduced synthesis of coagulation factors. Conventional coagulation tests (PT/INR and aPTT) poorly correlate with bleeding problems in patients with cirrhosis possibly because they do not adequately reflect the balance between procoagulant and anticoagulant factors. The INR was originally developed for use in patients under oral anticoagulation and may not adequately reflect the changes in the coagulation system in patients with cirrhosis of the liver, since it reflects only changes in Factor VII, X and II. Recently a test has become available which will make it possible to routinely measure the endogenous thrombin generation potential (ETP). Aims: To compare INR and ETP as predictors of synthetic and metabolic liver function (according to Child classification, serum-cholinesterase and 13C-aminopyrine breath test [ABT]). Patients: Patients with biopsy proven liver cirrhosis (n=112: 73 Child A, 21 Child B, 18 Child C) without known pre-existing coagulation abnormalities except liver disease, outside the setting of acute infections and without coagulation correction were prospectively included after informed consent. In these patients ETP, PT/INR and S-cholinesterase were measured. 13C Aminopyrine was performed in each patient. We used a fully automated assay for the determination of the endogenous thrombin potential (ETP) on the BCS® System (both Dade Behring, Marburg, Germany). Results: INR and ETP show good correlation with the Child score (P<0.05). There is no better correlation for ETP (R2:0.28) with S-cholinesterase than there is for INR (R2:0.10). ETP is a significant better predicting factor (P<0.05) for low S-cholinesterase in linear regression analyses than INR (p=0.53). ETP and INR show a good correlation with 13C-Aminopyrine breath test. A linear regression model ETP (β= 0.27, p<0.05) is the main predictive factor for decreasing thrombin generation. No such relationship can be identified for the INR (β= -0.16, p=0.083). Conclusion: Our results indicate that the integrity of the coagulation as measured through the thrombin generation decreases in line with deterioration of other liver functions in patients with liver cirrhosis. The ETP correlates significantly better with parameters of synthetic liver function compared to INR, currently being part of the MELD. ETP measurement is therefore a promising tool for assessing liver function impairment.

760 EARLY PRIMARY PROPHYLAXIS WITH BETA-BLOCKERS AND ROLE OF HEPATIC VENOUS PRESSURE GRADIENT ASSESSMENT IN PREVENTION OF GROWTH OF SMALL ESOPHAGEAL VARICES IN CIRRHOSIS

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Background: Efficacy of Beta-blockers (BB) and Efficacy of HVPG in early primary prophylaxis of variceal bleed in small varices is not clear. Methods: 85 cirrhotics with small varices (gr I or II) without previous bleeding were enrolled. Baseline HVPG was done. 43 randomized to receive propranolol [Gp A] (dose titrated to decrease resting HR to 55; median dose 120 mg/day, range 20-240) and 42 to placebo [Gp B]. Patients further randomized to either repeat HVPG after 1 yr or no repeat HVPG in both groups. Primary endpoints were progression of esophageal varices by 1 grade or hemodynamic response (≥20% reduction in HVPG) and secondary endpoints were bleeding and death. Endoscopy was done every 3 mo. Results: Both groups were comparable for baseline characteristics (table). Baseline Child score [Gp B=7.9, Gp A=7.6; p=0.45] and follow up score [Gp B=8.0, Gp A=7.6; p=0.40] were comparable. Baseline HVPG values were similar [Gp B=14.8, Gp A=15.1; p=0.77]. During a median follow up of 18 (range 3-60) mo, the rate of growth of varices did not differ between groups A & B (n=8 [19%] and n=9 [21%], P=0.9). Categorizing these patients on baseline HVPG value ≤10 and >10 mmHg, there were no differences in variceal progression (p=0.32), bleed (p=1.0) or mortality (p=1.0) in Gp B. Similarly in BB group variceal progression (p=0.32), bleed (p=0.40) or mortality (p=0.39) did not reach significance. In the placebo group, median HVPG change is 0 [range -1.4 to +1.1] and those in BB group median HVPG decreased by 3 mmHg [range -11 to +3] (p=0.07). Hemodynamic response achieved in 11/22 (50%) in Gp A and 5/17 (29%) in Gp B (p=0.32). Variceal progression in responders was similar to non responders (p=0.47). Outcome response by endoscopic method or by serial HVPG correlated closely and did not vary (p=ns) between groups A1 vs. A2 and B1 vs B2. Conclusions: Nonselective BB prophylaxis is ineffective in preventing growth of small esophageal varices, variceal bleeding, and mortality. While baseline HVPG helps in assessing the severity of portal hypertension, it did not reliably predict the growth or bleeding rate of small varices. Close endoscopic monitoring could reliably be used instead of serial HVPG measurements in these patients.

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SPLINE STIFFNESS MEASUREMENT BY MAGNETIC RESONANCE ELASTOGRAPHY IS AN INDEPENDENT PREDICTOR OF ESOPHAGEAL VARICES IN PATIENTS WITH COMPENSATED CIRRHOSIS

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Background: A strong relationship between spleen stiffness and portal pressure has recently been described. However, the relationship between spleen stiffness, liver stiffness, and esophageal varices remains unknown. Aim: 1) To identify the clinical factors associated with spleen stiffness in patients with chronic liver disease. 2) To determine the relationship between spleen and liver stiffness with esophageal varices. Methods: Individuals with chronic liver disease (n=38) and healthy volunteers (n=12) were studied. All MR elastography examinations used a 1.5 T whole-body imager. In the supine position, a 19 cm cylindrical passive pneumatic driver was placed against the anterior abdominal wall. Shear waves at 60 Hz were generated by the driver from an active speaker. Axial wave images of liver and spleen were obtained by GRE-based sequences. Quantitative shear stiffness images were obtained by LFE inversion algorithms. Results: Mean age was 56 yrs with 50 % women for liver disease patients. Average BMI was 29.7 kg/m2 (range, 18-42). Major diseases were hepatitis C (20%), NASH (20%), alcohol (11%), AIH (11%), and PBC (11%). Fibrosis stage was 0I in 36%, I-II in 18%, and IV in 45% of cases. Average MELD score was 6 (range, 6-8). Mean serum platelet count was 193,000/mm3 (range, 36,000-358,000). Prevalence of splenomegaly was 34%. Esophageal varices were identified in 9 of 38 (24%) patients (grade 1, 56%; grade 2, 22%; grade 3, 22%). Spleen elastograms were obtained in all subjects. Mean spleen stiffness was higher in patients compared to healthy volunteers (8.0 kPa vs 2.2 kPa, p<0.0001). Univariate analysis identified splenomegaly, liver stiffness, and platelet count as significant factors (p<0.01 for all) associated with spleen stiffness. Age, sex, BMI, and disease etiology did not influence spleen stiffness. Patients with esophageal varices had significantly higher median liver stiffness (7.5 kPa vs 4.1 kPa, p=0.009) and median spleen stiffness (11.7 kPa vs. 6.25 kPa, p=0.001) values compared to unaffected patients. Both spleen stiffness (p=0.005) and splenomegaly (p=0.001) were independent predictors of esophageal varices adjusted for platelet count and liver stiffness value. Conclusions: 1) Spleen stiffness, platelet count and liver stiffness value were associated with spleen stiffness. 2) Patients with esophageal varices had significantly higher median liver and spleen stiffness values compared to unaffected patients. 3) From this preliminary study, the spleen stiffness value and presence of splenomegaly were independent predictors of esophageal varices regardless of platelet count and liver stiffness value.

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LEUKOPENIA AND THROMBOCYTOPENIA PREDICT A POOR PROGNOSIS IN COMPENSATED CIRRHOSIS

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Background: Abnormal hematological indices (HI) occur in cirrhosis due to multiple factors including hypersplenism. A correlation between serum platelet count (PLT) and hepatic venous pressure gradient (HVPG) has been shown [Hepatology 2006; 44: A721]. The aim of this study was to determine if hemoglobin (Hgb) and white blood cell count (WBC) correlate with HVPG and whether abnormal HI have prognostic significance. Methods: Secondary analysis was conducted of a database of subjects with compensated cirrhosis without esophageal varices enrolled in a randomized, placebo controlled trial of beta-blockers in the prevention of varices. (NEJM 2005; 353:2254). Subjects were followed until the development of varices or variceal bleeding or completion of the study. 84 subjects developed varices. Patients were followed until 9/02. Baseline HVPG measurements, PLT, WBC and Hgb were analyzed. Abnormal HI was defined as the baseline occurrence of anemia (Hgb < 13.5 g/dl for men and 11.5 g/dl for women), leukopenia (WBC ≤ 4,000/ mm3) or thrombocytopenia (PLT ≤ 150,000/ mm3). HI were categorized into 4 groups (Table). The main event was death or transplant. Statistical analysis: ANOVA, Spearman correlation, Kaplan-Meier with log rank test and Cox proportional regression. Results: There was significant correlation of HVPG with both Hgb and WBC (Hgb: r = 0.35, p<0.0001, WBC: r = -0.31, p<0.0001). The Table shows median baseline HVPG (mm Hg) among different HI groups. A significant difference (p<0.0001) in death or transplant between patients who had thrombocytopenia and leukopenia compared to patients with normal HI was observed over a median follow-up of 4.2 years. Similarly, death only was more frequent in subjects with thrombocytopenia and leukopenia compared to normal HI group (p = 0.0128). The differences in death or transplant among the HI groups remained significant when adjusted for HVPG and Child-Pugh score (p=0.0048). Summary: There is a significant correlation between HVPG and WBC or Hgb. Occurrence of abnormal HI at baseline is associated with a markedly elevated HVPG and poor prognosis. Conclusion: Portal hypertension contributes to HI abnormalities in cirrhosis. The occurrence of leukopenia and thrombocytopenia in patients with compensated cirrhosis is a poor prognostic indicator.
Liver failure represents a major cause of death in alcoholic steatohepatitis (ASH). The extent to which the liver can regenerate is a key point in the outcome of liver diseases. Aim: To study the short-term effects of GCSF on mobilization of CD34+ hematopoietic stem cells, hepatocyte lineage proliferation, and biological parameters in patients with ASH and variable degree of liver failure. Patients and Methods: 24 consecutive patients with biopsy-proven ASH (age 54 yrs [34-69]; Maddrey’s score 36 [21-60]; Pugh’s score 10 [7-12]) were randomized to standard care alone (Group A: n=11; 7 received steroids) or associated to a 5-day mobilization course with GCSF 10 mcg/kg sc (Group B: n=13; 5 received steroids). The number of hematopoietic progenitor cells (HPC) and mature hepatocytes which entered into a proliferative phase were identified and manually counted on the entire biopsy specimen at baseline and repeat liver biopsy at day 7, using a double immunostaining method MIB1/CK7 and MIB1/CK18, respectively. Circulating CD34+ cells (flow cytometry) and cytokines involved in regeneration (HGF) and inflammation (IL-6), and liver function tests were also measured. Results: Groups were similar at baseline. Tolerance to GCSF was excellent. Table presented for comparison. Percent Death or Transplant (n=) 6 (2) 8 (1) 24 (28) 49 (23) Percent Death (n=) 6 (2) 8 (1) 18 (21) 28 (13)

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an alterations of the self-reactive antibody repertoire. CON-CLUSION: Acquired auto-immune PS-deficiency is probably central to the cause of NRH in HIV-infected patients, resulting in thrombotic venopathy, NRH, portal hypertension and sometimes to liver failure.

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SURVIVAL BENEFIT IN PATIENTS WITH ACUTE VARICEAL BLEEDING AND HEMODYNAMIC REPERCUSSIONS FOLLOWING EARLY ADMINISTRATION OF VAPRETIDE
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Purpose: Bleeding from esophageal varices is a life-threatening complication of portal hypertension in patients with cirrhosis. A subset of these patients experience hemodynamic repercussions, which increase the risk of organ failure and mortality. In the first major clinical trial conducted to evaluate the effectiveness of the somatostatin analog vapreotide administered early in combination with endoscopy in patients with cirrhosis and acute esophageal bleeding, treatment with vapreotide and endoscopic therapy improved bleeding control and 15-day mortality versus endoscopic therapy alone, but did not significantly affect 42-day mortality. The objective of this post hoc analysis was to determine if vapreotide treatment prior to endoscopy increased survival in a subset of patients with active variceal bleeding accompanied by hemodynamic repercussions. Methods: Consecutive patients aged 18 to 75 years diagnosed with cirrhosis, acute variceal bleeding, and hemodynamic repercussions between July 1997 and December 1998 were identified. Hemodynamic repercussions were defined according to the protocol-specified criteria for uncontrolled bleeding derived from the Baveno II criteria (ie, heart rate >100 beats per minute or systolic blood pressure <80 mmHg). Patients were administered an intravenous bolus of 50 µg followed by a continuous infusion of vapreotide 50 µg per hour, or placebo, for 5 days. Therapeutic endoscopy was conducted within 12 hours of initiation of infusion, and patients were followed for 42 days. Results: In the intent-to-treat population (N=196), 103 patients had hemodynamic repercussions upon hospitalization. Baseline demographic characteristics were similar among the vapreotide (n=45) and placebo (n=58) treatment groups. Vapreotide significantly reduced the number of patient deaths versus placebo at days 15 (7% vs 24%, respectively; P=0.02) and 42 (7% vs 31%, respectively; P=0.002). Vapreotide treatment also significantly decreased active bleeding at time of endoscopy (29%) versus placebo (50%; P=0.03). Vapreotide-treated patients required significantly fewer alternative interventions at day 42 (16%) versus patients receiving placebo (41%; P=0.005). Conclusions: Early administration of vapreotide significantly reduced active bleeding and mortality in patients experiencing acute variceal bleeding accompanied by hemodynamic repercussions. These findings identify a subgroup of cirrhosis patients with an increased risk of mortality that may benefit substantially from early vapreotide treatment combined with endoscopic therapy.

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NODULAR REGENERATIVE HYPERPLASIA: A CLINICO-PATHOLOGICAL EVALUATION

Background: Noncirrhotic portal hypertension may be secondary to several under-recognized disorders of the liver, with
potentially fatal complications. Nodular regenerative hyperplasia (NRH) is one such disorder that can result in noncirrhotic portal hypertension. Portal and hepatic vascular abnormalities have been postulated as a potential underlying etiology of NRH. Aim: To describe the histological characteristics and evaluate for the presence of nodular regenerative hyperplasia liver biopsies of patients with chronic granulomatous disease, sickle cell disease, systemic mastocytosis, and common variable immunodeficiency; all systemic diseases that have been reported to occur in conjunction with NRH. Methods: The liver biopsies of 180 patients enrolled in natural history protocols including chronic granulomatous disease, sickle cell disease, systemic mastocytosis, and common variable immunodeficiency at the National Institutes of Health were reviewed and evaluated for adequacy of tissue. H&E, Masson, Reticulin and anti-cytokeratin 7 (CK7) stains were evaluated. Biopsies were scored for the presence of fibrosis, regeneration, portal venopathy, hemorrhage, pigmented macrophages, sinusoidal dilatation, sinusoidal fibrosis, veno-occlusive changes, ductular proliferation, and CK7 expression in zone 3 hepatocytes. Associations between these scores were performed by disease and by histological findings using the chi squared test and Fisher’s test. Results: 129 biopsies were evaluated. Nodular regenerative hyperplasia was present in 13.6%, 16.7%, and 16.7% of cases of chronic granulomatous disease, sickle cell disease, and systemic mastocytosis, respectively. There was a statistically significant association between the presence of NRH and sinusoidal fibrosis (p<0.0001), NRH and CK7 ductular proliferation (p=0.05), and NRH and CK7 zone 3 hepatocyte expression (p<0.0001), but not with hemorrhage (p=0.172). There was also a significant association between NRH and portal venopathy (p=0.018) consistent with a currently accepted theory of obliterative portal venopathy as a potential underlying mechanism in the pathogenesis of NRH. Veno-occlusive changes were correlated with hemorrhage (p=0.002) and central CK7 expression (p<0.034), but not NRH (p=0.167). Conclusion: NRH appears to be prevalent in patients with systemic disorders which might affect the hepatic microcirculation. Significant histologic associations are apparent on systematic evaluation of liver biopsy specimens. In particular, NRH is correlated with vascular damage and evidence of regeneration. Current evaluation will correlate laboratory values, imaging characteristics, and histology.

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THE NEAR-INFRARED SPECTROSCOPY (NIRS) IS USEFUL IN THE DIAGNOSIS OF MINIMAL AND OVERT HEPATIC ENCEPHALOPATHY

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Background: Although some patients with chronic liver disease exert subclinical level of hepatic encephalopathy (HE), there is no established diagnostic tool for this minimal HE. Recently in psychiatric disease, near-infrared spectroscopy (NIRS) has been utilized for the evaluation of the brain functions. NIRS has advantage of noninvasive and bed side measurements of regional cerebral blood volume in terms of the relative concentrations of oxyhemoglobin (oxy Hb) and deoxyhemoglobin (deoxy Hb), with a high time resolution. The aim of the present study was to evaluate the usefulness of NIRS in the diagnosis of minimal hepatic encephalopathy in patients with liver cirrhosis. Methods: Fortyfive patients with chronic liver disease and 12 healthy control subjects were enrolled in the study after giving written consent. Sixteen patients had overt HE. Seven patients who had chronic liver disease and abnormality in electric encephalography (EEG) and did not have overt HE were defined as minimal HE. The relative concentrations of oxy Hb were measured every 0.1 sec during word fluency task, with 52 channel NIRS machine. We analyzed the channels 36-38 and 46-49 that were located in forehead which has been shown to be specific for mental disorder. Results: The overt HE patients were characterized by a smaller and slower oxy Hb increase during the task as compared to that of healthy control subjects. The average peak value of oxy Hb was 0.24±0.14 in overt HE patients compared to 0.68±0.41 in subjects without HE (P<0.001). The average peak value was significantly small in minimal or overt HE. The increase of oxy Hb concentration were also smaller and slower during word fluency task in 18 patients with abnormal EEG were smaller than that of 31 subjects with normal EEG (P=0.02). The patients with minimal or overt HE showed smaller increasing of oxyhemoglobin concentration than that of subjects without HE. The average of respective peak values of channel 36-38 and 46-49 were 0.45±0.35 in minimal or overt HE patients and 0.24±0.13 in subjects without HE (P=0.01). These results indicate that NIRS showed small increase in cerebral blood flow in response to a stimulation imposed in patients with minimal HE, as well as in overt HE. Conclusions: The patients of minimal HE has the characteristic time course of cerebral blood flow in the frontal lobe during the task as measured by NIRS. Simple and noninvasive measurement of cerebral blood flow by NIRS could be a new diagnostic modality for HE.

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GROWTH HORMONE-STIMULATED IGF-1 GENERATION IN CIRRHOSIS REFLECTS HEPATOCELLULAR DYSFUNCTION

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Background: Previous studies have demonstrated that serum IGF-1 generation correlate with the extent of hepatic dysfunction. Serum IGF-1 generation correlate may be an influential factor in determine whether basal and/or growth hormone (GH) stimulated IGF-1 levels are decreased in patients with cirrhosis. Aim: To determine whether basal and/or growth hormone (GH) stimulated IGF-1 generation correlate with the extent of hepatic dysfunction. Methods: 53 patients (56±2 yrs) with post viral and cryptogenic cirrhosis and normal renal function were enrolled in the study. Serum IGF-1 levels were measured byRIA before and 24 hours after a single sc injection of GH (0.06 mg/kg/body
SECONDARY PARENCHYMAL DAMAGES IN CIRRHOTIC LIVERS

Cirrhosis is associated with low serum IGF-1 levels and an attenuated response to exogenous GH. These findings correlate with the extent of hepatic dysfunction rather than the presence of portal hypertension or malnutrition.

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SECONDARY PARENCHYMAL DAMAGES IN CIRRHOTIC LIVERS ASSOCIATED WITH ACTIVATED PLATELET AGGREGATION IN SINUSOIDS

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Background and Aims: Circulatory disturbances occurring in cirrhotic livers have been believed to cause further hepatic damage. Previous histological studies have shown morphological evidence of parenchymal extinction associated with hepatic thrombosis. However, no study has shown potential contribution of thrombus formation/activated platelet aggregation to parenchymal extinction in cirrhotic livers. On the other hand, thrombocytopenia is one of the common systemic complications of liver cirrhosis. It has been explained by increased platelet destruction due to hypersplenism and by reduced thrombopoietin production in diseased livers. We have hypothesized that consumption of platelets related to parenchymal extinction may also contribute to thrombocytopenia in cirrhotics. To test this hypothesis, we performed the following immunohistochemical investigation.

Methods: Frozen liver samples of chronic hepatitis (n=4), cirrhosis (n=7) and normal livers (n=5) obtained at autopsy were thin-sliced and stained with immunoperoxidase using specific antibodies against platelet glycoprotein IIb/IIIa (GP IIb/IIIa) and P-selectin (both from Dako Corp., Carpinteria, CA) and examined under standard microscopy. The sections were then subjected to immunofluorescent staining with anti-GP IIb/IIIa and anti-P-selectin. Double positive areas, indicating the aggregation of activated platelets, were measured by computer-aided morphometry (NIH image, Bethesda, MD). The data were expressed as % tissue area and analyzed by non-parametric tests [Mann-Whitney U-test and Spearman rank correlation (Rs)].

Results: In normal livers, activated platelet aggregation, namely platelet thrombus, in sinusoids was rarely seen, and there was no parenchymal damage associated with sinusoidal thrombosis. In contrast, cirrhotic livers had many foci of activated platelet aggregation, which was frequently accompanied by patchy liver cell necrosis/degeneration. Areas of activated platelet aggregation were significantly (p<0.028) greater in cirrhotic livers (0.36±0.13%) than in normal livers (0.21±0.06%). Furthermore, the areas of activated platelet aggregation were correlated to peripheral platelet counts (R=0.54, p<0.037).

Conclusion: These findings suggest that secondary parenchymal damage in cirrhotic livers is closely associated with sinusoidal thrombosis characterized by activated platelet aggregation in sinusoids, and that increased platelet consumption in diseased livers is associated with thrombocytopenia in patients with cirrhosis. The results also indicate that cautious consideration be given to the possible role of platelet inhibition in early cirrhosis.

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CIRRHOSIS represents a serious and increasing burden of morbidity in the general population of the UK.

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CLOSTRIDIUM DIFFICILE INFECTION IS ASSOCIATED WITH PROTON PUMP INHIBITORS IN PATIENTS WITH CIRRHOSIS

Jasmohan S. Bajaj, Yelena Zadvornova, Muhammad Hafeezullah, Kia Saedian; Gastroenterology and Hepatology, Medical College of Wisconsin, Milwaukee, WI

Clostridium difficile-associated disease (CDAD) is a significant burden on the healthcare system. Proton pump inhibitor therapy (PPI) is associated with increased CDAD rates. Cirrhotic patients have a compromised immune status and are often prescribed PPI due to their propensity to develop upper GI symptoms and bleeding. The aim of this study was to describe whether PPI therapy increases the rate of CDAD in cirrhotics using a case-control method. Method: 985 cirrhotic inpatient charts were evaluated. PPI therapy was defined as PPI use as an outpatient prior to the admission. Antibiotic use was defined as clear evidence of antibiotic use before CDAD diagnosis either during or 7 days prior to admission. Charts without clear medication lists and suspected but not confirmed CDAD were excluded. 250 patients had suspected but only 54 had confirmed CDAD by toxin assay. These 54 were matched to 108 cirrhotics without CDAD admitted during the same time period by age. Demographics, antibiotic use, Child (CTP score) and PPI use were compared between groups and logistic regression with CDAD as the outcome was performed. Results: Both groups were similar with respect to demographic factors and reasons for admission. Antibiotic and PPI use were significantly higher in the CDAD group (Table). 85% of CDAD patients developed diarrhea during admission; the remaining had diarrhea on admission. The reasons for admission were mostly liver-related, but only <20% of admissions in both groups were because of spontaneous bacterial peritonitis. Pneumonia and suspected sepsis were the leading reasons (42% CDAD/36% other group) followed by variceal bleeding prophylaxis for antibiotic use. On univariate logistic regression with CDAD as the outcome, only antibiotic use (p<0.0001 OR: 6.7 CI: 3.2-14) and PPI use (p<0.0001 OR: 8; CI: 3.4-19) were significant predictors. On multivariate logistic regression PPI use (p<0.0001 OR: 6.1 CI: 3-14) and antibiotic use (p<0.0001, OR 7.8, CI: 3.2-19) were again significantly associated with CDAD. Conclusions: PPI use and, as would be expected, antibiotic use are independently associated with a higher risk of CDAD in cirrhotic patients. Trials to prospectively study this issue are warranted in the light of increasing rate of PPI therapy and CDAD.

<table>
<thead>
<tr>
<th>Age</th>
<th>CDAD (n=54)</th>
<th>no CDAD (n=108)</th>
<th>p value</th>
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<tr>
<td>54±10</td>
<td>55±10</td>
<td>0.7</td>
<td></td>
</tr>
<tr>
<td>CTP (CI)</td>
<td>54%</td>
<td>46%</td>
<td>0.6</td>
</tr>
<tr>
<td>Ethnicity (% white)</td>
<td>83%</td>
<td>78%</td>
<td>0.6</td>
</tr>
<tr>
<td>Reason for admission liver-related (%)</td>
<td>64%</td>
<td>52%</td>
<td>0.7</td>
</tr>
<tr>
<td>Antibiotic Rx</td>
<td>54 (63%)</td>
<td>28 (26%)</td>
<td>0.0001</td>
</tr>
<tr>
<td>SBP prophylaxis</td>
<td>6 (11%)</td>
<td>11 (10%)</td>
<td>0.8</td>
</tr>
<tr>
<td>Ox PPI</td>
<td>40 (74%)</td>
<td>38 (35%)</td>
<td>0.0001</td>
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</tbody>
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WHAT IS THE BEST NON INVASIVE METHOD FOR EARLY PREDICTION OF CIRRHOSIS IN CHRONIC HEPATITIS C? PROSPECTIVE COMPARISON BETWEEN FIBROSCAN AND SERUM MARKERS (LOK INDEX, APRI, AST/ALT RATIO, PLATELET COUNT AND FIBROTEST)

Laurent Castera1,2, Pierre-Henri Bernard2, Brigitte Le Bail3, Juliette Foucher1,2, Wassil Merrouche1, Patrice Couzigou1, Victor de Ledinghen1, 1Hepatology, Hospital Haut Leveque, CHU Bordeaux, Pessac, France; 2Hepatology, Hospital St Andre, CHU Bordeaux, Bordeaux, France; 3Pathology, Hospital Pellegrin, CHU Bordeaux, Bordeaux, France

Background: Several non invasive methods have been proposed for the diagnosis of cirrhosis in patient with hepatitis C. They include routinely available markers (platelet count, AST/ALT ratio (AAR), AST to Platelet ratio (APRI), scores (Fibrotest (FT), LOK index (platelet count, AST/ALT ratio, and INR), and lately transient elastography (Fibroscan (FS)). Aim: to prospectively compare the performance of these different methods for the early prediction of cirrhosis in the same population of patients with chronic hepatitis C. Methods: 305 consecutive HCV patients (males 57%, mean age: 52±12) who undergone a liver biopsy (>10 mm) were studied. All patients had FS, FT, APRI, LOK index, AAR and platelet count in the same lab the day of liver biopsy, taken as reference. Cut-offs used for each test were those defined in the original studies. Performances were compared in intention to treat analysis (FS failure was considered as need for liver biopsy). Results: Significant fibrosis (Metavir F2-F3-F4) was present in 228 (75%) and cirrhosis (F4) in 77 (25%) (Child-Pugh A 90%; B 10%, oesophageal varices 39%). The mean liver biopsy length was: 19±8 mm. FS failure was observed in 10 patients (3%). Performances of the different methods are shown in the table. 32 out of 77 (42%) cirrhotic patients had no clinical or biochemical or US signs suggestive of cirrhosis. Among these 32 patients, cirrhosis could have been detected in 5 (16%) using the LOK index, in 5 (16%) using the AAR, in 8 (25%) using the APRI, in 14 (44%) using FT, and in 22 (70%) using FS, respectively. In addition, at a cut-off of 21 kPa, the performance of FS for detecting oesophageal varices was: Se 79%; Sp 72%, PPV 65%, and NPV 84%. Conclusions: Fibroscan is the best non invasive method for prediction of cirrhosis in chronic hepatitis C, as compared with other available methods, saving the need for liver biopsy in 90% of cases. In patients without any signs of cirrhosis, early diagnosis can be made in 70% of cases.

<table>
<thead>
<tr>
<th>Cutoff</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>PPV (%)</th>
<th>NPV (%)</th>
<th>AUROC</th>
<th>Saved biopsies (%)</th>
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<tbody>
<tr>
<td>&gt;12.5 kPa</td>
<td>&gt;1.6</td>
<td>&gt;0.2</td>
<td>&gt;0.5</td>
<td>&lt;1.0</td>
<td>&gt;2.0</td>
<td>&gt;1</td>
</tr>
<tr>
<td>&gt;15</td>
<td>85</td>
<td>74</td>
<td>87</td>
<td>46</td>
<td>67</td>
<td>36</td>
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<tr>
<td>&gt;18</td>
<td>95</td>
<td>98</td>
<td>94</td>
<td>91</td>
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<td>88</td>
</tr>
<tr>
<td>&gt;21</td>
<td>96</td>
<td>92</td>
<td>93</td>
<td>55</td>
<td>68</td>
<td>54</td>
</tr>
<tr>
<td>&gt;27</td>
<td>95</td>
<td>92</td>
<td>94</td>
<td>88</td>
<td>84</td>
<td>86</td>
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<td>&gt;30</td>
<td>90</td>
<td>89</td>
<td>94</td>
<td>79</td>
<td>76</td>
<td>82</td>
</tr>
<tr>
<td>&gt;37.5</td>
<td></td>
<td></td>
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TABLE 1

<table>
<thead>
<tr>
<th>Parameter</th>
<th>With PHG (n=140)</th>
<th>Without PHG (n=114)</th>
<th>P value</th>
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<tr>
<td>HVPG (mmHg)</td>
<td>17.8 (±4.8)</td>
<td>15.3 (±5.5)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Mean arterial pressure (mmHg)</td>
<td>10.7 (±2.3)</td>
<td>9.0 (±2.3)</td>
<td>0.66</td>
</tr>
<tr>
<td>Pulmonary capillary wedge pressure (mmHg)</td>
<td>9.0 (±2.2)</td>
<td>8.2 (±2.5)</td>
<td>0.20</td>
</tr>
<tr>
<td>Cardiac index (L/min/m²)</td>
<td>5.3 (±3.5)</td>
<td>6.6 (±1.9)</td>
<td>0.01</td>
</tr>
<tr>
<td>Cardiac output (L/min)</td>
<td>8.9 (±3.0)</td>
<td>7.0 (±2.3)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Systemic vascular resistance (dyn.s/cm⁵)</td>
<td>929 (±449)</td>
<td>1073 (±485)</td>
<td>0.02</td>
</tr>
<tr>
<td>Pulmonary vascular resistance (dyn.s/cm⁵)</td>
<td>58 (±23)</td>
<td>83 (±24)</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

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LIMITED EFFICACY OF PROPRANOLOL ALONE OR IN COMBINATION WITH ISOSORBIDE-5-MONONITRATE FOR PRIMARY PROPHYLAXIS OF VARICEAL BLEEDING: A STEP-WISE HEMODYNAMIC EVALUATION

Praveen Sharma, Ashish Kumar, Smruti R. Mishra, Sanjeev K. Jha, Barjesh C. Sharma, Shiv Kumar Sarin; Gastroenterology, G B Pant Hospital, New Delhi, India

Introduction: Response to propranolol is seen only in a proportion of patients receiving beta-blockers (BB) for primary prophylaxis of variceal bleeding. Addition of isosorbide-5-mononitrate (ISMN) enhances the portal pressure reducing effects of propranolol. The percentage efficacy of BB alone or the combination has not been well studied in large clinical studies. Aims: To evaluate (i) the efficacy of propranolol alone in reduction of hepatic venous pressure gradient (HVPG) and prevention of first bleed, and (ii) whether addition of ISMN reduces the non-response to BB. Patients and Methods: Consecutive cirrhotic patients with large (>5 mm) varices, who had not bled in the past, were enrolled. HVPG was measured at baseline and 4 wk after BB administration when target heart rate of 55/min was achieved. Response was defined if HVPG decreased to <12 mm Hg or ≤20% compared with basal value. ISMN was added to a maximum of 40mg/d in non-responders and HVPG measurement was repeated after 4 weeks. Side-effects were recorded and patients followed for a mean of 24 mo. Results: Fifty one cirrhotics (age 47.5±13.7 yr, M:F 41:10, Child-Pugh A:B:C 14:27:10, etiology: hepatitis B [19], hepatitis C [13], alcohol [11], cryptogenic [7] and autoimmune [1]) were enrolled. Baseline HVPG was 18.1±5mmHg. The mean BB dose was 124±49 mg/d. On intention to treat analysis, seventeen (33%) patients responded with mean reduction in HVPG of 30±8%. With ISMN in a mean dose of 32±8mg, six (12%) additional patients responded, with HVPG reduction of 35±14% from baseline. One (4%) of 23 responders and 4 (14%) of 28 non-responders bled during follow up of 12 mo. (p<0.05). Ten (20%) patients developed severe side-effects to BB requiring dose reduction (7 [21%] non responders and 3 [18%] responders [p=ns]). Six patients (18%) developed side-effects to nitrates. We did not find any correlation of response to etiology (p=0.08) or Child’s status (p=0.46). Conclusions: Propranolol alone achieves hemodynamic response in 33% and in combination with nitrates in additional 12% of patients. 55% patients remain non-responsive to current pharmacotherapy and such patients bleed significantly more than responders. A step-wise approach involving serial HVPG measurements is necessary to identify this subset of patients by 8 weeks as alternative treatments need to be administered in them. 

Disclosures:
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CIRRHOTICS ADMITTED TO INTENSIVE CARE UNIT: THE IMPACT OF ACUTE RENAL FAILURE ON MORTALITY

Evangelos Cholongitas1, Marco Senzolo1, David Patch1, Steve Shaw1, James O’Beirne1, Andrew K. Burroughs1

Background: Although renal dysfunction is a well known risk factor of mortality in patients with cirrhosis, its exact role in critically ill cirrhotics admitted to an Intensive Care Unit (ICU), has not been assessed extensively. Aim: To evaluate the impact of acute renal failure (ARF) on 6 weeks mortality in cirrhotics admitted to ICU. Patients/methods: 312 cirrhotics (182 M, mean age 49.6±11.5yrs) were consecutively admitted during the study period. The patients (n=128, 40%) (group 1) with ARF on admission and/or during ICU were compared with the patients whose ICU-stay was not complicated with ARF (n=184, 60%) (group 2). At admission, 40 variables, including demographic, clinical and laboratory data, were available. Child-Pugh (CP), MELD, Apache II, SOFA and OSF scores on admission, were evaluated and compared by ROC curves. Results: Group 1, compared to group 2 patients, had longer period of stay in ICU (5 vs 4 days, p=0.04), were admitted less frequently for upper gastrointestinal bleeding (44% vs 73%) and required cardiovascular support more frequently with inotropes (90% vs 75%), (p<0.001). Mortality was significantly higher in group 1, compared to group 2 (91% vs 47%, p<0.001). At admission, group 1, compared to group 2, had significantly higher CP (12 vs 11), MELD (31 vs 21), SOFA (13 vs 9) and OSF (2 vs 1.5) scores (p<0.001). In the total cohort of patients (n=312), ARF was an independent factor of mortality (OR=5.4, 95 CI:2.4-12.5). In group 1, factors independently associated with mortality were: higher FiO2 (p=0.044), bilirubin (p=0.021) and creatinine (p=0.002) on admission. Predictive value of outcome in terms of ROC was best for SOFA score (AUC>0.75). Amongst group 1 patients, those with ARF on admission, compared to those who developed ARF during ICU stay, needed ventilatory (p=0.04) and haemofiltration (p=0.02) support more frequently and they had higher bilirubin (p=0.002) and prognostic scores (p<0.001) on admission. Conclusions: ARF at admission or during ICU stay is strongly predictive of mortality, which is high, despite supportive therapeutic interventions. Preventive measures are needed to prevent ARF, to improve prognosis.

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The following people have nothing to disclose: Evangelos Cholongitas, Marco Senzolo, David Patch, Steve Shaw, James O’Beirne, Andrew K. Burroughs

EVALUATION OF HEPATIC BLOOD FLOW BEFORE AND AFTER TREATMENT FOR ESOPHAGOGASTRIC VARICES BY XENON COMPUTED TOMOGRAPHY - MEASUREMENT OF QUANTITATIVE PORTAL VENOUS AND HEPATIC ARTERIAL TISSUE BLOOD FLOW

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Background: Xenon computed tomography (Xe-CT) provides substantial quantitave and visual information on hepatic blood flow (HBF), allowing separate evaluation of hepatic arterial and portal blood flow. We have previously reported that it was useful to measure HBF by Xe-CT in patients with chronic hepatitis C and nonalcoholic steatohepatitis, and noted significant decreases in portal blood flow with increasing hepatic fibrosis (Suzuki et al, 2003AASLD #545, Kobayashi et al, 2006AASLD #1064). Arterial blood flow increases in order to compensate for decreased portal blood flow in patients with liver cirrhosis. In the present study, we thus performed Xe-CT in patients with
esophagogastric varices before and after treatment, and evaluated HBF. Patients: Subjects comprised 36 patients with esophagogastric varices (26 men, 10 women, median patient age was 61 ± 11 years, range 37-77). Etiology was as follows: hepatitis B virus (B), n=1; hepatitis C virus (C), n=13; alcohol (Al), n=12, C+Al, n=5; B+C+Al, n=1; unknown, n=4. All patients received treatment with endoscopic injection sclerotherapy (n=32) or balloon-occluded retrograde transvenous obliteration (n=4). Method: The imaging devices, protocol and processing were the same as reported previously. Using the CT images obtained, portal venous tissue blood flow (PVTBF) and hepatic arterial tissue blood flow (HATBF) were separately calculated (ml/100ml/min) pixel by pixel. Total hepatic tissue blood flow (THBF) was obtained by adding PVTBF to HATBF. Xe-CT was performed before and after treatment. In addition, we measured indocyanine green retention rate after 15 minutes (ICG15) (%), total bile acids (nmol/ml) (TBA), ammonia (µg/dl) (NH3), prothrombin time (%)(PT), albumin (g/dl) (Alb) and total cholesterol (mg/dl) (TC) before and after treatment. Results: PVTBF, HATBF and THBF before treatment were 32.3 ± 14.0, 23.4 ± 13.4 and 55.7 ± 20.7, respectively. Those after treatment were 39.5 ± 13.7, 17.4 ± 7.1, and 56.9 ± 17.0, respectively. PVTBF after treatment was significantly higher than before treatment (p<0.001), and HATBF after treatment were significantly lower than those before treatment (p=0.037). ICG15R, TBA and NH3 after treatment were significantly lower than those before treatment (p=0.026, p=0.010, p=0.014, respectively). Although TC, Alb and PT after treatment was significantly lower than those before treatment, they immediately recovered similar values as before treatment. Conclusion: In evaluation of HBF using Xe-CT, we confirmed that HATBF decreased in response to increase of PVTBF after treatment for esophagogastric varices. Increase of PVTBF might lead ICG15R, TBA and NH3 to the improvement.

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781 PROSPECTIVE ASSESSMENT OF RENAL HISTOPATHOLOGICAL LESIONS PRIOR TO LIVER TRANSPLANTATION

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Terminal renal failure occurs in more than 10 % of liver transplant recipients after 10 years. We have previously shown that, beside calcineurin inhibitor nephrotoxicity, substantial renal lesions may be related to diabetes, arterial hypertension, accumulation of hydroxysterolstarch (HES), and the etiology of the liver disease. We made the hypothesis that these lesions may be already present at the time of liver transplantation (LT), a finding that could lead to adapt the perioperative management. This work investigated prospectively whether renal histopathological lesions were present before LT by performing a renal biopsy by endovenous route in 60 candidates to LT with end-stage liver disease. These patients were 58 ± 10 years old; 10 had a diabetes, and 21 an arterial hypertension; the liver disease was related to alcohol in 32 cases, HCV and HBV in 12 and 5 cases, and to a cholestatic disease in 7 cases. The biochemical parameters were : Child score 10 ± 2; MELD score 18 ± 4; prothrombin rate 60 ± 10 %; creatinin serum level 90 ± 6 umol/L; proteinuria 0.12 ± 0.04 g/24h. Severe side effects related to the procedure were limited to 2 cases of macroscopic hematuria, lasting less than 24 hours. In 10 cases, the material obtained during the procedure did not allow histological analysis. Among the 50 samples available, 21 were considered as normal or without significant lesions; in 29 cases, substantial lesions, mainly related to IgA glomerulonephritis (11 cases), glomerulosclerosis (12 cases), and HES accumulation (4 cases), were often combined; in 5 cases, the lesions were severe, leading to combined kidney/liver transplantation in 2 cases. The presence of histological lesions was associated with worse renal function, proteinuria, and more severe liver condition (Table). However, no clear threshold could be found between patients with and without significant lesions. In conclusion, significant renal lesions are detectable in more than 50 % of the candidates to LT. Histological findings often combined lesions related to the liver disease and to an associated cause (diabetes, previous treatment by HES). Significant proteinuria or renal failure should indicate a renal biopsy. Results of histological analysis could help to decide either to perform a combined renal/liver transplantation or to adapt the immunosuppressive regimen.

<table>
<thead>
<tr>
<th></th>
<th>No-lesion</th>
<th>Minimal/moderate lesions</th>
<th>Severe lesions</th>
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<tbody>
<tr>
<td>N</td>
<td>21</td>
<td>24</td>
<td>5</td>
</tr>
<tr>
<td>Creatinin serum level (umol/L)</td>
<td>71 ± 3</td>
<td>93 ± 5</td>
<td>166 ± 44</td>
</tr>
<tr>
<td>Creatinin clearance (ml/min)(Cockcroft)</td>
<td>110 ± 9</td>
<td>98 ± 5</td>
<td>51 ± 13</td>
</tr>
<tr>
<td>Proteinuria (g/24h)</td>
<td>0.044 ± 0.011</td>
<td>0.108 ± 0.024</td>
<td>0.557 ± 0.406</td>
</tr>
<tr>
<td>Prothrombin rate (%)</td>
<td>69 ± 4</td>
<td>60 ± 5</td>
<td>59 ± 10</td>
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Disclosures:
The following people have nothing to disclose: Yvon Calmus, Dominique Nochy, Philippe Cluzel, Corinne Antoine, Olivier Scatton, Olivier Saoubrane, Filomena Conti, Denis Glotz

782 THE USEFULNESS OF MEASURING LIVER STIFFNESS IN PREDICTING THE DEVELOPMENT HEPATOCELLULAR CARCINOMA

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Background/Aims: The degree of liver fibrosis is closely correlated with the development of hepatocellular carcinoma (HCC). A new noninvasive method—transient elastography (FibroScan®)—can assess liver fibrosis by measuring liver stiffness and fibrosis. We investigated the effectiveness of FibroScan® in predicting HCC development. Subjects & Methods: Between July 2005 and June 2006, liver stiffness was measured in 273 patients with chronic hepatitis B (n=232), chronic hepatitis C (n=41), alcoholic liver disease (n=11), or other liver disease (n=22). This cohort was followed for the occurrence of HCC. Results: The median follow-up duration was 13.6 months (8.1–21.9) and the median interval from the day of FibroScan® assessment to HCC occurrence was 13.0 months (10.8–20.0). The mean age of the patients without and
with HCC development was 52.6 and 58.4 years, respectively (P=0.296). Male gender predominated (male:female, 211:102). In comparing the two groups, biochemical tests, AFP, HBV DNA levels, and clinical diagnosis (chronic hepatitis/cirrhosis) did not differ significantly, while liver stiffness (16.0 kPa for no HCC occurrence, 26.2 kPa for HCC occurrence, P=0.040) and Child–Pugh class A/B (301/5 for no HCC occurrence, 6/1 for HCC occurrence, P=0.016) differed significantly. The area under the receiver operating curve (AUROC) for stiffness was 0.793. Considering the optimal sensitivity and specificity, the cutoff value for predicting the development of HCC from our data was 16.0 kPa. Using a cutoff of 16.0 kPa, the sensitivity and specificity were 0.86 and 0.65, respectively. Conclusions: Our data suggest that transient elastography is effective in predicting the development of HCC and that patients with an initial liver stiffness score >16.0 kPa have a greater chance of developing HCC than those with a score <16.0 kPa. Keywords: Hepatocellular carcinoma, Prediction, Liver stiffness measurement

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783 USEFULNESS OF LIVER STIFFNESS MEASUREMENT IN DISCRIMINATING BETWEEN CHRONIC HEPATITIS B AND COMPENSATED CIRRHOSIS

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Background/Aim: It is difficult to differentiate between chronic hepatitis and early compensated cirrhosis solely by laboratory and ultrasonographic features. The aim of this study was to assess the usefulness of liver stiffness measurement (LSM) in discriminating between chronic hepatitis B and compensated liver cirrhosis. Patients/Methods: Between May 2005 and Dec 2006, a total of 124 patients with hepatitis B virus-related liver disease underwent liver biopsy and LSM. Of these, 31 patients had definite clinical evidence of cirrhosis. Thus, 93 patients who did not fulfill the clinical criteria for liver cirrhosis were recruited in this study. The clinical criteria for liver cirrhosis were as follows: 1) history of overt complication of cirrhosis such as ascites, variceal bleeding, hepatic encephalopathy, or 2) platelet count <100,000/mm3 and ultrasonographic findings suggestive of cirrhosis such as blunted, nodular liver edge accompanied by splenomegaly (>10 cm). The cut-off value of LSM for the diagnosis of cirrhosis was set as 10.5 kPa. Results: There were 71 males and 20 females with a mean age of 40 years. The mean aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels were 36 and 46 IU/L, respectively. The mean platelet count, albumin, total bilirubin, and prothrombin time (PT) were 177,000/mm3, 4.5 g/dl, 0.8 mg/dl, and 96 sec, respectively. The median LSM score was 9.1 kPa (4.2–36.3). All the patients were divided into two groups by liver biopsy result; group A (cirrhosis, n=39) and group B (chronic hepatitis B, n=52). In comparison of two groups, the mean age of group A was significantly higher than group B (47 vs. 35 years, P<0.001). The platelet count and PT in group A were also statistically different from group B (147,000/mm3 vs. 201,000/mm3, P<0.001; 91% vs. 99%, P=0.02). The median value of LSM in each group was 11.8 kPa (6.8–36.3) and 7.6 kPa (4.2–28.4), respectively (P<0.001). In group A, the number of patients with LSM >10.5 kPa was 23 (sensitivity, 23/39=0.59). In group B, the number of patients with LSM ≤10.5 kPa was 41 (specificity, 41/52=0.78). The positive predictive and negative predictive value (NPV) was 0.68 and 0.72, respectively. The area under receiver operating characteristics (AUROC) curve was 0.78, whereas the AUROC of AST/ALT and APRI ([AST/upper normal limit]/platelet) was 0.54 and 0.74, respectively. Conclusion: LSM showed an acceptable diagnostic accuracy, especially high NPV, in the discrimination between chronic hepatitis B and compensated liver cirrhosis.

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784 URINARY LIPOCALIN2 REFLECTS HEPATIC DYSFUNCTION IN PATIENTS WITH CHRONIC LIVER DISEASE

Jin-Wook Kim, Sook-Hyang Jeong; internal medicine, Seoul National University Bundang Hospital, Seongnam, South Korea

Lipocalins form a large family of small extracellular proteins with a binding property to small hydrophobic molecules and cell surface receptors. Lipocalin 2 (LCN2), also known as neutrophil gelatinase-associated lipocalin (NGAL), forms complex with matrix metalloproteinase-9 (MMP-9) and inhibits auto-degradation of MMP9. LCN2 / MMP-9 complex was shown to be present in the urine of breast cancer patients, suggesting its role in the progression of cancer. We hypothesized that urinary LCN2 would be increased in advanced cirrhosis and/or hepatocellular carcinoma (HCC) in which MMP-9 might contribute to the pathogenesis of disease progression. Urinary LCN2 was quantified by ELISA in patients with fatty liver (n=7), chronic HBV carrier / chronic viral hepatitis (n=24) and liver cirrhosis (n=5) with or without HCC and corrected for urinary concentration of creatinine. Urinary lipocalin2 level was significantly increased in patients with liver cirrhosis (30.1 ± 5.6 mg/mg creatinine) compared to fatty liver (2.7 ± 1.0) or non-cirrhotic HBV carrier / chronic viral hepatitis patients (6.7 ± 2.4) (P = 0.006, Fig. 1). Associated HCC tended to increase the level of urinary lipocalin2 levels in cirrhotic patients and real-time quantitative PCR showed increased LCN2 mRNA in HCC compared to background cirrhotic tissues. However, LCN2 level was not significantly different between HCC and non-HCC patients (13.4 vs. 11.9, p = 0.732). Serum albumin (r = -0.52), total bilirubin (r = 0.50), prothrombin time (INR; r = 0.34), Child–Pugh score (r = 0.56), and MELD score (r = 0.48) correlated significantly with urinary LCN2. AST and ALT did not show significant correlation with urinary LCN2. Among the non-invasive predictors of hepatic fibrosis, FORNS score (r = 0.29) and FIB-4 score (r = 0.35) significantly correlated with urinary LCN2. In multivariate analysis, hepatic encephalopathy, serum albumin and bilirubin were independent predictor or urinary LCN2 levels. In conclusion, urinary lipocalin2 increases as liver disease progresses from simple steatosis / chronic hepatitis to advanced liver cirrhosis and correlates with known indicators of hepatic dysfunction and fibrosis. We postulate that dynamic remodeling of hepatic extracellular matrix is associated with increased urinary excretion of LCN2 in patients with advanced chronic liver disease.

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Background/Aim: Model for end-stage liver disease with incorporation of serum sodium (MELD-Na) was suggested to provide better survival prediction than MELD alone for patients with end stage liver disease in Western countries. However, there is no data verifying the usefulness of MELD-Na for predicting short term mortality of cirrhotic patients in Asian countries where the etiology and the course of chronic liver diseases may be different. This study was aimed to determine whether MELD-Na would be more accurate in the prediction of short term mortality than other prognostic models such as Child-Turcotte-Pugh (CTP) and MELD in an Asian country as well. Methods: Medical records of patients with liver cirrhosis who admitted at Korea University Ansan Hospital between January, 1996 and September, 2006 were retrospectively reviewed. Patients with hepatocellular carcinoma, chronic renal failure, serious cardiopulmonary disease, sepsis or other malignant tumors were excluded. The cumulative survival rates according to the respective scores of three prognostic models at admission were decided with Kaplan-Meier presumed values, and the differences of survival were verified with log rank test. Predictability on mortality for three months and one year were analyzed using the area under receiver operating characteristics (AUC). Results: Data from 355 patients (male, 266; female, 89) were analyzed. Mean age was 55.9 years old. Major causes of liver cirrhosis were alcohol (177 patients, 49.9%), chronic HBV infection (142 patients, 40.0%), and chronic HCV infection (20 patients, 5.6%). Miscellaneous causes (16 patients, 4.5%) included autoimmune hepatitis, nonalcoholic fatty liver disease, and primary biliary cirrhosis. Mean follow up period was 18.9 months. One hundred patients (28%) died during the study period. All of the three models showed significant differences in the cumulative survival rate according to the scores at admission (p<0.001). The AUC of CTP, MELD, and MELD-Na in predicting the three-months mortality were 0.828, 0.845, and 0.862, p<0.05) and the AUC of each score system for death within one year were 0.792, 0.800, and 0.831, respectively (p>0.05). The AUC of MELD-Na in predicting short term death was the highest, although not statistically significant. However, multivariate analysis showed that only MELD-Na was significantly related to three-month mortality among three prognostic models (p=0.012). Conclusion: It was shown that MELD-Na is more appropriate in predicting short term mortality, but larger scale studies are needed to confirm the superiority of MELD-Na to MELD or CTP in patients with liver cirrhosis.

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786 LIVER INJURY IS ASSOCIATED WITH MORTALITY IN SICKLE CELL DISEASE

Jordan J. Feld1, Tammy Shields2, Mark T. Gladwin2, Mariana E. Hildesheim2, James S. Nichols2, David Kleiner3, T. Jake Liang1, Jay H. Hoofnagle1, Gregory J. Kato2, Theo Heller1; 1Liver Diseases Branch, NIDDK, NIH, Bethesda, MD; 2Vascular Diseases Branch, NHLBI, NIH, Bethesda, MD; 3Liver Diseases Branch, NHLBI, NIH, Bethesda, MD

Background: Sickle cell disease (SCD) is a blood disorder leading to progressive multi-organ damage. Although hepatic abnormalities have been described in SCD, it is unknown if they relate to disease outcome. Aims: To determine if markers of liver involvement affect survival in SCD. Methods: We evaluated a large cohort of well-characterized patients with SCD followed at the NIH. Cox proportional hazards regression was used to assess factors associated with mortality. Univariate and multivariate logistic regression were performed to determine factors associated with identified predictors of mortality. Results: A total of 247 patients (148F/99M) with a mean age of 36.2 (18-74) years were followed for a median of 26.7 (0.3-49) months. Twenty-two patients died during that period. One patient with hepatitis C infection and iron overload underwent liver transplantation. Liver parameters, markers of iron overload and factors previously identified to be associated with mortality in SCD (age, pulmonary hypertension, WBC and creatinine) were evaluated by Cox regression. By multivariable analysis, direct bilirubin (HR 2.6 95% CI 1.4-4.8, p=0.002) and log ferritin (HR 1.6 95% CI 1.1-2.5, p=0.018) were independently associated with mortality. Pulmonary hypertension and WBC contributed to the final mortality model but were not independently statistically significant. To determine factors associated with predictors of mortality, logistic regression was performed. By multivariable analysis, increasing number of blood transfusions, WBC and ALT associated with a serum ferritin above 1,000 µg/l while increasing inferior vena cava diameter, mean arterial blood pressure, AST and LDH were associated with an elevation of direct bilirubin. Interpretation/Conclusion: Serum ferritin and direct bilirubin are independently associated with mortality in SCD. Although ferritin reflects transfusion-related iron overload, it may also be a marker of liver inflammation. Elevation of direct bilirubin is associated with evidence of hepatic congestion. The additional association with markers of hemolysis suggests that the inability to handle increased bilirubin loads in the face of chronic congestion may indicate subtle but clinically important impairment of hepatic function. Monitoring of serum ferritin and direct bilirubin levels may be helpful in assessing prognosis in patients with SCD.

Disclosures: The following people have nothing to disclose: Jordan J. Feld, Tammy Shields, Mark T. Gladwin, Mariana E. Hildesheim, James S. Nichols, David Kleiner, T. Jake Liang, Jay H. Hoofnagle, Gregory J. Kato, Theo Heller
SPUR CELL ANEMIA IN CIRRHOTIC PATIENTS AS PROGNOSTIC INDEX OF SEVERITY OF LIVER DISEASE AND SURVIVAL

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1Gastroenterology and Hepatology Department, 2nd Propaedeutic Internal Medicine Clinic, Hippokration Hospital, Aristotle University of Thessaloniki, Thessaloniki, Greece;
2Hematology Department, 2nd Propaedeutic Internal Medicine Clinic, Hippokration Hospital, Aristotle University of Thessaloniki, Thessaloniki, Greece

Introduction: Spur cell anemia (SCA) is a rare cause of hemolytic anemia in patients with advanced cirrhosis, more often alcohol-related. Diagnosis is made by the morphological appearance of red blood cells in peripheral blood smear. It is associated with bad prognosis and is considered reversible after liver transplantation. Aim: To evaluate the incidence of spur cell anemia in hospitalized patients with advanced cirrhosis and to correlate it with disease severity as well as overall survival.

Methods: From March 2004 to April 2006 all patients with advanced cirrhosis who were hospitalized for various conditions were examined for spur cells. Excluding criteria were: hepatocellular carcinoma at the time of examination, hemoglobinopathies, direct Coombs positive, chronic renal failure (not hepatorenal syndrome), sepsis, active bleeding and cirrhosis due to cholestatic liver disease. 48 patients, median age 66 years old (range 29-84) and male/female ratio 40/8 were included. The etiology of cirrhosis was: viral-17 patients (35.42%), alcohol-21 patients (43.75%) and cryptogenic-10 patients (20.83%). Patients were divided in two groups: patients without spur cells (Group A, n=26) and with spur cells (group B, n=22). Results: Overall 22 out of 48 patients (45.83%) had evidence of spur cells in blood smear. Seven out of 48 patients (14.58%) had >5% spur cells. The presence of spur cells was associated with more advanced liver disease (Child C 90.9% vs 62.5% and MELD score 24.5 vs 18 for group B vs A, p<0.001 respectively) and mainly alcohol-related (54.55 vs 34.62%). Patients with spur cells had lower hemoglobin level (8.8 vs 11.2 g/dL, p=0.001), higher bilirubin level (total/unconj-5.3 vs 3.78 vs 2.35/1.58 mg/dL, p<0.001), higher reticulocyte count (3.5% vs 1.6%, p<0.001), number of RBC transfused (12 vs 0, p<0.001) and shorter survival [90 vs 270 days, p<0.001]. When we compared patients with 1-5% (n=15) vs >5% spur cells (n=7), we found statistical difference in MELD score (21 vs 30, p=0.012), bilirubin level (total/unconj 4.4/3.3 vs 14.7/7.35mg/dL, p=0.026) and survival (150 vs 25, p=0.002). Three patients with spur cells underwent liver transplantation, one died during the operation from uncontrolled bleeding, one died 5 days later due to multigorgan failure and one is still alive with no spur cells detected in blood smear.

Conclusion: The presence of spur cells in not an uncommon finding in patients with advanced liver disease, however a poor outcome correlates with increasing number of spur cells. This entity is reversible only with liver transplantation.

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The following people have nothing to disclose: Themistoklis Vassiliadis, Sofia Vakalopoulou, Alexander Mpoumpounaris, Vasilis Perifanis, Dimitrios Gkisakis, Lemonia Mathiopoulou, Konstantinos Theodoropoulos, Nikolaos Grammatikos, Nikolaos Nikolaidis, Vasilina Garipidou, Olga Giouleme, Nikolaos Evgenidis.

THE IMPACT OF SERUM POTASSIUM CONCENTRATION ON MORTALITY FOLLOWING LIVER TRANSPLANTATION: A COHORT MULTI-CENTRE STUDY

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BACKGROUND: Potassium plays a key role in human metabolism in both health and disease. The impact of recipient serum potassium concentration [K] on mortality following liver transplantation has not been previously described. METHODS In this cohort multi-centre study, we used Cox proportional hazards modelling to assess the effect of recipient [K], measured immediately before transplantation, on the survival of all adults with chronic liver disease who received a first single organ liver transplant in the United Kingdom and Ireland between March 1994 and June 2005 (n=5,262), adjusting for a wide range of recipient, donor and graft characteristics. RESULTS Post-transplant mortality significantly varied by [K], being highest among recipients with [K] of greater than 4.4mmol/L and lowest among those with [K] of 3.5-3.9mmol/L (log-rank test for trend: p<0.0001). Compared to those with [K] of 3.5-3.9 mmol/L (n=1,332, reference category), the overall 5-year risk-adjusted mortality was higher among hyperkalaemic ([K]>5mmol/L) recipients (n=477, HR 1.35 95%CI 1.07-1.72) and those with [K] of 4.5-5.5mmol/L (n=914, HR 1.40 95%CI 1.18-1.67). Compared to the reference category, the excess mortality was however confined to the first post-transplant year among recipients with [K] of 4.5-5.5mmol/L (≤90 days: HR 1.52 95%CI 1.15-2.00; 90days-1year: HR 1.72 95%CI 1.20-2.45) with no significant difference thereafter (HR 1.09 95%CI 0.81-1.46, p=0.6). This was also true for hyperkalaemic ([K]>5mmol/L) recipients (≤90 days: HR 1.28 95%CI 0.87-1.89, 90 days-1year: HR 2.04 95%CI 1.27-3.29; >1 year: HR 1.15 95%CI 0.75-1.75). In contrast, hypokalaemic ([K]<3.5mmol/L) recipients (n=318) and those with [K] of 4-4.4mmol/L (n=1,829) had similar risk-adjusted mortality at the above time-points.

CONCLUSIONS Recipient serum potassium concentration is an independent predictor of death following liver transplantation. This finding could be of clinical utility in the management, risk stratification, selection and prioritisation of appropriate candidates for transplantation among patients with end-stage liver disease.

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The following people have nothing to disclose: Muhammad F. Dawwas, James D. Lewsey, Christopher J. Watson, Alexander E. Gimson
COMPARISON OF CONVENTIONAL TESTS WITH THROMBOELASTOGRAPHY (TEG) IN ASSESSING BLEEDING RISK IN DECOMPENSATED CIRRHOSIS PATIENTS

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Background – Recent studies have raised questions about the International Normalized Ratio (INR) in estimating bleeding risk in cirrhosis. Thromboelastography (TEG – Haemoscope, Niles, Ill) is an automated measure of multiple parameters of hemostasis including rate of clot formation (r), which reflects clotting factor availability, alpha angle (α), which reflects assembly of fibrin monomers, maximum amplitude (ma), which reflects platelet function, coagulation index (CI), which reflects hyperviscosity, clot strength (G), and amplitude 3 minutes after ma (A) which reflects clot lysis. We assessed the utility of PT/INR vs. TEG in hospitalized cirrhosis patients. Methods – We evaluated 14 consecutive hospitalized cirrhosis patients (BF, age 54.3 ± 10.7) with PT/INR, platelets, and TEG. TEG was performed on recalculated citrated specimens (allows study within 2 hours of collection) by a single operator on a computerized device and allowed for run for at least one hour after achieving maximum amplitude. Results – Mean PT/INR was 2.0 ± 1.0 (1.1-5.1) and mean platelet count was 70,571 ± 38,790 (13,000-156,000). Mean and ranges of the TEG were for = 4.7 ± 1.8 (2.3-8.7), k = 2.7 ± 1.3 (1.0-6.3), a = 58.1 ± 15.3 (15.7-75.4), ma = 45.8 ± 15.9 (12.2-74.8), CI = -1.2 ± 3.4 (-7.3-4.2), G = 5.15 ± 3.61 (0.7-14.9), A = 39 ± 17 (0.1-6.5). We could not detect any association between INR and TEG although trends were evident for k and α (p<0.06). Platelet count was associated with a and ma (p<0.05) by regression. Each parameter was then compared for INR ≥ 1.5 (n=9) vs. INR < 1.5 (n=5). We discerned differences only in k (p=0.05) and ma (p<0.01) between these groups. One patient (INR = 1.4 and plt = 51,000) had intractable vaginal bleeding and evidence of hyperfibrinolysis by TEG (A=0.1), indicating normal maximal clot amplitude but rapid dissolution by 30 minutes. Another patient (INR=1.5, plt 36,000) had only mild defect by TEG (ma 44.4, ref=51-69 with normal r). He received prophylactic plasma for central line exchange by conventional practice and developed clinically evident transfusion-related acute lung injury (TRALI). Conclusion – Conventional tests (INR and platelets) failed to detect the most significant hemostatic defect that we encountered (hyperfibrinolysis in 1 of 14 patients (7%). Plasma use based on conventional parameters was associated with suspected TRALI in a patient whose TEG showed only mild platelet dysfunction. Our findings demonstrate the limitations of conventional measures of hemostasis in cirrhosis and the need for larger trials to define the potential role of alternative methods.

Disclosures:

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Heart rate variability (HRV) is a measure of the physiological fluctuation of the cardiac cycle over time. Decreased HRV has been reported in a variety of clinical settings associated with increased production of inflammatory cytokines and neuropsychiatric impairment (Griffin et al., 2005; Vigo et al., 2004). HRV is decreased in patients with cirrhosis (Fleischer et al., 2000) but its relationship to the impairment of neuropsychiatric performance, commonly observed in these patients, has not been explored. The aim of this study was to determine the relationship between HRV, hepatic encephalopathy (HE) and inflammatory cytokines, in patients with cirrhosis. Seventy-five patients (51 males; age [mean±SD] 55±9 yr; Child A 43, B 12, C 20) with biopsy-proven cirrhosis, were classified as neuropsychiatically unimpaired (n=37) or as having minimal (8) or overt (30) HE, using clinical (Conn et al., 1977), psychometric (Weissenborn et al., 2001) and EEG criteria (Amodio et al., 1999).

HRV was assessed by applying linear (SDNN [Niskanen et al., 2004]) and non-linear methods (SD1 and SD2 from Poincaré plots [Tulppo et al., 1996]; Sample Entropy [Richman et al., 2000]) to the R-R interval series on a five-minute ECG. Reference data were obtained from eight healthy subjects (3 males; 40±12 yr). Inflammatory cytokines (TNFα, IL6, IL10, IL12) were measured in a subgroup of 22 patients. Differences between normally [non-normally] distributed variables were examined by ANOVA/ANCOVA (age adjustment) [Kruskal-Wallis ANOVA]; post-hoc comparisons were performed using the Tukey [Dunn's] test. Factorial ANOVA was used to examine the interacting effects of hepatic/neuropsychiatric impairment on HRV. Correlations were tested using the Spearman's R. HRV was significantly decreased in the patients with cirrhosis on both linear (SDNN 19.4±9.5 vs. 36.2±11.3 p<0.01) and non-linear indices (SD1 11.3±7.8 vs. 23.4±12.1 p<0.05; SD2 31.8±15.5 vs. 56.1±11.8 p<0.001; Sample Entropy 2.25±0.61 vs. 2.90±0.33 p<0.001). HRV indices decreased in parallel with the degree of neuropsychiatric impairment (p<0.000) independently of the degree of hepatic dysfunction (SD2: Child F[2,66]=0.27 p=0.76; HE F[2,66]=4.8 p=0.01; Child*HE F[2,66]=0.42 p=0.79). Significant correlations were found between HRV and EEG/psychometric variables. Plasma levels of IL6 correlated significantly with both indices of HRV and neuropsychiatric performance. The changes in HRV and neuropsychiatric status observed in patients with cirrhosis are linked, most likely via a common pathogenic mechanism mediated by inflammatory cytokines. These data support the hypothesis that systemic inflammation plays a role in the genesis of HE.

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The following people have nothing to disclose: Ali R. Mani, Sara Montagnese, Clive Jackson, Robert C. Stephens, Kevin P. Moore, Marsha Y. Morgan
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INCREASED RESTING ENERGY EXPENDITURE (REE) IN CIRRHOSIS. RELATIONSHIP WITH BODY COMPOSITION AND INSULIN RESISTANCE

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Aim: Factors influencing REE in cirrhosis are poorly understood. This study evaluated REE and its relationships with body composition, intermediary metabolism and its regulatory hormones and calorie intake in male cirrhotics. Patients and Methods: Forty-six male cirrhotic patients were evaluated (Child: A:17, B:20, C:9). REE was evaluated by indirect calorimetry and is expressed as % of the estimated normal value for each patient. Body composition was studied by air-displacement plethysmography (Bod-Pod). Serum Insulin, glycogen, adiponectin and leptin were measured by RIA. Calorie intake was estimated by a dietary questionnaire. Results: REE was normal (85-115% of theoretical value) in 18 patients (39%) and high (116-160%) in 28 (61%). Patients with increased REE exhibited as compared with those with normal REE: lower Child-Pugh index (p<0.05), lower total body fat (p<0.001), lower percent of body fat (p<0.003), higher percent of lean body mass (p<0.003), higher insulin resistance as evaluated by the HOMA index (p<0.01) and lower serum leptin (p<0.015). Calorie intake was reduced in both groups as compared with that expected for matched healthy subjects but intake deficiency was more severe in patients with normal REE (p<0.02). Conclusions: Hypercatabolism is present in 60% of male cirrhotics. These patients are characterized by less advanced disease, a lower fat mass-to-lean mass ratio, higher insulin resistance and a less severe undernutrition. These results suggest that cirrhosis is a hypercatabolic state even from early stages of the disease. This may be related to an abnormal utilization of fuel substrates. Further investigation is needed to define whether individual-specific dietary or pharmacological interventions would be of metabolic benefit in cirrhosis.

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BENEFICIAL EFFECTS OF TIPS ON PARTIAL PORTAL VEIN THROMBOSIS (PVT) IN PATIENTS WITH CIRRHOSIS

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PURPOSE: Partial PVT is a common finding in cirrhotic patients. In patients listed for liver transplantation (LT) the extension of PVT may be a contraindication for LT. Our study investigated the effect of TIPS in cirrhotic patients with partial PVT. MATERIALS AND METHODS: From January 2002 to January 2006, 166 consecutive cirrhotic patients underwent TIPS placement at a single transplant center. A total of 33 patients with partial thrombosis of main portal vein (MPV), and/or superior mesenteric vein (SMV), and/or splenic vein (SV) was included in this study. Luminal occlusion was estimated using a multidetector CT before and after TIPS creation (mean follow-up 22±14 months). Twenty-one patients received bare stent, 12 patients PTFE-covered stent. Patients with PVT ≥50% underwent mechanical thrombolysis using a balloon catheter. RESULTS: TIPS was placed in all patients without procedure-related complications. Portal pressure decreased from 24.4±6.0 mmHg to 8.9±4.2 mmHg (p<0.001). MPV thrombosis which was detected in 31 patients decreased from 49% (range: 10-90%) to 3% (0-20%) (p<0.001) after TIPS creation. SMV thrombosis which was detected in 20 patients decreased from 46% (range: 10-90%) to 5% (0-15%) (p<0.001) after TIPS. Two patients with SV thrombosis of 80% and 15%, had a complete resolution and no change, respectively. In overall 23 patients had a complete resolution of PVT, 8 patients had a marked reduction (PVT <20%), and 2 patients had no changes. Seventeen patients had PVT ≥50%; after TIPS placement, 12 patients had a complete resolution of thrombosis and five a significant reduction (<20%). A total of 30 episodes of TIPS stenosis occurred in 17 patients at the follow-up. TIPS stenosis rate was greater using bare stent vs. PTFE-covered stent (1.33 vs. 0.16 episodes/patients, p<0.001). Nine patients underwent LT 21±14 months after TIPS and none of them had PVT. No patient has been removed from the waiting list due to extension of PVT. Kaplan Mayer survival at 2-years was 90%. CONCLUSION: Our study showed that in cirrhotic patients with partial PVT, TIPS is safe and causes complete resolution or reduction of thrombosis. In patients eligible for LT, TIPS may avoid the extension of the clot and reduce the risk for LT. Additional studies should investigate if partial PVT can be a primary indication for TIPS placement.

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IS VON WILLEBRAND FACTOR A PREDICTIVE PARAMETER FOR CLINICALLY SIGNIFICANT PORTAL HYPERTENSION IN PATIENTS WITH LIVER CIRRHOSIS?

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Introduction: In patients with liver cirrhosis elevated levels of von Willebrand factor antigen (vWF-Ag) are found frequently. vWF-Ag plays an important role in primary haemostasis and development of thrombotic vascular obliteration is discussed as a possible mechanism leading to portal hypertension. Invasive measurement of hepatic venous pressure gradient (HVPG) is the current gold standard for the diagnosis of portal hypertension. We therefore investigated if vWF-Ag levels in plasma correlate with portal pressure, indicated by HVPG. Further, we investigated if vWF-Ag is a predictive parameter for clinically significant portal hypertension. Methods: Forty eight patients (mean age 55 y, 36 m and 12 w) with alcoholic, viral (chronic hepatitis C), and cryptogenic liver cirrhosis were included. Portal hemodynamics were assessed indirectly by measurement of HVPG, vWF-Ag levels were measured by ELISA. Results: Forty one patients had clinically significant portal hypertension (PH) (i.e. HVPG>or=10). In these patients HVPG was 19 mmHg (mean, 95%CI: 17-21 mmHg), and in seven patients without PH HVPG was 5 mmHg (mean, 4-6 mmHg). vWF-Ag was signifi-
cantly increased in patients with portal hypertension: 375% (334-417%) compared to vWF-Ag in patients without portal hypertension: 230% (173-288%); p<0.001. Further, HVPG correlated significantly with vWF-Ag (R=0.65; p<0.0001). Patients with vWF-Ag levels >300% had a markedly greater risk of PH (odds ratio: 15.3). Conclusion: In patients with liver cirrhosis vWF-Ag is elevated and can discriminate between patients with clinically significant portal hypertension and those without with a cut-off vWF-Ag >300%. This cut off of value for vWF-Ag level as a non invasive parameter indicating clinically significant portal hypertension has to be confirmed in an independent larger patient sample.

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796 MOLECULAR MECHANISMS UNDERLYING MUSCLE LOSS IN CIRRHOTIC PATIENTS

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Liver cirrhosis (LC) is frequently accompanied by malnutrition and muscle loss (ML), which in turn negatively affect quality of life, morbidity and mortality. Increased protein degradation or decreased protein synthesis are mechanisms which may be involved in ML. Increased gene expression of MuRF and MAFbx, the so called “atrogins”, or of Myostatin (MSTN), a negative modulator of skeletal muscle growth, have been found to be associated with protein degradation in different clinical conditions. On the other hand depletion in the expression of Insulin Like Growth Factor-1 (IGF-1) may document an impairment in protein anabolism. The study was aimed at exploring the molecular mechanism underlying ML in CLF. Muscle biopsies from rectus abdominis were obtained intraoperatively in 12 patients undergoing orthotopic liver transplantation (LC) and in 4 well-nourished subjects undergoing elective surgery (C). Total RNA was extracted and m-RNA for atrogins (MuRF-1, MAFbx), MSTN, and IGF-1 was assayed (arbitrary units) by semiquantitative PCR. Patients were classified with anthropometry (Mid Arm Muscle Area : MAMA < 5th) and Subjective Global Assessment (SGA 0 or 1-2). Results are shown in Table 1. Overall, no significant differences were observed between LC and C in m-RNA levels. When patients were stratified according to nutritional status or ML, MuRF-1 tended to be increased but the difference did not reach statistical significance. These preliminary data suggest that the molecular pathways underlying ML in LC might be different from those involved in other chronic diseases, such as cancer, and deserve further research.

Table 1 - MuRF-1, MAFbx, MSTN and IGF-1 m-RNA expressed in arbitrary units

<table>
<thead>
<tr>
<th>Condition</th>
<th>MuRF-1</th>
<th>MAFbx</th>
<th>MSTN</th>
<th>IGF-1</th>
</tr>
</thead>
<tbody>
<tr>
<td>LC ALL (n=12)</td>
<td>0.23±0.08</td>
<td>0.36±0.02</td>
<td>0.39±0.06</td>
<td>0.46±0.12</td>
</tr>
<tr>
<td>LC SGA 1-2 (n=6)</td>
<td>0.29±0.07</td>
<td>0.38±0.09</td>
<td>0.40±0.05</td>
<td>0.50±0.09</td>
</tr>
<tr>
<td>LC SGA 0 (n=6)</td>
<td>0.17±0.02</td>
<td>0.35±0.05</td>
<td>0.36±0.08</td>
<td>0.40±0.13</td>
</tr>
<tr>
<td>LC MAMA &lt; 5th (n=3)</td>
<td>0.25±0.04</td>
<td>0.35±0.13</td>
<td>0.40±0.05</td>
<td>0.51±0.05</td>
</tr>
<tr>
<td>LC MAMA &gt; 5th (n=9)</td>
<td>0.21±0.09</td>
<td>0.36±0.05</td>
<td>0.38±0.07</td>
<td>0.44±0.14</td>
</tr>
<tr>
<td>CONTROLS (n=4)</td>
<td>0.20±0.09</td>
<td>0.38±0.08</td>
<td>0.42±0.02</td>
<td>0.41±0.05</td>
</tr>
</tbody>
</table>

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797 PARTICULATE MATTER AS POTENTIAL TRIGGER FOR ESOPHAGEAL VARICEAL BLEEDING IN PATIENTS WITH CIRRHOSIS OF THE LIVER

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BACKGROUND: Particulate matter (PM10), a consequence of air pollution, has recently been identified to have an impact on the etiology of several pulmonary and cardiovascular diseases. Recent studies have shown that exposure to PM10 is associated with oxidative stress, inflammatory responses, and endothelial dysfunction. These mechanisms have also been identified to play a role in the pathophysiology of esophageal varical bleeding in patients with the portal hypertensive syndrome. AIM: Aim of the study was to investigate a possible correlation of levels of particulate matter in the city of Vienna, Austria, and the incidence of esophageal varical bleeding in patients with cirrhosis of the liver. PATIENTS AND METHODS: All data of patients with varical bleeding were collected prospectively (May 2004–May 2006) within an European multicenter study investigating patients with cirrhosis and varical bleeding. Data of the current study were retrieved retrospectively from the database. All patients of the Viennese sample with cirrhosis and esophageal varical bleeding were included into the study. During the study period, the Viennese Emergency Medical Services were to transfer all patients with a suspected varical bleeding to the General Hospital. For the study period, daily levels of ambient air pollution parameters were provided by the Federal Environment Agency. These included levels of NO2, CO2, CO, PM10, O3, and air pressure. The data were analyzed by a multivariate logistic regression analysis with a nonlinear time trend (restricted cubic spline) as well as covariables to model the potential influence of the winter season (heating period), the day of the week, and holidays. RESULTS: 52 patients were included into the study. A significant correlation between PM10 levels and occurrence of varical bleeding was observed (p=0.014; odds ratio=1.018). On days with PM10 concentrations exceeding the maximum permissible limit (50μg/m3), the probability of varical bleedings was significantly increased (p=0.002, odds ratio 2.83). No such correlations were found for NO2, CO2, CO, O3, and air pressure. Probability for varical bleeding was independent from season, day of the week, and holidays. CONCLUSIONS: The incidence of varical bleeding in patients with cirrhosis and portal hypertension correlates with levels of PM10. An increase of 10μg/m3 PM10 approximately increases the probability of varical bleeding by 20%. On days with PM10 higher than the maximum permissible limit, the risk for varical bleeding is approximately 2-fold increased. PM10- and therefore air pollution- seems to be a hitherto unknown trigger for varical bleeding.

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INCREDIBLE MORTALITY IN PATIENTS WITH PORTOPULMONARY HYPERTENSION: HAVE WE LEARNED THE LESSON IN THE MELD ERA?
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Background: Severe portopulmonary hypertension (PoPH) has a poor patient outcome with a decreased survival and is a contraindication to liver transplantation (LT). Patients with advanced liver disease appear to have a high mortality from PoPH. We sought to identify the survival of patients with PoPH based on the MELD score. Methods: Patients undergoing pre-LT evaluation in Mount Sinai Hospital from 2003-2006 were retrospectively analyzed for the presence of PoPH. The diagnosis of PoPH was established by the clinical diagnosis of portal hypertension and right heart catheterization showing mean pulmonary artery pressure (mPAP)>25 mmHg, pulmonary capillary wedge pressure<15 mmHg and pulmonary vascular resistance>240 dyne/sec/cm5. MELD score at initial presentation was calculated for each patient. Death or LT was considered as the primary outcome. In patients with PoPH, the outcome was compared between subjects having a MELD score of >15 and <15. The survival data for patients with PoPH and a MELD>15 were further compared with age and sex-matched patients who had MELD>15 without PoPH and did not undergo LT. Results: 52 patients fulfilled the criteria for the diagnosis of PoPH within the study period. The mean MELD score was 17 (6-35). The mean mPAP was 49 (26-80) and pulmonary vascular resistance was 560 (240-1300). PoPH was more common in female gender (F: M 1.7:1). The mean age of diagnosis was 48 yrs (18-74). Forty three (83%) had liver disease due to HCV or alcohol. Twenty two patients (41%) were excluded from the study in view of age<18 yrs, poor compliance to therapy or inadequate follow up. Out of 30 patients, 20 (66%) received treatment for pulmonary hypertension. With a mean follow up of 39 months, 12 patients are still alive, 3 were transplanted, and 15 patients died within a mean period of 11 (1-26) months of presentation. There was no difference in mPAP between the survivor and non-survivor groups (45.8 vs. 50.5 mmHg, p=0.37). Age, gender and drug therapy for PoPH did not differ between the two groups. Of the 15 patients who died, only 3 had MELD<15, compared with 12 with MELD>15. The number of patients without PoPH with MELD>15 who did not undergo LT was 964 (mean follow up 18 months). When they were compared with patients having MELD>15 with PoPH, there was a statistically significant difference in mortality (46% vs. 85%). Conclusions: Patients with PoPH with a MELD score>15 have a significantly increased mortality when compared with patients with lower MELD score.

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PREVALENCE OF PORTAL HYPERTENSIVE GASTROPATHTY AT DIAGNOSTIC UPPER GI ENDOSCOPY
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Background: Portal hypertensive gastropathy (PHG) is a common complication of portal hypertension. PHG has been reported to affect up to two-thirds of patients with cirrhosis and portal hypertension, with an increasing incidence in higher Child-Pugh grade. However, despite a consensus of specific, diagnostic endoscopic features from the New Italian Endoscopy Club (1), the frequency with which PHG is a presenting feature of portal hypertension remains undefined. Aims and Methods: The aim of this study was to determine the prevalence of PHG in an unselected population attending for diagnostic upper GI endoscopy in a tertiary endoscopy centre. Furthermore, the proportion of patients with no history of liver disease, in whom PHG was the first manifestation of portal hypertension, was determined. PHG was defined as a snake-skin or mosaic-like appearance of the gastric mucosa, with or without the presence of red point lesions, cherry-red spots and black-brown spots. Results: A total of 17320 upper GI endoscopies were performed between January 2001 and January 2007. PHG was diagnosed in 254 (1.5%) patients. Esophageal varices (EV) were present in 152 patients, and 102 patients had PHG with no evidence of EV. Of these 102 patients, 36 (35.3%) had no previous history of portal hypertension or liver disease. The indications for endoscopy in these 36 patients were: anemia (27.8%), abdominal pain (25.0%), dyspepsia (22.2%), dysphagia (11.1%), vomiting (5.6%), weight loss (2.8%), oesophagitis (2.8%) and hiccoughs (2.8%). The majority of patients with EV and PHG had a history of portal hypertension or liver disease. Conclusions: One-third of de novo cases of portal hypertension are diagnosed in patients without a history of underlying portal hypertension or liver disease. Despite these limitations, this study confirms that PHG remains a rare clinical entity for the general endoscopist. Nevertheless, physicians and endoscopists must be aware of the endoscopic diagnostic features of PHG, since de novo PHG at diagnostic upper GI endoscopy may be a common mode of presentation of portal hypertension and underlying liver disease. 1. Carpinelli L, Primignani M, Pretoni P, et al. Portal hypertensive gastropathy: reproducibility of a classification, prevalence of elementary lesions, sensitivity and specificity in the diagnosis of cirrhosis of the liver: a NIHC multicentre study. Ital J Gastroenterol Hepatol 1997;29:533-40.

COUNTRY STIFFNESS PREDICT THE PRESENCE OF HEPATOCELULAR CARCINOMA?
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Background/Aims: The degree of liver fibrosis is one of the strongest risk factors for hepatocellular carcinoma (HCC) development. Several studies have validated the correlation between stiffness and fibrosis, and a new noninvasive method—transient elasticity. 1. n vivo
er, hepatic and portal venous blood of 103 subjects whose hepatic disease classification spanned the spectrum from healthy controls to patients with type 2 HRS. Groups included healthy controls (N=6), organ donors (N=11), compensated cirrhotics (N=17), uncomplicated ascites (N=24), refractory ascites, (N=9), Type 2 HRS (N=19), and CRF without liver disease (N=17). Results. A progressive rise in plasma NOx and L-Arg, but not ET-1, was seen in decompensated cirrhosis as a function of renal insufficiency and worsening azotemia. The highest levels of NOx and L-Arg were observed in patients with type 2 HRS (P < 0.001 vs all groups). Patients with CRF had peripheral NOx levels significantly lower than patients with type 2 HRS, but significantly higher than healthy controls. There were no quantitative differences in NOx and ET-1 in blood collected from peripheral vs portal or hepatic veins. In general, plasma levels of L-Arg tended to follow NOx levels which were the closest when L-Arg was measured in portal vein. Univariate analysis showed significant correlations between Child-Pugh score, MELD score, and Platelet counts for NOx and L-Arg, but not ET-1. Conclusion. Patients with decompenated cirrhosis show progressive elevation in plasma NOx that correlates with the severity of renal dysfunction from compensated cirrhosis to type 2 HRS at the end of the disease spectrum. In our data, this occurred with only minor changes in ET-1 and was not a function of renal failure per se. These findings, from a large series of patients, support the hypothesis that unopposed NO vasodilation as a result of worsening liver disease is a major contributor to renal failure in patients with decompenated cirrhosis.

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COVERED-STENT TIPS OFFERS A BETTER SURVIVAL FOR CIRRHOTIC PATIENTS WITH REFRACTORY ASCITES

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Background/aim: Transjugular intrahepatic portosystemic shunt (TIPS) is used to treat refractory ascites (RA). The self-expanded polytetrafluoroethylene covered-stent-graft (cs TIPS) gives a better long-term patency rate. Our aim was to study whether cs-TIPS might improve survival. Methods: We compared in a prospective cohort study from 1992-2006 in 222 consecutive cirrhotic patients with RA the long-term outcome of 126 patients with a non-covered stent TIPS (ncs-TIPS) versus 96 with a cs TIPS. Liver transplantation (OLT) and/or death were the end points of the follow-up. Results: The baseline characteristics were similar: age 55±11 years, alcoholic cirrhosis (73% ncs-TIPS/80% cs-TIPS), Child-Pugh (9±2.0 ncs-TIPS/9.2±1.3 cs-TIPS) and MELD (15±6 ncs-TIPS/15±4.9 cs-TIPS). Successful bridge to OLT was reached in 10% (n=13) of the ncs-TIPS after a median of 3.7 months vs. 20% (n=19) of the cs-TIPS patients after a median of 3 months. One year shunt dysfunction occurred in 49% (n=63) of the ncs-TIPS vs. 19% (n=18) of the cs-TIPS (P<0.0001) and post TIPS encephalopathy in 56% (n=70) of the ncs-TIPS vs. 22% (n=22) in the cs-TIPS group (however, 10 patients needed a reduction stent) (P<0.0001). The overall OLT free survival was better in the cs-TIPS (P=0.0071). The gain in survival in the cs-TIPS patients occurred especially if they had a baseline MELD score <16 (P<0.0001). The one year OLT free survival of patients with MELD score <16: ncs-TIPS [63% (n=44)] vs. [80%

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INCREASED PLASMA NITRIC OXIDE AND L-ARGININE IN CIRRHOTIC PATIENTS WITH PROGRESSIVE RENAL DYSFUNCTION

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Vasodilatation in the peripheral and splanchnic vasculature is a prelude to the development of ascites and azotemia. Nitric oxide (NOx), a potent vasodilator, and endothelin-1 (ET-1), a vasoconstrictor, have been reported to be elevated in cirrhosis, however, whether alterations in the plasma balance of these mediators correlates with the development of progressive renal dysfunction in human cirrhosis is unclear. The purpose of this study was to compare plasma levels of NOx, the NOx precursor L-Arginine (L-Arg), and ET-1 in splanchnic and post-hepatic circulation in patients with cirrhosis, decompensated liver disease, and HRS as compared with healthy controls and patients with chronic renal failure (CRF) without hepatic disease. Methods: Serum NOx, L-Arg and ET-1 were measured in the peripheral, hepatic and portal venous blood of 103 subjects whose hepatic disease classification spanned the spectrum from healthy controls to patients with type 2 HRS. Groups included healthy controls (N=6), organ donors (N=11), compensated cirrhotics (N=17), uncomplicated ascites (N=24), refractory ascites, (N=9), Type 2 HRS (N=19), and CRF without liver disease (N=17). Results. A progressive rise in plasma NOx and L-Arg, but not ET-1, was seen in decompensated cirrhosis as a function of renal insufficiency and worsening azotemia. The highest levels of NOx and L-Arg were observed in patients with type 2 HRS (P < 0.001 vs all groups). Patients with CRF had peripheral NOx levels significantly lower than patients with type 2 HRS, but significantly higher than healthy controls. There were no quantitative differences in NOx and ET-1 in blood collected from peripheral vs portal or hepatic veins. In general, plasma levels of L-Arg tended to follow NOx levels which were the closest when L-Arg was measured in portal vein. Univariate analysis showed significant correlations between Child-Pugh score, MELD score, and Platelet counts for NOx and L-Arg, but not ET-1. Conclusion. Patients with decompenated cirrhosis show progressive elevation in plasma NOx that correlates with the severity of renal dysfunction from compensated cirrhosis to type 2 HRS at the end of the disease spectrum. In our data, this occurred with only minor changes in ET-1 and was not a function of renal failure per se. These findings, from a large series of patients, support the hypothesis that unopposed NO vasodilatation as a result of worsening liver disease is a major contributor to renal failure in patients with decompenated cirrhosis.

Disclosures:
The following people have nothing to disclose: Zeid Kayali, Jason Hanson, Pedro Baron, Olekkukow Ojojogo, Edson Franco, Jason Smith, Gregory Watkins, David Smith, Victor Lamin, Thanh Hoang, Meleah Mathahs, Robert Sowers, Kyle Brown, Warren N. Schmidt
803 PROGNOSTIC VALUE OF ALGORITHMS COMBINING FIBROTEST®/FIBROSURE® (FT) AND ASHTEST® (HT) IN COMPARISON WITH MELD AND PUGH PROGNOSTIC INDEXES IN PATIENTS WITH SEVERE CIRRHOSIS

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Background. FT and HT have been validated as biomarkers of fibrosis and acute alcoholic hepatitis. The prognostic value of FT has been validated at 5 years only for chronic hepatitis C (Clin Chem 2006). Aim. It is clinically useful to combine diagnostic and prognostic markers for the short-term prognosis of patients with severe cirrhosis. We prospectively validated the prognostic value of FT and HT at 2 and 6 months and their combination with prognostic markers in a multicenter prospective randomized trial of pentoxifylline vs placebo. Methods. The usual baseline clinical and biochemical characteristics plus FT, MELD, Pugh, HT (only for alcoholic liver disease - ALD) were assessed. The most discriminant algorithms combining FT and independent biomarkers were constructed according to multivariate analysis for the 2- and 6-month-prognosis. The prognostic values of FT and HT alone or in combination with other biomarkers were assessed and compared with the standard prognostic markers (Pugh and MELD) using univariate (log-rank test) and multivariate prognostic analysis (Cox model), and area under the ROC curves (AUROCs) for predicting death at 2 and 6 months (mo). Results. A total of 224 patients were included, all Pugh C, age 55 years, 78% male, 84% alcoholic cirrhosis. The mortality was 20% (44/224) and 33% (75/224) at 2 and 6 months. Baseline Pugh was 11.3 (0.07) m(se), MELD 19.8 (0.6), FT 0.94 (0.06), and HT 0.62 (0.02). The best algorithms for all causes of cirrhosis combined FT, creatinine and natremia (FTprog). In the total cohort, the best AUROCs at 2 and 6 mo were for FTprog 0.82 (0.04) and 0.78 (0.03); this was equivalent to MELD 0.78 (0.04) P=0.38 and 0.70 (0.04) P=0.11, higher than FT 0.75 (0.06) P=0.07 and 0.64 (0.04) P=0.001, and higher than the Pugh score 0.63 (0.05) and 0.59 (0.04), respectively. Pugh was significantly lower than FTprog and MELD P<0.01. Sensitivity analysis showed that the results were the same after excluding patients with non alcoholic cirrhosis, hepatocellular carcinoma, and those treated or not with pentoxifylline or steroids. Among the 189 patients with ALD, the combination of HT slightly increased the prognostic value of the algorithm (regression coefficient Z value P=0.04) but without significant difference in the AUROCs. Cutoffs with Se>90% and Sp>90% permitted to identify a group (n=102) with 95% 2month survival (95%CI 91-99%), one (n=83) with 79% survival (95%CI 70-88%), and one (n=41) with 41% survival (95%CI 26-56%). Conclusion: Algorithms combining Fibrotest, creatinine and natremia have prognostic values at 2 and 6 months similar to those of MELD and better than Pugh in patients with severe cirrhosis.

Disclosures: The following people have nothing to disclose: Nelson Antonio Perez Gutierrez, Sylvie Evrard, Geert Maleux, A. Mroue, Olivia Le Moine, Frederik Nevens

804 MONITORING OF LOW MOLECULAR HEPARINS IN PATIENTS WITH LIVER CIRRHOSIS

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Background: The use of low molecular heparins (LMH) in patients with advanced liver diseases is frequently avoided, due to coagulopathy and therefore an enhanced risk of bleeding complications under the medication. However, many patients with impaired liver function are at enhanced high risk of thrombosis (immobilization, presence of HCC) or even have an indication for therapeutic anticoagulation (portal vein thrombosis). Therefore the aim of this study was to evaluate the safety and efficacy of low molecular weight heparin (LMH) in patients with liver cirrhosis and impaired liver function. Methods: 52 consecutive patients (28 males; 24 females) with proven liver cirrhosis and the clinical indication for prophylactic LMH treatment were included. Enoxaparin (40mg/d) was applied subcutaneously. Anti-Xa-activity was assessed on two consecutive, four hours after drug application. Severity of the liver disease was quantified using the Child-Pugh-criteria and correlated with the anti-Xa-activity and the occurrence of complications. Results: Three out of 52 patients suffered from endoscopically treatable variceal bleeding during LMH treatment, most likely unrelated to treatment. No patient died of complications. A negative correlation between Child score and anti-Xa-activity was observed (Child A = 0,24 ±0,08 p<0,05; Child B = 0,15 ±0,09 p<0,05; Child C= 0,18 ±0,09 p<0,05). Also, anti-Xa-activity and extent of ascites were negatively correlated. Conclusion: Prophylactic use of Enoxaparin in patients with liver cirrhosis is safe and does not increase the risk of bleeding. A decreased anti-Xa-activity in cirrhotic patients and a negative correlation with the degree of liver function impairment indicates that common doses of LMH might be too low in this subgroup of patients. Low levels of AT-III due to reduced hepatocellular synthesis in cirrhosis are the most likely cause of this observation and currently further investigated.

Disclosures: The following people have nothing to disclose: Matthias Sichau, Lars Bechmann, Guido Gerken, Knut Kroeger, Philip Hilgard

805 THE ROLE OF B TYPE NATRIURETIC PEPTIDE (BNP) AS A MARKER OF STRUCTURAL CARDIAC DISEASE IN PATIENTS WITH CIRRHOSIS

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Cardiac complications of chronic liver disease are well documented. Electrocardiogram (EKG) and echocardiographic
CIRRHOSIS

INTRODUCTION:

Cirrhotic patients admitted to the intensive care unit (ITU) have a poor survival. Previous studies have addressed the ability of both liver specific (MELD, Child-Pugh) and ITU disease severity and organ failure (SOFA, APACHE 11) parameters to discriminate between survivors and non-survivors. These studies have shown that SOFA is superior to MELD and Child-Pugh (CPS) score however to our knowledge the impact of cirrhosis aetiology on ITU mortality has never been evaluated.

PATIENTS AND METHODS:

A total of 524 autopsies were reviewed and three groups of patients were identified. 1. A total of 364 patients with advanced liver disease had undergone autopsy and 47 were excluded. In only 93(29%) patients were the clinical and post-mortem diagnoses in complete agreement. Discrepancy between pre-mortem clinical and postmortem pathological diagnoses. Agreement between pre-mortem clinical and postmortem pathological diagnoses. Agreement between pre-mortem and postmortem diagnoses were compared using the Goldman system. Studies with limited autopsy and missing clinical diagnoses were excluded. Results: A total of 524 autopsies were reviewed and three groups of patients were identified. 1. A total of 364 patients with advanced liver disease had undergone autopsy and 47 were excluded. In only 93(29%) patients were the clinical and post-mortem diagnoses in complete agreement. Major missed diagnoses included: gastrointestinal bleeding-73, superficial variceal bleeding with or without esophageal ulcer-50, gastritis/ulcers-8, intraperitoneal bleeding-6, diffuse bleeding-7, pneumonia-23, fatal acute pancreatitis-6. Unusual findings included death due to hemorrhage...


808 VWF FUNCTIONS, ADAMTS-13 ACTIVITY AND ANTIGEN LEVELS IN PATIENTS WITH LIVER CIRRHOSIS

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Introduction. Liver cirrhosis is one of the major causes of the acquired haemostatic disorders. There are a lot of alterations, which could result in a reduced haemostatic capacity, but many compensatory mechanisms have also been detected. Methods. Patients with cirrhosis of different origin (n=94; m/f:45/49; Child A/B/C: 40/44/16%) and healthy volunteers (n=57) were examined. Platelet poor plasma was obtained then stored at -80°C and tested within 1-2 weeks. Von Willebrand factor (VWF) antigen (VWF:Ag), ristocetin cofactor activity (VWF:RCo), collagen binding activity (CBA) and ADAMTS-13 antigen levels were 140±44 %, 144±42 % and 169±53 %, respectively. VWF:Ag (220±60 %), VWF:RCo (144±42 %) and CBA levels (169±53 %) were significantly elevated in patients with cirrhosis as compared to the control group. However, the functional activities were not that high as the antigen level. The VWF:RCo/VWF:Ag and the CBA/VWF:Ag ratios were significantly reduced as compared to the control, respectively (0.65 vs.0.94 and 0.77 vs.0.92; p<0.01). Both functional activities were reduced to the same extent, the CBA/VWF:RCo ratio was 1.17 (control: 1.04, p=NS). Multimeric distribution was characterized by the presence and significant elevation of all molecular weight fractions with the significant predominance of the low molecular weight fractions. The activity and antigen levels of ADAMTS-13 showed a high variability, both substantially elevated and low values. The plasma level of ADAMTS-13 activity in cirrhotic patients was 135±71 % and in controls 126±45 % (p=NS). The ADAMTS-13 antigen levels were 140±83 vs. 124±44 %. Discussion. Plasma VWF:Ag level and its functional activities were significantly elevated in patients with liver cirrhosis. However, the decreased activity/antigen ratios indicate a relative loss of function of the molecule. This is also suggested by the altered multimeric structure i.e. the reduced polymerization. These changes might be the consequences of endothelial perturbation in liver cirrhosis which results in the alteration of the endothelial synthesis of VWF. Bacterial infection, frequent in cirrhosis, may contribute to this. ADAMTS-13 probably has no significant role in the structural and functional alteration of VWF in patients with liver cirrhosis. Elevated VWF might compensate the defect in primary haemostasis.

Disclosures:
The following people have nothing to disclose: Istvan Tornai, Papp Maria, Miklos Udvardy, Jolan Harsfalvi

809 RELATIONSHIP BETWEEN SEVERITY OF LIVER DISEASE AND RISK OF GALLSTONES IN MEN WITH CHRONIC HEPATITIS C: A CLINICOPATHOLOGICAL STUDY

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Background: Pigment cholelithiasis is common in patients with chronic liver disease and cirrhosis. Its pathogenesis is incompletely understood. Our specific aims were 1) to assess the relationship between liver histology and risk of gallstone disease in a relatively homogeneous population of men with chronic hepatitis C; and 2) to examine the role of demographic, viral, clinical and laboratory features as determinants of gallstone risk. Methods: We retrospectively analyzed data from two groups of male veterans with hepatitis C whose liver disease spanned the spectrum from normal to end-stage cirrhosis. Group A consisted of 493 male veterans with hepatitis C who underwent both liver biopsy and ultrasonography at our institution in anticipation of antiviral therapy. Liver biopsies were reviewed by two experts, and activity and fibrosis were scored according to Ishak. Group B included 548 additional male veterans with hepatitis C and established cirrhosis referred from outside institutions for consideration of liver transplantation. Findings: In men undergoing liver biopsy for hepatitis C, gallstone disease (gallstones on imaging or history of cholecystectomy) was present in 23%. Prevalence of gallstone disease increased with severity of hepatic fibrosis. Lowest prevalence was seen in patients with Ishak fibrosis stage 0-2 (15%), increasing at Ishak fibrosis stages 3 and 4 (27%, p<.01 compared to stage <2), and a further increase was seen at Ishak stages 5-6 (34%). Gallstone disease was associated with platelet count <130k (39% vs. 19%, p<.01) and with increasing splenomegaly (absent=18%, mild=30%, moderate to severe=40%, p=.014). Hemoglobin, erythrocyte indices and reticulocytes were similar in patients with and without gallstones. Gallstone prevalence did not vary with history of alcoholism, obesity or diabetes mellitus, was similar in whites and African-Americans, and was unrelated to hepatitis C genotype or viral load. Gallstone disease and fibrosis severity both increased with age, but age and fibrosis stage were independently associated with gallstones by logistic regression. In group B, prevalence of gallstones by CTP class was A=47 %, B=50 % and C=50 % (differences NS) and was not significantly associated with MELD or CTP score. Conclusions: Age, hepatic fibrosis, and presence of splenomegaly and thrombocytopenia are the key determinants of gallstone risk in men with
810 SURVIVALS OF HEPATIC ENCEPHALOPATHY: RELEVANCE TO ETIOLOGY AND INITIAL COMPLICATION OF CIRRHOSIS

Chang Wook Kim, Jong Young Choi, Won Young Tak, Sung Kyu Choi, Jeong Heo, Mong Cho, Jeong Won Jang, Seung Kew Yoon, Joon Yeol Han, Young Sok Lee, Chang Don Lee. 

Background/Aims: Despite numerous studies concerning natural history and prognostic factors of cirrhosis, there are few reports about epidemiology and prognostic factors focused on hepatic encephalopathy (HE) as well as the relationships between survival of HE and several factors such as initial complication of cirrhosis, etiology of cirrhosis, and model for end-stage liver disease (MELD). Methods: A total of 223 consecutive liver cirrhosis patients diagnosed as the first episode of HE with flapping tremor (1st HE) were included in this study from 4 medical centers of Korea from January 2001 to December 2003. Initial complication of cirrhosis is defined as first event of cirrhosis complication such as ascites, variceal bleeding, HE, and hepatocellular carcinoma (HCC) in his or her life. Survival times from 1st HE were calculated using the Kaplan-Meier method. The prognostic significance of variables such as demographic data, etiology of cirrhosis, clinical laboratory data, initial complication of cirrhosis, Child-Pugh (CP) score, CP classification, MELD score and MELD classification was analyzed using Cox regression model. MELD classification is categorized as MELD-A, MELD-B, and MELD-C according to MELD score (≤19, 20-39 and ≥40, respectively). Results: The major etiology of cirrhosis was virus such as HBV (61%) and HCV (11%). The median interval (median ± SE) between initial complication and 1st HE was 9.0 ± 1.9 months. The interval between first variceal bleeding and 1st HE was longer than intervals between other initial complications and 1st HE. The median survival time from 1st HE was 86 ± 29 months (37 ± 9 months if HCC cases were included). The 1 year, 3 years, survival rate after 1st HE was 71% and 56%, respectively. On univariate and multivariate analyses, etiology of cirrhosis, CP score and MELD classification remained independently significant for survival. HE associated with HBV showed low hazard ratio compared to HE associated with HCV (hazard ratio of HBV vs. HCV 3.813, 95% CI 1.439-10.10, p = 0.007). Conclusions: The median survival time (86 months) after 1st HE in HBV endemic area was longer than those of previous reports in non-HBV endemic area. Etiology of cirrhosis, CP score and MELD classification are the most important prognostic factors in patients with 1st HE. These findings suggest that survival of HE may be improved after anti-viral agent such as lamivudine has been available in HBV-endemic area.

Disclosures:

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Layla Hajjafar1,2, Mendel Singer2, Anthony Post1; 1Gastroenterology and Hepatology, Case Medical Center, Cleveland, OH; 2Epidemiology and Biostatistics, Case Western Reserve University, Cleveland, OH

Study Aim: There is no gold standard treatment for refractory ascites in cirrhotics, so we conducted a cost-effectiveness analysis comparing transjugular intrahepatic portosystemic shunt (TIPS) vs. repeated large volume paracentesis (LVP) with albumin infusion in this population. Methods: We performed a decision analysis using a Markov model in a hypothetical cohort of identical 40 year old cirrhotics with newly-diagnosed refractory ascites. The two treatment modalities compared were TIPS and repeated LVP with albumin infusion. Patients failing one treatment were automatically switched to the other. Using Monte Carlo simulation, these patients were followed until death. Estimates of treatment outcomes were obtained from 3 randomized controlled trials (RCTs). Costs were determined by Medicare reimbursement and health state utilities were assigned based on previous data in the literature when available. Both costs and utilities were discounted at a rate of 3% per year. We calibrated our model to produce 1 and 2-year mortality rates consistent with those defined by the 5 RCTs in the literature: 38% and 50%, respectively for TIPS and 43.5% and 57%, respectively for LVP. Results: The incremental cost-effectiveness ratio (ICER) was $36,451 per additional quality adjusted life-year (QALY) for TIPS when compared to repeated LVP at a rate of 1.5 LVPs/month (refer to table). When compared to repeated LVP at a frequency of 1 LVP/month, the ICER increased to $52,335/QALY. Conclusion: TIPS is cost-effective in the treatment of refractory ascites when compared to repeated LVP with albumin infusion at a frequency of greater than 1 LVP/month.

Baseline Analysis at 1.5 LVPs/month per patient lifetime

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Cost ($)</th>
<th>Cost Effectiveness (QALY)</th>
<th>ICER (QALY)</th>
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<tr>
<td>TIPS</td>
<td>261,641</td>
<td>3.34</td>
<td>36,451</td>
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<tr>
<td>LVP</td>
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814 PULMONARY DYSFUNCTION AND THE HEPATOMONARY SYNDROME (HPS) IN CIRRHOSIS: PREVALENCE AND EFFECTS OF OXYGEN INHALATION
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Background/Aims. Circulatory and pulmonary complications with gas exchange abnormalities are common in patients with cirrhosis. The prevalence of the hepatopulmonary syndrome (HPS) is variably reported from 8 to 20% but its precise relation to the liver function is unclear. The aims of the present study were therefore 1. to assess the prevalence of the HPS in consecutive patients with cirrhosis in relation to patient characteristics and 2. during a 100% oxygen test, to assess the cardiopulmonary effects and peripheral oxygenation in patients and in matched controls. Patients and Methods. Fifty patients (19 women and 31 men) with alcoholic cirrhosis (Child class A/B/C: 18/21/11) and 12 healthy matched controls were entered in the study. All underwent haemodynamic and pulmonary investigations including measurement of lung diffusion capacity (TLCO), a contrast-enhanced echocardiography (CEE), and a lung scan with determination of the extrapulmonary shunt fraction (EPSF) to diagnose HPS. A 100% oxygen test was performed with the assessment of PaO2, the alveolar-arterial oxygen gradient (AaPO2), and peripheral transcutaneous oxygen tension (tcPO2). Results All patients had portal hypertension and a hyperdynamic circulation (p<0.05). The EPSF was normal in the controls. Five patients had an EPSF fraction above 6% and a positive CEE. Three of these patients had a PaO2 below 10.7 kPa giving a frequency of HPS of 6%. PaO2 was reduced below 10.7 kPa in 39% and AaPO2 above 2.7 kPa in 60% of the patients and PaO2 correlated with EPSF (r=0.31, p<0.05). TLCO was reduced in 72% of the patients and correlated significantly with markers of metabolic liver function (p=0.01), splanchnic haemodynamics (p=0.004) and systemic haemodynamics (p=0.006). After oxygen inhalation, the change in heart rate and MAP were smaller in the patients (p<0.02) and PaO2 and AaPO2 increased to only 75% of the level in the controls.
TERLIPRESSIN THERAPY FOR RENAL DYSFUNCTION AND HYponatREMIA IN PATIENTS WITH LIVER FAILURE

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Background: Indications for terlipressin use other than hepatorenal syndrome (HRS) and variceal hemorrhage are poorly defined. Therefore, we performed an analysis of terlipressin therapy for renal dysfunction and hyponatremia in patients with liver failure. Methods: A retrospective analysis was performed on all patients at a large single tertiary referral centre, who received terlipressin for a minimum of 24 hours. Biochemical response was defined as: (a) the return of creatinine to ≤133 µmol/L in HRS, (b) normalization of creatinine (≤110 µmol/L) in renal failure other than HRS, and (c) an increase in serum sodium level by 5 mmol/L from baseline in hyponatremia (Na <130 mmol/L) without renal failure. Results: Between August 2003 - February 2007, 85 patients (61 male, 24 female) with median age of 52 years (range 27-78) were treated with terlipressin. Seventy-six patients received 1 course of terlipressin, 6 received 2 courses, and another 3 received 3 courses (total terlipressin courses = 97). Indications for terlipressin were HRS1 (13), HRS2 (41), renal failure other than HRS (18), and hyponatremia without renal failure (25). Median duration of treatment was 6 days (range 1-60). Baseline MELD score was >25 in 79% of patients. Etiology of liver failure was mostly viral hepatitis (42%) and alcohol (41%). For all indications, terlipressin resulted in an overall improvement in creatinine from a median of 164 to 116 µmol/L (p<0.05) and an increase in serum sodium from a median of 128 to 134 mmol/L (p<0.05). Response rates for HRS, renal failure other than HRS and hyponatremia without renal failure were 43%, 39% and 76% respectively. There were 26 adverse events (19 pulmonary edema, 2 cyanosis, 3 chest pain, 1 livedo reticularis and 1 bleeding ischaemic gastric ulcers). After the initial terlipressin therapy, the follow-up duration was 27 days (range 1-1248). Thirteen (15%) patients underwent liver transplantation and 56 (66%) died including 2 who were transplanted. The 30-day survival rates were 31%, 52%, 31% and 67% for HRS1, HRS2, renal failure other than HRS and hyponatremia without renal failure respectively. Conclusions: Terlipressin therapy resulted in improved renal function and increased serum sodium in those with advanced liver failure. However, its use is also associated with significant side effects. This is the largest study to date of terlipressin use and the first to demonstrate the efficacy of terlipressin in treating hyponatremia without renal failure in advanced liver failure.

MONOCYTE HLA-DR EXPRESSION IS A PROGNOSTIC MARKER FOR MORTALITY IN PATIENTS WITH ACUTE ON CHRONIC LIVER FAILURE

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Background: Acute on chronic liver failure (ACLF) is associated with a poor prognosis and an increased susceptibility to infections. Recently, we showed that monocyte HLA-DR expression is decreased in patients with ACLF compared to healthy controls and subjects with stable liver cirrhosis (Wasmuth et al. J Hepatol 2005; 42: 195-201). However, the prognostic value of HLA-DR expression has not been systematically investigated
of plasma VEGF and sVEGF R1 was observed in liver cirrhosis of various etiologies by ELISA. Results. The significant increase in growth factor and its soluble receptors: sVEGF R1 and sVEGF R2 levels in all cirrhotic patients were increased respectively to the degree of liver insufficiency. It was demonstrated through a significant positive correlation with Child-Pugh score: r=0.38, P<0.001 for VEGF and r=0.77, P<0.001 for VEGF R1. The similar relationship was observed in respect to MELD classification: r=0.41, P<0.01 for VEGF and r=0.56, P<0.001 for sVEGF R2. In conclusion, our study suggests that serum VEGF and VEGF R1 may reflect the hepatic function impairment in liver cirrhosis and seems to be associated with portal hypertension symptoms. Disclosures: The following people have nothing to disclose: Jerzy Jaroszewicz, Robert Flisiak

818 CIRCULATING VASCULAR ENDOTHELIAL GROWTH FACTOR AND ITS SOLUBLE RECEPTORS IN PATIENTS WITH LIVER CIRRHOSIS: POSSIBLE ASSOCIATION WITH HEPATIC FUNCTION IMPAIRMENT

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Recent studies provided in vivo evidences of an increased angiogenesis in animal model of portal hypertension and cirrhosis which was linked to increased expression of vascular endothelial growth factor. The aim of present study was to evaluate the plasma concentration of VEGF and its receptors in liver cirrhosis of various etiologies and the possible association with the degree of liver insufficiency. Methods. Vascular endothelial growth factor and its soluble receptors: sVEGF R1 and sVEGF R2 were measured in plasma of 78 patients with liver cirrhosis of various etiologies by ELISA. Results. The significant increase of plasma VEGF and sVEGF R1 was observed in liver cirrhosis compared to healthy individuals (153.1±51.9 pg/mL, P<0.05; 279.8±34.3 vs. 105.1±5.9 pg/mL, P<0.001, respectively). On the contrary VEGF R2 plasma concentration was lower in liver cirrhosis then in control group. Differences in plasma VEGF as well as its receptors R1 and R2 were not significant in patients with liver cirrhosis of various etiologies. Plasma VEGF and foremost sVEGF R1 showed significant associations with biochemical indices of liver function. Among clinical parameters of liver dysfunction only ascites revealed significant association with plasma VEGF and sVEGF R1. Plasma sVEGF R1 and VEGF, but not VEGF R2 levels in all ascites vs no ascites (§) p<0.05 ; pre vs post TIPS (±) p>0.05

819 ACCURATE ASSESSMENT OF CARDIAC FUNCTION AND MYOCARDIAL FIBROSIS IN LIVER CIRRHOSIS BY CONTRAST-ENHANCED CARDIAC MAGNETIC RESONANCE

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Liver cirrhosis is associated with vasodilatation and hyperdynamic circulation affecting cardiac function. Contrast-enhanced Cardiac Magnetic Resonance (CMR) is a new diagnostic tool for the evaluation of cardiac volumes, systolic and diastolic function and is also able to detect myocardial fibrosis (MF). We investigated 22 cirrhotic patients (all males, 56 ± 7 yrs, Child-Pugh A/ B-C 7/15 patients: 9 without ascites, 13 with ascites [actual]). Nine patients were re-evaluated 7 days after elective Transjugular Intrahepatic Portosystemic Shunt (TIPS). Six healthy males matched for age were used as controls. CMR study (Siemens Avanto 1.5 T, Germany) including cine steady state free-presssure (SSFP) imaging and velocity encoded cine MR (VENC) sequences was performed in all patients. Delayed Enhancement (DE) acquisitions were used for MF detection. Left End Dyastolic (LEDV), and Systolic Volume (LESV), Cardiac Output (CO), E/A ratio, Ejection Fraction (EF%), and Systemic Vascula r Resistances (SVR) were calculated. Plasma Renin Activity (PRA) was also measured. Statistical Analysis: mean ± SD, “t” test for unpaired and paired data. Cirrhotic patients showed increased CO, and decreased SVR and Mean Arterial Pressure than controls. MF was detected in 3 patients (13%). The behaviour of cardiac parameters in patients with and without ascites and before and after TIPS are shown in Table 1. Patients with ascites showed a significant reduction in ESV and E/A ratio, while PRA was higher. Seven days after TIPS procedure CO, LEDV, E/A ratio and EF significantly increased, while SVR and PRA decreased. CMR allows an accurate assessment of cardiac function (particularly in volumes acquisition) in patients with liver cirrhosis and may be a useful in the evaluation of patients before and after TIPS procedure.

Table 1

<table>
<thead>
<tr>
<th>CO (L/min)</th>
<th>Ascites</th>
<th>No ascites</th>
<th>Pre TIPS</th>
<th>Post TIPS</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.2 ± 0.9</td>
<td>5.1 ± 0.3</td>
<td>5.4 ± 1</td>
<td>6.6 ± 1.1</td>
<td></td>
</tr>
<tr>
<td>SVR (dyne/cm²)</td>
<td>1367 ± 363</td>
<td>1406 ± 366</td>
<td>1516 ± 365</td>
<td>787 ± 124</td>
</tr>
<tr>
<td>LEDV (ml)</td>
<td>106.4 ± 28.1</td>
<td>126 ± 19.9</td>
<td>106.1 ± 23.5</td>
<td>120.7 ± 43</td>
</tr>
<tr>
<td>LESV (ml)</td>
<td>35.8 ± 12.7</td>
<td>51.7 ± 10.3</td>
<td>42.4 ± 13.3</td>
<td>36.1 ± 11.6</td>
</tr>
<tr>
<td>E/A</td>
<td>0.90 ± 0.01</td>
<td>0.96 ± 0.04</td>
<td>0.90 ± 0.05</td>
<td>1.0 ± 0.05</td>
</tr>
<tr>
<td>EF (%)</td>
<td>61.6 ± 9.4</td>
<td>59.1 ± 6.3</td>
<td>58.2 ± 6.9</td>
<td>72.2 ± 6.1</td>
</tr>
<tr>
<td>PRA (ng/mL)</td>
<td>19.6 ± 8.2</td>
<td>7.3 ± 10.7</td>
<td>22.7 ± 5.4</td>
<td>14.3 ± 9.7</td>
</tr>
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ascites vs no ascites (§) p<0.05 ; pre vs post TIPS (±) p>0.05
820

CLINICAL COURSE AND PROGNOSIS OF CULTURE-NEGATIVE NEUTROCYTIC ASCITES AND SPONTANEOUS BACTERIAL PERITONITIS

Nuria Cañete1, Eva Erice2, Isabel Cirera1, Anna Bargalló2, Rosa Maria Morillas2,Montserrat Garcia-Retortillo1, Susanna Coll1, Felip Bory1, Helena Masnou2, Maria Dolors Giménez1, Ricard Solà1, Ramon Planas2

1Liver Section, Digestive Service, Hospital del Mar, Barcelona, Spain; 2Liver Section, Digestive Service, Hospital Germans Trias i Pujol, Badalona, Spain

Spontaneous infections of ascitic fluid include the classical spontaneous bacterial peritonitis (SBP) and culture-negative neutrocytic ascites (CNNA), diagnosed when the ascitic fluid culture grows no bacteria, and the ascitic fluid polymorphonuclear cell count is >250 cells/mL. CNNA has been considered as a variant of SBP, with a similar short- and long-term course of patients with either condition. AIM: To assess the clinical course and prognosis of SBP and CNNA in patients prospectively followed up after their first ascites decompensation. PATIENTS AND METHODS: From August 1997 to December 2002, 263 consecutive cirrhotic patients who developed the first episode of ascites were prospectively followed for a median of 41±3 months. Analysis of ascitic fluid was performed in all patients presenting symptoms of infection or when their clinical condition deteriorated. Laboratory analysis of ascitic fluid included total and differential cell counts, protein levels and bacteria cultures obtained by bedside inoculation of 10 mL of ascitic fluid into culture blood bottles (BACTEC). RESULTS. Fifty-eight patients developed 83 episodes of ascitic infection, being community-acquired in 60 cases (72%). Forty-four episodes (53%) were CNNA and 39 (47%) culture-positive SBP. Escherichia coli was the most frequently isolated organism (25 cases), followed by Streptococcus (10), and Enterococcus, Klebsiella, Staphylococcus and Morganella (1 case each). At admission, hepatic and renal function in patients with CNNA were significantly better than those from SBP group as indicated by higher prothrombin index (62 vs. 49%; p<0.001) and serum albumin (33±0.9 vs. 29±0.9 g/L; p<0.02), lower incidence of encephalopathy (18 vs. 46%; p<0.05), as well as lower Child-Pugh score (8.9±0.3 vs. 10.1±0.3; p<0.01) and MELD score (13.3±1.1 vs. 18.9±1.2; p<0.001). Moreover, ascitic polymorphonuclear cell count was lower in CNNA group than in SBP group (1256±252 vs. 5513±1117 cells/mL; p<0.001), and ascitic fluid infection resolved more quickly in CNNA patients than in SBP patients (4.2±0.3 vs. 6.7±0.9 days; p<0.01). CNNA group had a lower incidence of infection-induced renal failure than SBP group (18.2 vs. 38.5%; p=0.04). Although hospital mortality was similar in both groups (27 vs. 41%; NS), there was a trend forward to a lower cumulative mortality at 1-year in CNNA group (34 vs. 57%; p=0.09). CONCLUSION: CNNA occurs mostly in cirrhotic patients with less severe disease and seems to have a better clinical course and prognosis than SBP.

Disclosures:
The following people have nothing to disclose: Nuria Cañete, Eva Erice, Isabel Cirera, Anna Bargalló, Rosa Maria Morillas, Montserrat Garcia-Retortillo, Susanna Coll, Felip Bory, Helena Masnou, Maria Dolors Giménez, Ricard Solà, Ramon Planas
822 HISTOLOGICAL PREDICTORS OF OESOPHAGEAL VARICES: A RETROSPECTIVE SINGLE CENTRE STUDY

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Background: Clinically significant portal hypertension (CSPH) is defined as a hepatic vein portal gradient of >12mm of Hg and may be complicated by the development of oesophageal varices. Studies have suggested that small nodularity and septal thickness correlates with CSPH 1 and a histological sub-classification of cirrhosis has been suggested. Aims: The aim of this study was to determine if the nodule size and septal thickness on histology were predictive variables for endoscopic presence of oesophageal varices. Methods: Patients with established cirrhosis were randomly selected from an endoscopy and histology database. Biopsies were retrospectively reviewed by two operators (TT & SM) who were blinded to the presence or absence of varices on endoscopy. Nodules were classified as small, mixed or large based on width of the nodule in relation to biopsy size. The septal thickness was classified as thin, medium or thick. Results: Of the 95 slides reviewed, 8 were excluded as they did not have histologically established cirrhosis leaving 87 slides (Males: 54, median age: 53 years (IQR: 48-59)) for the final analysis. In 49% of cases the etiology was alcoholic liver disease. The median biopsy size was 15mm (IQR: 12-20) and the median number of portal tracts was 3 (IQR: 2-4). The number of patients with small, mixed and large nodules was, 34, 32, and 21 respectively and the number with thin, medium and thick septae was 44, 22, and 21 respectively. There was a significant correlation between both nodule size (Rho 0.2, 95% CI 0.002-0.40, two sided p=0.05) and septal thickness (Rho 0.51, 95% CI, 0.34-0.65, two sided p=0.0001) with the endoscopic presence of varices. However the correlation between small nodule size and endoscopic presence of varices was weak. Conclusion: Small nodule size and thick septae are predictors of the presence of oesophageal varices on endoscopy. Patients with the above histological variables should be clinically prioritised to have an early endoscopy. 1. Nagula S, Danpat J, Grossmann RJ et al. Histological- haemodynamic correlation in cirrhosis: a histological classification of the severity of cirrhosis Journal of Hepatology 2006; 44: 111-117

Disclosures: The following people have nothing to disclose: Titus Thomas, Sindu Menon, David Semeraro, Gerry Mortimer, Vincent Lai, Andy S. Austin, Jan G. Freeman

824 MEDICATION ADHERENCE TO BETA-BLOCKER THERAPY FOR PRIMARY PROPHYLAXIS OF ESOPHAGEAL VARICEAL HEMORRHAGE IN A VETERAN POPULATION

Katarina Greer1, Claudia O. Zein2; 1Department of Medicine, Case Western Reserve University, Louis Stokes Cleveland VAMC and University Hospitals of Cleveland, Cleveland, OH; 2Division of Gastroenterology and Hepatology, Case Western Reserve University-Louis Stokes Cleveland VAMC, Cleveland, OH

Background: Non-cardioselective beta-blockers (BB) are the mainstay of primary prophylaxis (PP) of esophageal variceal hemorrhage (EVH). BB decrease the rate of EVH and EVH-related mortality. BB are also associated with adverse effects and adherence to therapy can be a challenge. Aims: To assess BB adherence and discontinuation among veterans prescribed BB for PP of EVH. Methods: All patients with EV diagnosis seen by medication possession ratios based on pharmacy refill records. Inadequate adherence was defined as possession ratio < 0.75. Statistical analyses were performed. Results: 340 patients with EV were identified; 98 were excluded because of EVH before or at EGD. Of the remaining 242, 138 (57%) were on BB. Mean follow-up was 2.9 years. Inadequate adherence was recorded in 64/137 (47%) of subjects. Patients with inadequate adherence were more likely to have subsequent EVH compared to those adherent to therapy (Log rank test, p=0.001), and more likely to have EVH or die (combined outcome)(p=0.003, Figure). By Proportional Hazards Fit this association was independent of severity of liver disease and other variables. Conclusions: 1. Inadequate adherence to BB therapy for PP of EVH is frequent in the veteran population; 2. In this cohort, patients with inadequate adherence to BB had higher rates of subsequent EVH and death; 3. Studies to identify ways to improve adherence to PP in this population, as well as of possible preferential use of other PP modalities.

823 SERUM-ASCITES ALBUMIN CONCENTRATION GRADIENT (SAAG) MORE THAN 1.5 G/DL PREDICTS ESOPHAGEAL VARICEAL HEMORRHAGE

Pises Pisesponsa, Suthinee Sripotong, Taned Chitapanarux, Apinya Leerapun, Satawat Thongsawat, Ong-ard Praisontarangkul; Department of Internal Medicine, Chiang Mai University, Chiang Mai, Thailand

Background: Although serum-ascites albumin concentration gradient (SAAG) was strongly correlated with portal hypertension, the significance of SAAG to predict complications of cirrhosis was less well defined. We conducted a retrospective cohort study to determine the correlation of SAAG level and complications of cirrhosis, namely esophageal variceal bleeding (EVB), spontaneous bacterial peritonitis (SBP), and hepatic encephalopathy (HE). Methods: One hundred and eight cirrhotic patients with ascites and high SAAG (>1.1 g/dl) were included. The patients were divided into 3 groups according to the degree of SAAG: Group A (SAAG 1.1-1.49 g/dl), Group B (SAAG 1.5-1.99 g/dl), and Group C (SAAG > 2 g/dl) and complications of cirrhosis were reviewed. Results: Twenty-six patients (29%) were alcoholic cirrhosis. Among the three groups (A, B, C), EVB was found in 5.9% (2 of 34), 30.8% (12 of 39), and 34% (12 of 35) respectively (p=0.008). SBP was also found in 17.6% (6 of 34) 30.8% (12 of 39), and 14.2% (5 of 35) respectively (p=0.013), but there was no significant difference in the rate of HE occurrence. Conclusion: Complications of cirrhosis were associated with the degree of SAAG. Patients with SAAG value of > 1.5 g/dl are likely to have EVB. Thus, prompt primary prophylaxis may be warranted. However the prospective study in larger number of patients is needed to confirm these results.

<table>
<thead>
<tr>
<th></th>
<th>Group A (34)</th>
<th>Group B (39)</th>
<th>Group C (35)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>EVB</td>
<td>2 (5.9%)</td>
<td>12 (30.8%)</td>
<td>12 (34%)</td>
<td>0.008</td>
</tr>
<tr>
<td>SBP</td>
<td>6 (17.6%)</td>
<td>12 (30.8%)</td>
<td>5 (14.3%)</td>
<td>0.003</td>
</tr>
<tr>
<td>HE</td>
<td>10 (29.4%)</td>
<td>14 (35.9%)</td>
<td>12 (34.3%)</td>
<td>0.23</td>
</tr>
</tbody>
</table>

Disclosures: The following people have nothing to disclose: Pises Pisesponsa, Suthinee Sripotong, Taned Chitapanarux, Apinya Leerapun, Satawat Thongsawat, Ong-ard Praisontarangkul.
Selective impairments of covert visual attention and movement preparation in minimal hepatic encephalopathy

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Background/Rationale: Detecting cognitive impairment in cirrhotic patients is challenging until crude psychomotor slowing and impaired vigilance manifest in advanced stages. We utilized N2pc and lateralized readiness potential (LRP) event-related potentials (ERPs) to enable eloquent temporal characterization of disordered visual attention and motor preparation in minimal hepatic encephalopathy (MHE). Methods: We recorded ERPs from 10 MHE subjects and 10 age-matched controls. ERPs were recorded from standard 10-20 placements in subjects performing a feature-conjunction visual-search task. Data analysis compared ERP waves from equivalent channels ipsilateral and contralateral to the target for N1, N2pc, LRP, and P3 waves. Results: LRP latency was significantly delayed in MHE (MHE=774ms, control=609.6ms, p<.01). N2PC onset latency was also significantly delayed in MHE using the jackknife-based scoring method (MHE=268.8ms, Control=217.6, difference=51.2ms; Fc=3.24, p<.05). N1 and P3 latencies were not significantly different between MHE and control subjects. Conclusions: Specific impairments in visual attention and movement preparation underlie MHE, sparing stimulus registration and categorization, implying selective cerebral dysfunction from regional alteration of neurotransmission or deposition of hepatic neurotoxins. N2pc and LRP ERPs deserve further study as potential biomarkers for MHE.

Disclosures: The following people have nothing to disclose: Katarina Greer, Claudia O. Zein

825

Survival fee of EVH in patients wit EV with inadequate adherence to BB primary prophylaxis compared to those with adequate adherence

Background:

(i.e. primary band ligation) in populations at high risk of inadequate adherence to BB are warranted.

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Lasting amelioration in the clinical course of decompensated alcoholic cirrhosis with boost infusions of mobilized peripheral blood stem cells

Dimitrios Kapetanos1, Evangelia Yannaki2, Achilles Anagnostopoulos2, Angeliki Xagorari2, Fotis Iordanidis2, Ioannis Batis2, Panayotis Kaloyannidis2, Evangelia Athanasiou2, Georgios Dourvas2, Athanasios Fassas2, George E. Kits1; 1Gastroenterology Department, George Papanikolaou Hospital, Thessaloniki, Greece; 2Hematology Department-BMT Unit and Gene and Cell Therapy Center, George Papanikolaou Hospital, Thessaloniki, Greece; 3Pathology Department, George Papanikolaou Hospital, Thessaloniki, Greece

Background and aims: Liver transplantation is the only definite cure for decompensated cirrhosis, but donor livers are lacking and many patients die while waiting for transplantation. There is accumulating evidence that hematopoietic stem cells (HSCs) contribute to liver regeneration and infusion of autologous mobilized HSCs may be an alternative or bridging procedure until transplantation. We studied the safety and efficacy of infusions of autologous mobilized HSCs in patients with decompensated alcoholic cirrhosis while waiting for liver transplantation. Methods: Ten patients with decompensated alcoholic cirrhosis with at least 6 months’ abstinence were screened. Four patients were enrolled and the rest were excluded due to excessive splenomegaly and thrombocytopenia. G-CSF 10 µg/kg/day was administered for 4-5 days before HSCs were collected. When more than 10 CD34+ cells/µL were detected, leukapheresis started, with target CD34+ cell yield at least 5x106/kg. Three mobilization courses were performed and mobilized stem cells were collected and reinfused to the patients. Performance status, Child-Pugh and MELD score as well as hospitalizations due to complications of cirrhosis were recorded. Results: Two patients (P1, P2) completed the three cycles, the third (P3) is on the third cycle and the fourth (P4) on the second. The procedure was well tolerated without serious toxicities. P1 and P2 had no hospitalization during follow up of 30 and 41 months respectively, in contrast to repeated admissions before the treatment, due to complications of cirrhosis. The Child-Pugh score dropped 3 and 1 points and the MELD score 6 and 5 points respectively. P1 underwent liver transplantation 30 months after enrollment but he suffered cardiac arrest and died. P2 is on steady state 41 months after enrollment. P3 and P4 have improved disease indices and performance status, 5 months after the second and first cycle respectively. VEGF-ELISA in the serum of P1 and CD34 count in a liver biopsy of P2 suggested that hepatic angiogenesis, induced by peripheral blood stem cells (PBSCs) or by G-CSF, might contribute to the observed improvement. Another possible explanation is direct transformation of PBSCs to hepatocytes. Conclusion: Our preliminary results suggest that infusion of autologous PBSCs may convert decompensated cirrhosis to compensated and may serve as a bridge to transplantation.

Disclosures: The following people have nothing to disclose: Dimitrios Kapetanos, Evangelia Yannaki, Achilles Anagnostopoulos, Angeliki Xagorari, Fotis Iordanidis, Ioannis Batis, Panayotis Kaloyannidis, Evangelia Athanasiou, Georgios Dourvas, Athanasios Fassas, George E. Kits
827 PROTECTIVE EFFECTS OF ERYTHROPOIETIN IN RATS WITH CIRRHOTIC CARDIOMYOPATHY

Hongquan Liu, Annie Feng, Samuel S. Lee; University of Calgary, Calgary, AB, Canada

Background: Erythropoietin (EPO) is a major regulator of erythropoiesis. In addition, EPO receptor 1 (EPOR1) is found in rat heart. EPO can protect cardiomyocytes from ischemic injury, attenuate myocardial inflammation by suppression of tumor necrosis factor (TNFα) and improve cardiac contractile function. Similar to ischemic cardiomyopathy, cirrhotic cardiomyopathy shows decreased beta-adrenoceptors (β-AR) and decreased systolic and diastolic responses to stress stimuli. To date, no medical therapies have been shown to improve contractile function in cirrhotic cardiomyopathy. Therefore, it is crucial to explore possible new therapeutic agents for this condition. Methods: Rats were divided into 4 groups, sham control; sham + EPO; bile duct ligation (BDL); and BDL + EPO (n=6 in each group), and were studied 4 wk after surgery. EPO (1000 U/kg body weight every other day, i.p.) or equivolumic normal saline control injections were administered starting 10 days before the study date. Samples were collected for the measurement of TNFα, and EPOR1 expression. In separate groups of rats, isolated cardiomyocytes were subjected to contractile and relaxation function studies. Results: TNFα was significantly increased in BDL heart compared with sham control (345 ± 48 vs 250 ±18 pg/mg protein) and sham+EPO group (p<0.01). EPO significantly decreased TNFα expression in the BDL group (345 ± 48 vs 257 ± 32 pg/mg protein, p<0.01). There was no statistical difference between the sham control, sham + EPO and BDL + EPO groups. Western blot analysis showed that EPOR1 protein expression was significantly increased in the left ventricle from BDL compared with those from sham controls (P < 0.01). Isoproterenol-stimulated maximal systolic velocity in isolated cardiomyocytes was significantly decreased in cirrhotic rats compared with sham control [4.26 ± 1.09 vs 7.82 ± 2.27 µm/s, p<0.05). EPO significantly reversed the depressed contractility in the cirrhotic cardiomyocytes (4.26 ± 1.09 vs 8.26 ± 1.30 µm/s, p<0.01). Maximal diastolic velocity was also slower in cirrhotic rats compared with the sham group (2.21 ± 0.88 µm/s vs 5.53 ± 1.57, p<0.05). EPO corrected the diastolic velocity in BDL hearts (2.21 ± 0.88 vs 6.06 ± 0.88 µm/s, p<0.01). Conclusions: EPO receptors were increased in cirrhotic hearts. EPO treatment significantly decreased TNFα concentration and reversed the impaired systolic and diastolic function in cirrhotic cardiomyocytes. These results suggest that erythropoietin mediates at least part of its cardiotrophic effect by inhibiting the TNFα pathway, and may be a potential treatment for cirrhotic cardiomyopathy.

Disclosures:
Hongquan Liu - Grant/Research Support: Ortho
Annie Feng - Grant/Research Support: Ortho
Samuel S. Lee - Grant/Research Support: Ortho

828 EFFECT OF LONG-TERM INHIBITION OF ACID GASTRIC SECRETION ON GASTRIC PH AND ON BACTERIAL TRANSLOCATION IN CIRRHOTIC RATS

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Background: Bacterial translocation, as a consequence of intestinal bacterial overgrowth, plays an important role in the pathogenesis of bacterial infections in cirrhosis. Long-term inhibition of acid gastric secretion promotes intestinal bacterial overgrowth and could favour bacterial translocation. Aim: to evaluate the effect of long-term inhibition of acid gastric secretion on bacterial translocation in cirrhotic rats with or without ascites. Material and Methods: Cirrhotic rats with and without ascites induced by oral carbon tetrachloride were randomized to be treated with a daily subcutaneous injection of placebo, ranitidine (100mg/kg) or pantoprazole (8mg/kg) during two weeks. A control non-cirrhotic group were also randomized. The first day of the study (basal) and the previous day to the laparotomy a continuous 2 hour pH-metry under anesthesia was performed to evaluate the effect of antisecretory therapy. A laparotomy was performed to obtain samples of blood, mesenteric lymph nodes, ascites, pleural fluid, spleen, liver and cecal stools for culture. Results: Mortality was significantly higher in cirrhotic rats with ascites than in nonascitic cirrhotic rats (p<0.01) and in control rats (p<0.001) during the study, but no differences were observed in rats treated with placebo vs antisecretory drugs. Ranitidine and pantoprazole significantly increased gastric pH compared to rats treated with placebo (p<0.001). Administration of antisecretory drugs did not modify the incidence of bacterial translocation in nonascitic rats and control rats. In contrast, a higher incidence of bacterial translocation was observed in ascitic rats treated with ranitidine (7/11, p<0.05), pantoprazole (7/12, p=0.1) or any antisecretory therapy (14/23, p<0.05) compared to placebo treated ascitic rats (3/14) and to nonascitic rats treated with the same antisecretory drug (p<0.05, p=0.06 and p<0.01, respectively). Despite gastric pH previous to laparotomy was significantly higher in cirrhotic rats treated with pantoprazole than with ranitidine (p<0.001), the incidence of bacterial translocation was similar in both groups. Conclusions: Inhibition of acid gastric secretion increased acid gastric pH in control and cirrhotic rats with or without ascites, but the incidence of bacterial translocation only in cirrhotic rats with ascites. A similar incidence of bacterial translocation was observed in ascitic cirrhotic rats treated with any antisecretory therapy.

Disclosures:
The following people have nothing to disclose: Elisabet Sanchez, German Soriano, Beatriz Mirelis, Begoña Gonzalez, Carlos Guarnier, Ingrid Ordas, Joan Mones, Carlos Guarnier

829 L-ORNITHINE PHENYLACETATE REDUCES ARTERIAL AMMONIA, IMPROVES BRAIN OSMOLYTES AND REDUCES BRAIN WATER IN A BILE DUCT LIGATED RAT MODEL OF CIRRHOSIS

Gavin A. Wright1, Nathan A. Davies 1, Lars M. Ytrebø 2, Vanessa Stadlbauer1, Ole-Martin Fuskvåg2, Claudia Zwingmann4, Stephen Hodgson3, Rajiv Jalan1; 1The Department of Medicine, The Institute of Hepatology, UCL, London, United Kingdom; 2The Department of Anaesthesiology, University Hospital of Northern Norway and University of Tromsø, Tromsø, Norway; 3The Department of Clinical Pharmacology, University Hospital of Northern Norway and University of Tromsø, Tromsø, Norway; 4Center of Research, Department of Medicine, University of Montreal, Montreal, QC, Canada

Introduction: Treatment of hyperammonemia and HE in cirrhosis are unmet clinical needs. In cirrhosis, the muscles can detoxify ammonia into glutamine. This concept has been exploited by the drug L-ornithine L-aspartate, but the glutamine produced regenerates ammonia in the gut and kidneys by the action of glutaminase. This study tests the hypothesis that L-ornithine-phenylacetate (OP) would reduce hyperammonemia by L-ornithine acting as a substrate for glutamine (Gln) synthesis (from ammonia) in skeletal muscle, and phenylacetate com-
bining with this Gln to form phenylacetylglutamine (PAG) which is then excreted in urine. **Methods**: Rats were studied 6 weeks after bile-duct ligation (BDL) or Sham-operation. The BDL rats were treated for 1 week with high protein/ammoniagenic diet. Three hours before termination, intraperitoneal administrations of either, phenylbutyrate (pro-drug; 0.3gm/Kg) or L-ornithine (0.3gm/Kg alone; 'OP' (0.3 gm/Kg of each) or placebo was given. Hourly arterial samples were collected for ammonia and urine for PAG. Samples collected at termination were measured for; Brain water (dry weight); Brain tissue osmolytes (1H-NMR spectroscopy); and muscle glutamine synthetase (GS) activity. **Results**: Compared to Sham-operated rats, BDL rats had significantly increased plasma ammonia (p<0.001) and glutamine/myo-inositol (Gln/mI) ratios (p<0.001); brain water content (p<0.01) and perivascular edema. Administration of OP to BDL rats significantly reduced plasma ammonia (2-way ANOVA, p<0.05) and brain water (p<0.05) versus the other 3 groups and ameliorated low-grade edema histologically. The Gln/mI ratio (p<0.01) was lowered to values not significantly different to Sham-operated rats which was not observed with L-ornithine or phenylbutyrate administration alone. These changes were associated with significantly increased urinary PAG and muscle GS activity (p<0.01 and p<0.05, respectively). **Conclusion**: The results show that OP reduces ammonia significantly in cirrhosis by increasing muscle ammonia detoxification and elimination of the ornithine related glutamine as urinary PAG. Also, by reducing plasma ammonia, OP leads to normalisation of brain osmolytes and brain water content suggesting a reduction of edema. These observations provide the rationale for further development of OP as a drug for clinical trials in patients with HE.

<table>
<thead>
<tr>
<th></th>
<th>Sham-operated (n=7)</th>
<th>BDL + placebo (n=7)</th>
<th>BDL + OP (n=8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brain water content (%)</td>
<td>76.1 ± 0.4</td>
<td>77.9 ± 0.4**</td>
<td>76.01 ± 0.6**</td>
</tr>
<tr>
<td>Gln/mI ratio</td>
<td>0.83 ± 0.06**</td>
<td>1.32 ± 0.02</td>
<td>0.95 ± 0.04**</td>
</tr>
<tr>
<td>Urinary PAG (mmol/L)</td>
<td>0.05 ± 0.16</td>
<td>0.5 ± 0.14</td>
<td>11.46 ± 3.26**</td>
</tr>
</tbody>
</table>

*p<0.05, **p<0.01 versus Sham-operated rats

Disclosures:
The following people have nothing to disclose: Junlan Zhang, Jo Morrison, Jeannette Doeller, Yongming Wang, Michael B. Fallon

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830 EVALUATION OF PULMONARY HYDROGEN SULFIDE GENERATION IN EXPERIMENTAL HEPATOPULMONARY SYNDROME (HPS)

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**Introduction**: Hydrogen sulfide (H₂S) has emerged as a third endogenous gaseous signaling transmitter and a vasodilator, alongside nitric oxide (NO) and carbon monoxide (CO). Deficient hepatic H₂S production, due to a reduction in cystathionine γ-lyase (CSE) the main enzyme forming H₂S in peripheral tissues and vascular system, in experimental biliary cirrhosis (common bile duct ligation, CBDL) has recently been postulated to contribute to portal hypertension. CBDL animals also develop intrapulmonary vasodilatation and HPS and NO and CO contribute to this effect. However, the potential role of H₂S in the pulmonary vascular abnormalities of HPS has not been explored. **Aim**: To characterize pulmonary and hepatic CSE expression and H₂S generation in experimental HPS in relation to non-cirrhotic portal hypertension (partial portal vein ligation, PVL). **Methods**: Control, 1, 2, 3 and 4 week CBDL and 3 week PVL Sprague-Dawley rats underwent evaluation. The physiologic features of HPS were evaluated using ABGs and microsphere shunting. Western blot analysis and quantitative RTPCR were used to assess CSE protein and mRNA expression in lung and liver samples. H₂S generation in tissue homogenates were measured using a respirometer chamber and recorded with a polarographic H₂S sensor (PHSS) connected to a multichannel analyzer (Apollo 4000, WPI, Sarasota, FL). **Results**: CBDL animals, but not PVL animals developed physiologic alterations of HPS beginning within 2 wk after ligation. Pulmonary CSE expression decreased significantly within 2 wk relative to control and progressed over time (70% reduction in 4 wk CBDL vs control, p<0.05). These changes were accompanied by parallel reductions in lung H₂S generation (control and 4 wk CBDL as 4.6 ± 0.9 and 1.6 ± 0.2 pmol/sec/mg protein, p<0.05). The decrease in CSE expression and H₂S production also occurred over a similar time frame in CBDL liver. In contrast, PVL animals had no decrease in either CSE expression or H₂S production in lung or liver relative to control, despite a similar degree of portal hypertension. **Conclusions**: Both pulmonary and hepatic CSE expression and H₂S production decline in experimental HPS after CBDL, an effect not seen in non-cirrhotic portal hypertension where HPS does not develop. These findings indicate that the dramatic changes in H₂S production in experimental cirrhosis is not due to the development of portal hypertension and are not confined to the liver. Exploring the mechanisms and consequences of reduced H₂S production in cirrhosis and the possible role in experimental HPS may provide potential new therapeutic strategies.

Disclosures:
The following people have nothing to disclose: Junlan Zhang, Jo Morrison, Jeannette Doeller, David Kraus, Bao Luo, Liping Tang, Joseph Barney, Yongming Wang, Michael B. Fallon

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831 POST-TRANSLATIONAL REGULATION OF SINOSUDBID ENDOTHELIAL CELL ENOS BY THE GPCR INTERACTOR, GIT1

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After liver injury, endothelial nitric oxide synthase (eNOS) activity is reduced in sinusoidal endothelial cells (SECs) and appears to result in reduction of nitric oxide (NO) synthesis, typical of portal hypertension. Previous studies of eNOS link post-translational abnormalities of Akt activation to GPCR signaling; therefore, we hypothesized that a GPCR kinase interacting protein (GIT1) might regulate eNOS activity. **METHODS**: SECs were isolated from normal or injured livers (BDL). eNOS, phospho-eNOS, and GIT1 expression were detected by immunoblotting. SECs were transduced with GIT1 cDNA and GIT1 siRNA. NO production was measured using the Griess reaction; a potential eNOS and GIT1 interaction was examined by immunoprecipitation. Adenovirus encoding GIT1 (1x10^15 pfu/kg) was transduced into SECs in vivo. **RESULTS**: Here, we report that GIT1 co-localizes and interacts with eNOS in SECs. GIT1 overexpression significantly increased NO production and enhanced Ser 1177 phosphorylation and Thr495 dephosphorylation without affecting total eNOS expression. The GIT1 and eNOS
interaction increased in response to ET-1. Importantly, the calcium/calcmodulin (CaM) inhibitor, W13, had no effect on GIT1 induced NO production, while inhibition of Src blocked GIT1 mediated eNOS phosphorylation and NO production (See Figure). GIT1 knockdown led to reduced NO production (normal SECs: 4.46±0.59, scramble siRNA:4.93±0.29, GIT1 siRNA:2.21±0.51, p<0.01 for control vs. GIT1 siRNA). After liver injury, both GIT1 expression and the GIT1-eNOS interaction were reduced. Finally, expression of GIT1 in injured livers normalized portal pressure. These data identify GIT1 as a novel post-translational regulator of eNOS in SECs.

832 LIVER ADAPTATION TO ISCHEMIA: NOVEL EVIDENCE FOR AN ALTERNATIVE ATP-GENERATING METABOLIC PATHWAY
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INTRODUCTION: Hepatocyte cell death and ensuing liver atrophy are observed in almost every chronic liver disease. These events can be reproduced experimentally in the left lateral and medial lobes of the liver by selectively occluding the left branch of the portal vein (LPVL). Using this model in the rat, we observed that cellular injury was preceded by a lag phase during which time cell death was absent. We hypothesized that this lag phase could be due to metabolic adaptations that were triggered to maintain the bioenergetic status of the ischemic liver. Using LPVL in male Sprague-Dawley rats, we studied metabolic changes in the ischemic lobe using high-resolution 13C and 1H NMR spectroscopy, HPLC, laser Doppler flow studies and oxygen tension measurements. RESULTS: Intrahepatic oxygen tension and blood flow were decreased almost immediately after LPVL. Despite this, the bioenergetic status of the liver was maintained for up to 6 hours as evidenced by only a marginal decrease in the ATP/ADP ratio. To investigate the flux of glucose-derived metabolites through the tricarboxylic acid (TCA) cycle, [U-13C]glucose was infused for 45 minutes before harvesting the tissue. Although total glucose levels were slightly increased, the fractional enrichment of intrahepatic glucose did not change at any time point studied. In comparison to the SHAM group, the entry of glucose into the TCA cycle was impaired as evidenced by a decrease in the incorporation of 13C-label into succinate (65±7%) (intermediate of the TCA cycle) and glutamate (49±13%) (derivative of the TCA cycle). Despite this observation, total succinate (127±14%) and total glutamate (119±12%) levels were markedly increased. In other words, the activity of the TCA cycle became less dependent on the metabolism of glucose. Furthermore, a significant decrease in the intrahepatic aspartate (80±4%) content was observed. CONCLUSION: Together, these results suggest that, in the presence of liver ischemia, ATP generation can be maintained by the transamination of aspartate into glutamate coupled with the metabolism of alpha-ketoglutarate into succinate, thus generating GTP which can be transphosphorylated into ATP (Weinberg et al. PNAS 97: 2826-2831). To our knowledge, this is the first reported evidence of this form of mitochondrial ATP generation in vivo. The maintenance of hepatic bioenergetics by this pathway may eventually be exploited to delay hepatocellular death in disease. This research was supported by a grant from the Canadian Institutes of Health Research (CIHR) and salary support from the CIHR, Canadian Association for the Study of the Liver and Fujisawa Inc.

Disclosures:
The following people have nothing to disclose: Tom S. Chan, Claudia Zwingmann, Sven Gottschalk, Valerie-Ann Raymond, Peter Darby, David Mazer, Marc Bilodeau

833 REDUCED BRAIN CONCENTRATIONS OF A POTENT NEGATIVE ALLOSTERIC MODULATOR OF GABAERGIC TRANSMISSION IN HUMAN HEPATIC ENCEPHALOPATHY: A NOVEL EXPLANATION FOR "INCREASED GABAERGIC-TONE"
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Autopsied brain tissue from cirrhotic patients who died in hepatic coma contains increased concentrations of the neurosteroid allopregnanolone (Abboucha et al., Ann Neurol, 58:61-62, 2005). Allopregnanolone is a positive allosteric modulator of the GABA-A receptor with well established sedative properties. As part of a series of studies of neurosteroids in relation to the pathogenesis of hepatic encephalopathy (HE) in chronic liver failure, concentrations of dehydroepiandrosterone sulfate (DHEAS) were measured by radioimmunoassay in samples of frontal cortex obtained at autopsy from 11 cirrhotic patients who died in hepatic coma compared to an equal number of control subjects free from hepatic or neurological diseases at the time of death and who were matched for age, gender, and autopsy delay intervals. Patient material contained a significant reduction of DHEAS (5.81±0.88 µg/g tissue) compared to controls (9.70±0.79 µg/g tissue, p<0.01). Brain levels of DHEAS in four patients with chronic liver disease who died without HE (11.43±1.71 µg/g tissue), and in a patient who died in uremic coma (12.56±0.03 µg/g tissue) were within control range. In a separate series of experiments, DHEAS concentrations were found to be significantly reduced in CSF of rats following end-to-side portacaval anastomosis (PCA; 0.13±0.02 µg/ml) compared to sham-operated controls (0.21±0.03 µg/ml, p<0.01). In contrast to allopregnanolone, DHEAS is a potent negative allosteric modulator of GABAergic neurotransmission; reduced brain concentrations of DHEAS therefore have the potential to lead to a net stimulation of GABAergic neurotransmission; reduced brain concentrations of DHEAS therefore have the potential to lead to an increased GABAergic tone. Administration of Ro15-4513, a partial
834 THE SOMATOSTATIN ANALOGUE OCTREOTIDE INHIBITS ANGIOGENESIS AND VEGF EXPRESSION IN EARLY, BUT NOT LATE, STAGES OF PORTAL HYPERTENSION IN RATS. ROLE OF SOMATOSTATIN RECEPTOR SUBTYPE 2 DOWNREGULATION DURING THE EVOLUTION OF THE PORTAL HYPERTENSIVE SYNDROME

Marc Mejias, Ester Garcia-Pras, Jaime Bosch, Mercedes Fernandez; IDIBAPS, CIBEREHD, Hospital Clinic, University of Barcelona, Barcelona, Spain

Angiogenesis, the development of new blood vessels, is an important determinant of the pathophysiology of portal hypertension, contributing to the formation of portosystemic collateral vessels and the hyperdynamic splanchnic circulation associated to this syndrome. Somatostatin and its analogues, like octreotide, have been shown to be powerful inhibitors of experimental angiogenesis in vitro and in vivo. The purpose of our study was to determine the effects of octreotide on splanchnic neovascularization, expression of angiogenesis mediators, portosystemic collateralization and hemodynamics in portal hypertensive rats. The expression of the somatostatin receptor subtype 2 (SST2), which mediates the antiangiogenic effects of octreotide, was also assessed in mesentry from sham-operated rats and 4 and 7 days after induction of portal hypertension. Rats with portal hypertension induced by partial portal vein-ligation (PPVL) were treated with octreotide or vehicle during 4 or 7 days, starting immediately after PPVL. Splanchnic neovascularization (CD31 expression) as well as VEGF and SST2 expressions were determined by western blotting. Formation of portosystemic collaterals was measured by radioactive microspheres. Hemodynamic studies were performed by flowmetry. Octreotide treatment during 4 days significantly decreased splanchnic neovascularization and VEGF expression in portal hypertensive rats. Portal pressure and heart rate were also significantly reduced by the 4-days octreotide treatment, whereas portosystemic collateralization and splanchnic blood flow and resistance were not significantly modified. After one week of daily octreotide injection, starting on the day of PPVL, portal hypertensive rats escaped from octreotide therapy with regard to inhibition of angiogenesis. The only significant effect observed after 7-days octreotide treatment was a reduction in portal pressure. The mechanism underlying the escape phenomenon could be related to the finding that expression of the SST2 receptor decreased progressively during the evolution of portal hypertension. In conclusion, octreotide may be an effective therapy in early stages of portal hypertension by reducing splanchnic neovascularization, VEGF expression and portal pressure, but not in late stages most likely due to SST2 down-regulation during the progression of portal hypertension in rats.

Disclosures:
The following people have nothing to disclose: Marc Mejias, Ester Garcia-Pras, Jaime Bosch, Mercedes Fernandez.
Results: In cirrhotic rats CPG12177 decreases portal pressure, mainly caused by intrahepatic vasodilation, despite simultaneous splanchic vasodilatation. The additional application of propranolol blunted the splanchic blood flow and further decreased portal pressure. The selective blockade of beta-3-adrenoceptors by SR59230A led to a splanchic and hepatic vasoconstriction, and a slight decrease in portal pressure. The previous application of propranolol increased the splanchic vasodilatation and decreased markedly portal pressure. Discussion: Our data show for the first time that beta-3-adrenoceptor stimulation elicits intra- and extrahepatic vasodilatation with a drop in portal pressure. The combination with a non-selective beta-blocker (beta-1 and beta-2) further decreases portal pressure, suggesting that beta-1 and beta-2-adrenoceptor blockade together with beta-3 stimulation may have highest portal pressure lowering effect by decreasing portal inflow as well as hepatic resistance.

Disclosures: The following people have nothing to disclose: Jonel Trebicka, Andrea Schulze Pröbsting, Martin Hennenberg, Tilman Sauerbruch, Jörg Heller

837 PORTAL HYPERTENSION TRIGGERS PERIPHERAL SIGNALS THAT INDUCES VASCULAR ENDOTHELIAL GROWTH FACTOR (VEGF) AND ENDOTHelial NITRIC oxide SYNTHASE (eNOS) EXPRESSION AT THE INTESTINAL MICROCIRCULATION IN CIRRHTIC RATS

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Introduction: Portal pressure is an important factor that induces peripheral signals in different parts of the vasculature. Using a surgically induced model of portal hypertension in rats, it was shown that mild portal hypertension was enough to upregulate VEGF production and subsequent eNOS expression in the intestinal microcirculation. This upregulation of eNOS expression was partially reversed by the administration of VEGF receptor 2 blocker, suggesting that eNOS upregulation at the intestinal microcirculation was in part mediated by VEGF. In this study we hypothesized that the intestinal microcirculation may serve as an effector that senses changes in portal pressure in cirrhosis.

Thus we investigated how the severity of cirrhosis influences protein expression of VEGF and eNOS in intestinal microcirculation in cirrhotic rats. Methods: We generated rats (n=15) with different stages of cirrhosis using carbon tetrachloride (CCl4) inhalation in different duration: 6, 8, and 11 weeks, at which time points that rats were the beginning of, intermediate, and fully-established cirrhosis, respectively. Age-matched control rats (n=15) were also prepared as a control. Severity of cirrhosis was determined by portal pressure and Sirius red staining as a measurement of fibrosis. Results: Fibrosis was significantly increased at 8 weeks (3 %; microscopically: incipient cirrhosis) and 11 weeks (9 %; micronodular cirrhosis) of CCl4 treatment. Rats with 11 weeks of CCl4 inhalation developed ascites and showed significant increases in portal pressure (18.02±1.07 mmHg) and spleen weight (2.24±0.21 g), while those rats with 6 weeks (7.26±0.58 mmHg; 0.88±0.04 g) and 8 weeks (8.55±0.33 mmHg; 0.9±0.06 g) did not. At 11 weeks when portal hypertension was developed, both VEGF and eNOS expressions in the intestinal microcirculation significantly increased to 40 % and 3 folds (P<0.05), respectively, compared to the control group. Furthermore, rats with 11 weeks of CCl4 inhalation showed more than 2 fold elevated angiogenic cytokines such as TNF-alpha, Interleukin (II)-2, IL-6 and granulocyte-colony stimulating factor in the intestine, as evaluated by a cytokine array. These observations suggest that portal hypertension triggers peripheral signals at the intestine, which induces angiogenic cytokines and NO production, known mediators of complications observed in cirrhosis. Conclusion: Intestinal microcirculation senses the changes of portal pressure in cirrhosis and may serve as reservoir for angiogenic cytokines. The up-regulation of eNOS at the intestinal microcirculation contributes to the development of the hyperdynamic circulation in cirrhosis.

Disclosures: The following people have nothing to disclose: Yasuko Iwakiri, Juan G. Abraldes, Omar Haq, Supatsri Sethasine, Mauricio Loureiro-Silva, Roberto J. Groszmann

838 INHIBITION OF MYOSTATIN REVERSES THE LOSS OF SKELETAL MUSCLE MASS ASSOCIATED WITH IMPAIRED PROTEIN SYNTHESIS IN THE PORTACAVAL ANASTOMOSIS RAT

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The portacaval anastomosis (PCA) rat is a model for skeletal muscle loss in human cirrhosis. Our previous data showed an increased expression of myostatin (inhibitor of skeletal muscle protein synthesis) and lower expression of myoD and proliferating cell nuclear antigen-PCNA (impaired satellite cell function). Satellite cells are myogenically committed precursor cells that maintain muscle mass. In the present study, we examined the functional consequences of these alterations by directly quantifying skeletal muscle protein synthesis and using follistatin, a myostatin antagonist. Methods: Male Sprague Dawley rats with an end to side portacaval anastomasis were compared with pair fed sham operated rats (n=5 each). At 2, 4 and 6 weeks after PCA, protein synthesis in the skeletal muscle was quantified using a flooding dose of labeled phenylalanine. Fractional synthesis rate (FSR) and synthesis of total skeletal muscle protein were quantified. The expression of myostatin, PCNA and myoD were quantified by real time PCR. The response to follistatin was examined in PCA and sham rats and compared with the response to vehicle alone. Results: PCA rats had significantly lower body weight, lean body mass, grip strength, and skeletal muscle weight and these changes were reversed with follistatin. The FSR of skeletal muscle protein in the sham rats increased while it did not change in the PCA rats. The FSR in the sham rats was higher than that in the PCA rats at week 2 and subsequently was similar in the 2 groups despite a lower muscle mass in the PCA rats. Therefore, the synthesis rate of the total skeletal muscle protein was lower in the PCA compared to sham rats at each of the times examined (p<0.05). The expression of myostatin was higher and that of PCNA and myoD were lower at 4 and 6 weeks after PCA. Discussion. Lower skeletal muscle mass in PCA rat was accompanied by an inability to mount an increased fractional synthesis rate response after surgery in contrast to that observed in the sham rats. This inappropriate compensatory response in the skeletal muscle protein compartment of the PCA rat contributes to the failure to gain skeletal muscle mass. The mechanism of this impaired compensatory protein synthetic response in the skeletal muscle was a consequence of an increased expression of myostatin as well as the lower myonuclear accretion as a result of impaired satellite cell function as evidenced by a reversal of
the alteration in skeletal muscle mass by follistatin. These observations are of clinical relevance in developing strategies to reverse skeletal muscle loss in human cirrhosis.

Disclosures: The following people have nothing to disclose: Srinivasan Dasarathy, Sean Muc, Prabhu S. Parimi, Milan Dodig, Satish C. Kalthan, Arthur J. McCollough

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APELIN, A NOVEL CYTOKINE REGULATING INFLAMMATION AND ANGIOGENESIS, IS UPREGULATED IN DECOMPENSATED HUMAN CIRRHOSIS

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Apelin has recently been demonstrated to play a role in water homeostasis through the hypothalamic inhibition of ADH secretion. Several studies have also observed that this substance, expressed in a wide array of tissues, plays a role in inflammation and angiogenesis. In addition, apelin has been shown to be involved in ischemic heart disease and in intermediate metabolism regulation. Previous investigations demonstrated a close relationship between hepatic dysfunction and urinary excretion of apelin in experimental cirrhosis. In the present work we investigated whether the production of this peptide is also activated in patients with cirrhosis and portal hypertension. Two studies were performed. First, we assessed whether the concentration of apelin was higher in human blood during compensated cirrhosis. In the second study, we assessed mRNA expression of apelin and apelin receptor in different organs of cirrhotic rats with ascites to determine which tissue/s is/are responsible for the altered production of apelin in cirrhosis. Plasma concentration of apelin was measured in 64 patients with decompensated cirrhosis of different etiologies and in 10 individuals with no previous history of cardiovascular or chronic inflammatory disease and normal BMI. Apelin concentration was around three times higher in cirrhotic patients than in human controls (855±75 vs. 282±12 pg/ml, p<0.005). We then induced cirrhosis and ascites in 18 Wistar rats that were compared with 18 age matched control animals. Paralleling the results in human cirrhotics, apelin plasma concentration was significantly higher in cirrhotic rats than in control animals (1250±204 vs. 958±198 pg/ml, p<0.0001). Liver, lung, heart and kidney were extracted from both control and cirrhotic animals to assess apelin and apelin receptor mRNA expression by real-time PCR. Apelin gene expression showed a fourfold rise only in the liver (4.06±0.59 vs. 1.03±0.17 arbitrary units, p<0.0001), while no significant differences were found in any other organ. Interestingly enough apelin receptor mRNA levels were found to be almost 300 times higher in the liver of the cirrhotic animals than in controls (11.08±2.13 vs. 0.04±0.01 arbitrary units, p<0.0001), while a significant apelin receptor down regulation was observed in both heart (4.97±1.30 vs. 13.30±2.92 arbitrary units, p<0.05) and lung (43.53±14.28 vs. 91.82±13.74 arbitrary units, p<0.05). These results indicate that the hepatic apelin system is markedly and selectively activated in cirrhosis and suggest that this peptide could be involved in the angiogenic and fibrogenic processes occurring in this disease.

Disclosures: The following people have nothing to disclose: Alessandro Principe, Luis Ruiz del Arbol, Pedro Melgar-Lesmes, Manuel Morales-Ruiz, Vicente Arroyo, Wladimiro Jimenez

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CARBON MONOXIDE REGULATES THE INTRAHEPATIC VASCULAR TONE IN NORMAL AND CIRRHOTIC RATS

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Background/aim: Besides nitric oxide (NO), carbon monoxide (CO) produced by heme oxygenase isoforms (inducible HO-1 & constitutive HO-2) is involved in the regulation of the vascular tone of the systemic circulation. Our aim was to elucidate the role of CO in the vasoregulation of the hepatic microcirculation both in normal and thioacetamide (TAA) cirrhotic rat livers. Methods: Through immunohistochemistry (n=6) and Western blot (n=5), the topography and level of HO expression was examined. Total HO enzymatic activity (n=4) was measured spectrophotometrically. The contribution of CO vs NO in the regulation of the intrahepatic vascular resistance (IHVR) was evaluated in an in-situ liver perfusion model, using oxygenated human hemoglobin (HbO2) (a CO and NO intra- and extracellular trapping reagent) and methemoglobin (metHb) (a control tool that scavenges NO but not CO) (n=5). To further define the role of CO, the HO-inhibitor Zinc protoporphyrin IX (ZnPP-IX) and a CO-releasing molecule (COR-2) (n=5) were added to the liver perfusion system. Sensitivity of hepatic stellate cells (HSC) to CO-mediated relaxation was studied by a stress-relaxed-collagen-lattice model. Results: HO-1 was predominantly expressed in Kupffer cells, HO-2 in endothelial cells and hepatocytes. Western blot showed a decreased expression of both isoenzymes (HO-1 P<0.04; HO-2 P<0.001) in cirrhotic rat livers, which was confirmed by the HO activity assay (P=0.004). In normal rat liver, in-situ perfusion with HbO2 caused a strong elevation in IHVR, while metHb caused a significantly but smaller increase (P<0.05), suggesting an important role for CO in the intrahepatic microcirculation. The role of CO in the normal liver was further emphasized by a dose-related increase in IHVR after incubation with ZnPP-IX (P<0.001). The cirrhotic rat liver, basal IHVR was increased compared to control (0.22±0.01 vs 0.13±0.01 mmHg.min.mL-1, P<0.001). Administration of HbO2 and metHb caused similar small increases in IHVR, suggesting a smaller amount of hemodynamically important CO in cirrhotic rat livers. Perfusion with ZnPP-IX caused a similar pattern of increase in perfusion pressure as in normal rat livers (P=0.003). Perfusion with COR-2 attenuated the increased IHVR (P=0.016). In vitro, preincubation with COR-2 10-4M significantly decreased fetal calf serum (FCS)-promoted HSC contraction (P=0.013). Conclusion: These data suggest a role, probably mediated via HSCs, for CO in regulation of the normal hepatic and cirrhotic microcirculation, wherein reduced CO producing activity was associated with an increased IHVR.

Disclosures: The following people have nothing to disclose: Lien Van Langdeghem, Wim Laleman, Marcel Zeegers, Ingrid Vander Elst, Jos van Pelt, David Cassiman, Frederik NVeens

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AMMONIA AND HYPONATREMIA IMPAIR NEUTROPHIL FUNCTION BY INDUCING SWELLING AND IS MODULATED BY THE P38-MAPK PATHWAY

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Background: Hyperammonemia and hyponatremia can affect cell hydration and are associated with increased risk of infection and mortality. Alteration in cell volume alters intracellular...
signalling through effects on p38-MAPK. Neutrophil phagocytic dysfunction and increased spontaneous oxidative burst (OB) in cirrhosis has been shown to be associated with increased infection risk and mortality (Hepatology 2007, in press). The aims of this study were to explore in vitro and ex-vivo, the effect of hyperammonemia and hyponatremia on neutrophil phagocytosis and OB, and determine the role of p38-MAPK. Methods: Neutrophils were isolated from whole blood of healthy volunteers and incubated with either 75µM ammonia or PBS (control group). Both groups were further challenged with hyponatremia (induced by buffering blood with PBS containing 124 mM sodium chloride) and/or the addition of p38-MAPK modulators. Phagocytosis was analysed by fluorescent activated cell sorting (FACS) using FITC-labelled E. coli and quantification of OB was determined using a test which measured the % of neutrophils producing reactive oxidants at rest and after stimulation with E. coli and analysed by FACS. In rats fed an ammonia-rich diet (HD) and controls, neutrophil phagocytosis and OB were analysed by FACS. Cell size was quantified by forward scatter on FACS. Results: In healthy isolated neutrophils, phagocytosis was impaired on incubation with ammonia (p<0.0005), hyponatremia (p<0.05) or both (p<0.05) compared to controls. These effects were abrogated by the addition of isoproterenol, a p38-MAPK agonist. Cell size increased by 2 +/- 0.4, 3.4 +/- 0.6 or 5.7 +/- 1.2% respectively, with ammonia, hyponatremia or both, compared to controls (p<0.02) and was abrogated by p38-MAPK agonist and exacerbated by the addition of SB203580, a p38-MAPK antagonist. In healthy unstimulated isolated neutrophils the resting OB was increased compared to controls (p<0.001). This was abrogated by p38-MAPK antagonist. In isolated neutrophils from HD rats we observed impaired phagocytosis (55.4 +/- 7 vs 82.7 +/- 3.3%; p<0.05) and increased resting OB (31.2 +/- 5.3 vs 8.1 +/- 1.9%; p<0.001) compared to naïves. Conclusion: Hyperammonemia and hyponatremia impair neutrophil phagocytosis by inducing cell swelling in which the p38-MAPK pathway plays an important role. We hypothesise that p38-MAPK is activated by cell swelling which acts as an osmoregulator, thereby reducing swelling. However, activation of p38-MAPK also induces spontaneous OB which can be reversed with a p38-MAPK inhibitor. Prompt treatment of hyperammonemia and hyponatremia may prevent neutrophil dysfunction and reduce infection risk.

Disclosures: The following people have nothing to disclose: Debbie L. Shawcross, Gavin A. Wright, Vanessa Stadlbauer, Stephen Hodges, Rajiv Jalan

842 KUPFFER CELL (KC) ACTIVATION BY BETA-GLYCANS: GUANYLATE CYCLASE AND HEME OXYGENASES (HO) COUNTERACUTE INCREASE OF PORTAL PRESSURE (PP)

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Introduction: Recently we have shown that KC activation increases PP in normal and fibrotic livers via thromboxane A_2 [DOI: 10.1016/j.jhep.2007.03.019]. PP is the interplay of vasoconstrictors and vasodilators. Therefore we now investigated the role of the vasodilating NO and CO condition on PP after KC activation. Methods: Livers of male Sprague-Dawley rats were perfused for 100 minutes. KC were selectively activated by infusion of Zymosan (150 µg/ml). L-NAME (0.3 mM), NS 2028 (1.0 µM), and 8-Br-cGMP (50 µM) were administered to Zymosan-treated livers. CO generation was blocked by ZnPP IX (1 µM). Zymosan/ZnPP IX administration was combined with CO (2.5 µM), 8-Br-cGMP (50 µM), Indomethacin (50 µM), BM 131.77 (20 µM), Glutathione (2 mM) or Catalase (150 U/ml) to elucidate mechanisms limiting PP increase by HO. Thromboxane B_2, the stable degradation product of thromboxane A_2 was measured in effluent perfusate by RIA.

Results: (±SD) Activation of KC by Zymosan resulted in a transient increase of (PP, cm H_2O) from 4.0±0.8 to maximally 19.2±3.1 followed by a long-lasting elevation until end of perfusion (6.6±0.7) (p<0.05). The inhibition of NO-synthase by L-NAME or the inhibition of soluble guanylate cyclase by NS 2028 further raised the initial increase of PP. This could be abolished by 8-Br-cGMP indicating the NO-cGMP-pathway as important vasodilating mechanism following KC activation. Inhibition of HO by ZnPP IX caused a significant (p<0.05) amplification of initial (24.4±2.6) and prolonged increase of PP (14.1±1.4) in Zymosan-treated organs. Interestingly, CO prevented prolonged increase (11.7±0.9) by cGMP-independent mechanisms. Indomethacin, the thromboxane A_2 receptor antagonist BM 131.77 and also the antioxidants glutathione and catalase prevented maximal and long-lasting increase of PP about 60-80% in Zymosan/ZnPP- treated livers. In order to test the pathway of reactive oxygen species and thromboxane we measured hepatic efflux rates of thromboxane B_2 upon Zymosan infusion. Indeed the amplification of PP by ZnPP was accompanied by increased hepatic efflux rates of thromboxane B_2: 1.997±1.43 (Zymosan) vs. 4.500±529 pg/min x g liver (Zymosan/ZnPP). Interestingly, the additive infusion of glutathione or catalase lowered efflux of thromboxane B_2 significantly (1.443±243 and 1.351±265). Conclusion: NO restricts the initial PP increase after KC activation by guanylate-cyclase mediated cGMP. CO from heme degradation limits the initial and the long lasting increase of PP after KC activation by cGMP-independent mechanisms. Interestingly, reactive oxygen species from KC is blocked by HO leading to lowered thromboxane B_2 efflux which terminates increase of PP.

Disclosures: The following people have nothing to disclose: Christian J. Steib, Markus Bystron, Christine Opelz, Josef M. Härzl, Ingrid Liss, Burkhard Göke, Manfred Bilzer, Alexander L. Gerbes

843 UPREGULATION OF RHOA- AND RHO-KINASE-EXPRESS AND RHO-KINASE ACTIVITY IN LIVERS FROM RATS WITH CCL4-INDUCED MICRONODULAR CIRRHOSIS

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Background: In cirrhosis, increased resistance of the intrahepatic vasculature and intrahepatic hyperresponsiveness to vasoconstrictors contributes to portal hypertension. We have previously shown that upregulation of RhoA and Rho-kinase and a subsequently increased Rho-kinase activity critically participate in the development of increased intrahepatic vascular resistance in rats with secondary biliary cirrhosis after bile-duct occlusion [Gut2006;55(9)]. Moreover, Rho-kinase activity is increased in livers from patients with alcohol-induced cirrhosis [Gut2006;55(9)]. In contractile cells, the small monomeric GTPase is activated by vasoconstrictors through G-protein coupled receptors. RhoA in turn activates Rho-kinase, which mediates vasoconstriction via inhibition of myosin-light-chain phosphatase. Here, we investigated the expression of RhoA and Rho-kinase, as well as Rho-kinase activity in livers of rats with CCl4-induced micronodular cirrhosis. Methods: Micronodular cirrhosis in male rats was induced by chronic application of CCl4. Livers from cirrhotic rats and non-cirrhotic controls
were shock-frozen and homogenized. Expressions of RhoA and Rho-kinase were studied by Western-blot analysis of liver homogenates. Rho-kinase activity in liver homogenates was assessed as phosphorylation of the Rho-kinase substrate, moesin (Thr-558), by Western-blot analysis with a site- and phosphospecific antibody. Results: Expression of RhoA and Rho-kinase were upregulated in livers from CCl4-rats, compared to livers from non-cirrhotic rats. Levels of phospho-moesin (Thr-558) were increased in homogenates of cirrhotic livers, compared to non-cirrhotic controls. Conclusion: Upregulation of RhoA and Rho-kinase expression, and increased Rho-kinase activity obviously contribute to increased intrahepatic vascular resistance in cirrhosis of different etiologies.

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EFFECTS OF BILE ACIDS ON PULMONARY MICROVASCULAR ENDOTHELIAL CELL PROLIFERATION: IMPLICATIONS FOR EXPERIMENTAL HEPATOPULMONARY SYNDROME (HPS)
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Introduction: HPS occurs in 10-30% of patients with cirrhosis and increases mortality. Preliminary studies support that angiogenesis may be an important pathophysiologic process in experimental HPS induced by biliary cirrhosis (CBDL) which is not found in non-biliary cirrhosis (thioacetamide, TAA) where HPS does not develop. Whether circulating bile acids contribute to angiogenesis in CBDL animals is not known. Aim: To evaluate the effects and mechanisms of bile acids on endothelial proliferation in pulmonary microvascular endothelial cells.

Methods: Plasma bile acid concentrations were measured in CBDL and TAA cirrhosis and the effects of CBDL plasma on in vitro angiogenesis was assessed by measuring capillary tube formation on Matrigel. The effects of CDCA (100µM) on endothelial proliferation (MTT assay, 24hrs) and specific signaling pathways were assessed in rat pulmonary microvascular endothelial cells (RPMECs, VEC Technologies, Inc.).

Results: Plasma bile acid concentrations (µM, mean ± SE) were significantly increased in both 3 wk CBDL and 8 wk TAA cirrhosis relative to control (control 17 ± 3.4, CBDL 124 ± 8.4, TAA 35 ± 5.2, both p < 0.05). The addition of plasma from 3 wk CBDL animals significantly stimulated RPMEC tube formation, an effect not seen with the addition of control plasma. CDCA triggered a dose dependent increase in endothelial cell proliferation in RPMECs (2.1 ± 0.35 fold-control, p< 0.05). This effect was accompanied by an increase in VEGF expression (mRNA 2.9 ± 0.4 fold-control, protein 2.5 ± 0.6 fold-control, p< 0.05) and phosphorylation of Flik-1 and Akt. The proliferative response could be inhibited by VEGF neutralizing antibodies, a Flik-1 kinase inhibitor (SU1498) and by wortmannin (Akt inhibitor). Similar effects on VEGF expression and endothelial cell proliferation were also seen with GW474064 and accompanied by increased SHP mRNA levels (2.6 ± 0.4 fold-control, p< 0.05) supporting that the effects were in part FXR-dependent. However, time course analysis demonstrated that Akt phosphorylation occurred within 1 hour after CDCA administration and prior to upregulation of SHP supporting that the effects were in part FXR-independent.

Conclusions: Plasma bile acid levels are increased in experimental HPS and plasma from CBDL animals stimulates endothelial tube formation in vitro. In RPMECs, CDCA increases VEGF expression and Akt-dependent endothelial cell proliferation that is in part FXR-dependent. In addition, CDCA appears to have rapid FXR-independent effects on Flik-1 and Akt activation. These results support the hypothesis that circulating bile acids may contribute to the pulmonary angiogenic response in experimental HPS.

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The following people have nothing to disclose: Liping Tang, Junlan Zhang, Bao Luo, Yongming Wang, Joseph Barney, Michael B. Fallon

845
PROTEIN MICROARRAY STUDY REVEALS TOXIN-SELECTIVE CYTOKINE PROFILES IN EXPERIMENTAL ACUTE LIVER FAILURE: BENEFICIAL EFFECTS OF MILD HYPOThERMIA AND N-ACETYLCYSTEINE
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Hepatic encephalopathy and cerebral edema are serious neurological complications of acute liver failure (ALF). The precise pathophysiologic mechanisms responsible for these complications have not been fully established but recent studies suggest the involvement of proinflammatory cytokines. In order to address this issue, protein array technology was used to investigate changes in the expression profile of 62 pro- and anti-inflammatory cytokines and related chemokines and growth factors in mice with ALF due to toxic liver injury. Male C57BL6 mice were treated with azoxymethane (AOM) (100µg/g; i.p.) or aceniamophen (APAP) (300µg/g; i.p.); control mice received an equal volume of saline. At equivalent stages of ALF, determined by measurement of serum transaminases and verified post-mortem by histopathology (hematoxylin-phloxine-saphron staining of paraffin-embedded liver sections), mice were killed and blood drawn for cytokine measurement. AOM-treated comatosc mice manifested significant brain edema (measured by a sensitive gravimetric technique) and increased expression of a wide range of cytokines including interferon-gamma (IFN-γ), interleukins (IL-1β, IL-3, IL-4, IL-5, IL-6, IL-10, IL-12, IL-17), tumor necrosis factor-alpha (TNF-α) as well as monocyte chemoattractant proteins MCP-1 and MCP-5. Cytokine profiles from APAP-treated mice showed some similarities but also major differences from those associated with AOM-induced ALF (in particular with regard to the interleukins IL-1β, IL-5 and IL-6). Treatment of AOM mice with mild hypothermia (35°C) or N-Acetylcysteine (NAC) (1,200 mg/kg; i.p.) led to reduced hepatic damage and improvement in neurological function (brain edema prevention). Both hypothermia and NAC led to selective attenuation in expression of IFN-γ, IL-10, IL-12 and IL-17. Hypothermia caused additional decreases in expression of IL-3, IL-4 and IL-6. These findings demonstrate that 1) Cytokine profiles in ALF due to toxic liver injury are both selective and toxin-dependent; 2) Treatment such as mild hypothermia or NAC have distinct actions on cytokine expression profiles in ALF. These findings suggest that novel treatment strategies using anti-cytokine or immunoneutralization therapy aimed at reduction of hepatic injury or stimulation of hepatic regeneration may need to target cytokines specific to the etiology of the ALF. [Funded by CIHR and CASL, Canada]

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The following people have nothing to disclose: Chantal Bémeur, Javier Vaquero, Paul Desjardins, Roger F. Butterworth
MECHANISM OF B19-INDUCED ACUTE FULMINANT HEPATIC FAILURE: PARVOVIRUS B19 HELICASE-INDUCED DNA DAMAGE LEADS TO APOPTOSIS IN PRIMARY HEPATOCYTES AND HEP G2 CELLS

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PURPOSE: Parvovirus B19 can cause hepatic enzyme elevation, clinical hepatitis, and acute fulminant liver failure (AFLF).

BACKGROUND: B19 causes a restricted infection in non-erythroid cells (e.g., primary hepatocytes and Hep G2) with expression of nonstructural protein (NS1) but not viral capsid proteins. NS1 is a superfamily 3 helicase, typical of small DNA viruses, that plays a key role in initiation of viral genome replication. In hepatocytes, we demonstrated B19-induced apoptosis was mediated by caspase 9 and caspase 3 activation. We used an enhanced green fluorescent protein (eGFP) fusion protein system to confirm the role of NS1 in apoptosis in primary hepatocytes and Hep G2 cell lines, and to identify its molecular mechanisms.

METHODS: NS1 was cloned into an inducible plasmid or baculovirus expression system, and eGFP/NS1 or parental eGFP was expressed in the presence of inducer. Western blotting was performed with anti-GFP polyclonal rabbit antiserum. Apoptosis induction was detected with annexin V-Alexa-fluor 594. NS1 was immunoprecipitated with anti-GFP polyclonal antibody and protein-G agarose beads. Cellular DNA was labeled with α32P-dTTP. Antibodies specific for the single strand DNA recognition protein, replication protein A (RPA), or proliferating cell nuclear antigen (PCNA) were used in colocalization experiments. Poly(ADP ribosyl) polymerase (PARP) activity was determined by western blotting. Apoptotic subcellular particles were collected through filtration, and total protein or anti-GFP immunoprecipitate was examined on western blots. RESULTS: eGFP/NS1 expressed in either the plasmid or baculovirus system precipitated from purified subcellular apoptotic bodies contained NS1 fusion protein. CONCLUSIONS: B19 NS1 forms bulky adducts with cellular DNA, nicks cellular DNA, and induces apoptosis through DNA repair pathways that lead to mitochondrial stress. B19 may persist in tissues, including liver. Expression of NS1 in hepatocytes may lead to hepatic dysfunction, including acute fulminant hepatic failure through cell loss by apoptosis.

INCIDENCE OF BACTERAEMIA AND OUTCOMES IN PATIENTS WITH ACUTE LIVER FAILURE

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INTRODUCTION: Patients with acute liver failure (ALF) are susceptible to infections secondary to multiple immunological deficits and the high dependence of care required. Previous studies have demonstrated a high incidence of infection, early bacteremia (day 3) and significant attributable mortality. Methods: Data collected prospectively and entered onto a dedicated physiology database for all patients admitted to a specialist liver intensive care unit (LICU) was retrospectively examined for the period January 2003 to July 2005. The incidence and outcomes of bacteremia was examined in patients with ALF. Results: 206 patients were defined with ALF; 72 (35%) suffered bacteremia (BALF) on 133 occasions (163 isolates). In contrast, 134 patients had no bacteremia (NBALF). Aetiology of ALF was not different between the two groups and equal numbers fulfilled King’s College hospital criteria for transplant (BALF 54% vs NBALF 51%). Gram-positive organisms were observed in 44% of isolates, gram-negatives in 52% and candida in 4%. Median time to first bacteremia was 10 (7-16) days. On admission BALF patients had higher SIRS score (2 vs 1, p<0.001). No differences were seen for other biochemical markers on admission. While in LICU, BALF patients had higher degrees of encephalopathy (HE) (maximum HE grade >2: 81% vs. 63%, p=0.008). They also had greater requirements for hemofiltration (RRT) (92% vs. 65%), mechanical ventilation (100% vs. 77%) and significantly longer LICU stays (26 vs. 7 days, p<0.001 for all). Numbers undergoing transplant (OLT) were BALF=26% and NBALF=36% respectively (p=NS). Survival to hospital discharge was not different between the two groups (62% vs 64%). Subgroup analysis for those undergoing OLT (BALF=26%, NBALF=33) and medical management (non-OLT) (BALF=46%, NBALF=101) was performed. In the OLT and non-OLT subgroups, admission SIRS score, requirement for RRT, ventilation and length of stay in LICU were all significantly greater in BALF patients (p<0.05 for all). For the total group, multivariate analysis demonstrated OLT (OR 4.0) and maximum HE grade <3 (OR 5.4) to be important determinants of survival (p<0.001 for both); bacteremia was not significant (p=NS). Significant predictors of bacteremia were maximum HE grade >2 (OR 1.6), admission SIRS score > 1 (OR 2.7) and requirement for RRT during LICU stay (OR 8.9, p<0.02 for all). Conclusion: In this study bacteremia was observed later than reported previously. Bacteremia was associated with increased SIRS markers on admission, an increased requirement for organ support and increased length of LICU stay, but did not significantly impact on mortality.

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848 CEREBRAL HYPEREMIA AND INTRACRANIAL HYPERTENSION INDUCED BY CO-ADMINISTRATION OF ENDOTOXIN AND AMMONIA IN THE RAT ARE MEDIATED BY CYCLOOXYGENASE

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Background/Objective: Systematic inflammation and hyperammonemia acts synergistically in increasing cerebral blood flow (CBF) and intracranial pressure (ICP). The aim of this study was to determine if the increase in CBF and ICP seen after co-administration of lipopolysaccharide (LPS) and ammonia, can be prevented by inhibition of cyclooxygenase (COX) and by the unspecific anti-inflammatory hormone dexamethasone. Methods: Fifty-four male Wistar rats, 6 in each group, were divided into the following groups: Saline + saline; LPS (2 mg/kg) + saline; LPS + indomethacin (10 mg/kg); LPS + diclofenac (10 mg/kg); LPS + dexamethasone (2 mg/kg) in experiment A. Experiment B included the following groups: LPS + ammonium acetate (NH3) (140 μmol/kg/min) + saline; LPS + NH3 + indomethacin; LPS + NH3 + diclofenac and LPS + NH3 + dexamethasone. ICP was monitored via a catheter placed in cisterna magna, while the changes in CBF were recorded by laser Doppler flowmetry. Results: LPS with and without NH3 induced an increase in 6-keto-prostaglandin-F1α (6-keto PGF1α) together with a concomitant rise in CBF and ICP. Indomethacin and diclofenac both significantly prevented the increase in ICP by LPS alone, and with the addition of NH3 the increase in both CBF and ICP. There was no increase in the plasma 6-keto PGF1α concentration. Dexamethasone only reduced the LPS induced increase in ICP, and partly the 6-keto PGF1α plasma concentration. Conclusion: Cerebral cyclooxygenase seems of central importance for development of cerebral hyperemia and high ICP during systemic inflammation and hyperammonemia.

Disclosures: The following people have nothing to disclose: Hans R. Pedersen, Helmer Ring-Larsen, Fin Stolze Larsen

849 ACETAMINOPHEN THERAPEUTIC MISADVENTURE: A PROSPECTIVE STUDY

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Development of severe acute liver failure (SAF) related to acetaminophen use at therapeutic dose (≤60g/day) as the so-called “acetaminophen therapeutic misadventure (ATM)”, was reported in 1 registry but never evaluated in a prospective cohort. Aims: I/ describe and compare prospectively SAF-ATM to SAF with acetaminophen overdose (SAF-AO) II/ confirm the deleterious impact of chronic drinking in SAF-AO. Methods: All patients admitted for SAF (PT<50%) were asked for daily dose and duration of acetaminophen and alcohol intakes. SAF-ATM was defined as: a) acetaminophen use at therapeutic dose (≤4 g/day) together with a concomitant rise in CBF and ICP. Indomethacin and diclofenac both significantly prevented the increase in ICP by LPS alone, and with the addition of NH3 the increase in both CBF and ICP. There was no increase in the plasma 6-keto PGF1α concentration. Dexamethasone only reduced the LPS induced increase in ICP; and partly the 6-keto PGF1α plasma concentration. Conclusion: Cerebral cyclooxygenase seems of central importance for development of cerebral hyperemia and high ICP during systemic inflammation and hyperammonemia.

Disclosures: The following people have nothing to disclose: Alexandre Louvet, Benoît Quesnel, Jeanne Boitard, Sébastien Dharancy, Faustine Wartel, Valérie Canva, Pierre Deltenre, Philippe Mathurin

850 BRAIN CORTEX ACCUMULATION OF GLUTAMINE CORRELATES WITH LACTATE–PYRUVATE RATIO IN PATIENTS WITH ACUTE LIVER FAILURE

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Aims/Objectives: The pathogenesis of brain edema and high intracranial pressure (ICP) in acute liver failure (ALF) is not fully understood, but it is suggested that accumulation of glutamine compromises mitochondrial function within the brain parenchyma. The aim of this study was to test if cerebral glutamine concentrations is associated with a rise in the lactate concentration and the lactate-pyruvate ratio in patients with ALF. Methods: By using in vivo brain microdialysis technique together with ICP monitoring in 13 ALF patients (8 F / 5 M; median age 46 (range 18 to 66) years) the extracellular concentrations of lactate, pyruvate and glutamine were measured together with ICP and cerebral perfusion pressure. Results: The median cerebral glutamine concentration was 4396 (range 1011-9712) μmol/l, lactate 2.15 (1.1-4.45) mmol/l, pyruvate 101 (43-255) μmol/l and the lactate to pyruvate ratio 21.3 (15.8-40.1). No correlation was found between the glutamine and the lactate levels. A correlation of glutamine concentration and lactate-pyruvate ratio was found (r=0.89, p<0.05). In patients with a glutamine concentration above 6 mmol/l the lactate to pyruvate ratio was higher than compared to those patients with a glutamine concentration below 6 mmol/l (p=0.02). ICP was 20 (2-28) mmHg and cerebral perfusion pressure 72 (56-115) mmHg. Conclusion: Our results show that...
cerebral accumulation of glutamine correlates to the lactate-pyruvate ratio in patients with ALF, which indicates impairment of mitochondrial function as cerebral perfusion pressure was sufficient.

Disclosures: The following people have nothing to disclose: Peter N. Bjerring, Hans-Jørgen Frederiksen, John Hauerberg, Fin Stolze Larsen

851 CONTRIBUTION OF MICROGLIAL ACTIVATION TO HEPATIC ENcephalopathy AND BRAIN EDEMA IN EXPERIMENTAL ACUTE LIVER FAILURE: BENEFICIAL EFFECT OF MINOCYCLINE

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Encephalopathy and brain edema are serious complications of acute liver failure (ALF). The precise pathophysiologic mechanisms responsible have not been fully elucidated but a previous report (Hepatology 44:366A, 2006) provided evidence that brain-derived proinflammatory cytokines are involved. However, these findings have so far failed to lead to new therapeutic approaches in ALF management. In order to address this issue, we investigated the effects of the anti-inflammatory drug minocycline on the progression of hepatic encephalopathy and brain edema in rats with ALF resulting from hepatic devascularization (portacaval anastomosis followed by hepatic artery ligation). ALF rats were administered saline or minocycline (22.5mg/kg) on days 2, 1 and day 0 of surgery. ALF rats were sacrificed at precoma (loss of righting reflex) and coma (loss of corneal reflex) stages of encephalopathy along with their appropriate sham-operated controls. Minocycline-treated animals were sacrificed in parallel with saline-treated comatose rats. IL-1β in serum and brain was measured by ELISA. IL-1β mRNA in the brain was assessed by real-time PCR. Microglial activation was analyzed by immunohistochemistry using anti-OX42 antibody. Minocycline delayed the onset of coma and significantly reduced brain water content (sham vs. ALF-saline vs. ALF-minocycline: 78.09±0.11% vs. 80.78±0.09% vs. 79.38±0.15%; p<0.001). Minocycline treatment also attenuated the increase of IL-1β protein in serum (sham vs. ALF-saline vs. ALF-minocycline: 15.5±2 pg/ml vs. 66±6 pg/ml vs. 27±2 pg/ml; p<0.001), together with IL-1β mRNA expression (sham vs. ALF-saline vs. ALF-minocycline: 49.2±6.7 vs. 178±25 vs. 51±13; p<0.001) and IL-1β protein (sham vs. saline vs. minocycline: 4.3±0.4 ng/g protein vs. 8.4±0.8 ng/g protein vs. 2.7±0.7 ng/g protein; p<0.001) in cerebral cortex of ALF animals. Furthermore, induction of OX42 immunoreactivity observed in the frontal cortex of ALF rats was suppressed by minocycline treatment. These findings demonstrate that minocycline treatment delays the onset of precoma and coma in experimental ALF due to suppression of microglial activation and decreased IL-1β expression in brain. These results further support a role for brain-derived cytokines in the pathogenesis of brain edema and its complications in ALF and suggest that minocycline may be beneficial in their prevention. [Funded by CIHR and CASL, Canada]

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852 EVIDENCE OF ENDOTOXIN TOLERANCE AND SEVERELY IMPAIRED T-HELPER 1 RESPONSE IN ACUTE HEPATIC FAILURE

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Introduction: Both acute(ALF) and acute on chronic liver failure (AoCLF) are associated by monocyte deactivation and immunoparesis, characterised by diminished monocyte HLA-DR expression and elevated serum levels of interleukin-10 (IL-10). This phenomenon has striking similarities with the endotoxin tolerance model described in septic shock where impaired antigen presentation is of pathogenic importance. Aim: To determine monocyte pro-and anti-inflammatory cytokine profile following endotoxin challenge and assess immune responses to a panel of recall antigens in acute hepatic failure. Methods: TNF-α and IL-10 secretion was evaluated using Elispot in peripheral blood mononuclear cells (PBMC) at baseline and after stimulation with lipopolysaccharide (LPS) in 20 patients with acute hepatic failure (AHF), 8 ALF and 12 AoCLF, and compared them with 14 healthy controls. Th1 specific responses were evaluated by IFN-γ Elispot in peripheral blood mononuclear cells (PBMC) at baseline and after stimulation with a panel of recall antigens (tetanus toxoid antigen [TTAg], purified protein derivative [PPD], a peptide pool of viral epitopes [CEF]) in 20 AHF and compared to 14 healthy and 28 pathological (HCV infected) controls. Results are expressed as median of specific spot forming cells/10⁶ PBMC [spSF/10⁶ PBMC]. Results: IL-10 secretion was higher in AHF patients compared to healthy controls following LPS challenge (529 vs 77.5, p<0.001). After LPS stimulation no significant change in TNF-α secretion was detected (83 vs 61, p=0.9), while there was a highly significant increase in IL-10 secretion compared to unstimulated cells (205 vs 529, p<0.001). There was an inverse correlation between TNF-α and IL-10 secretion following LPS challenge, the monocytes with the highest IL-10 response having the lowest TNF-α secretion (r=-0.6, p=0.014). Following exposure to TTag, PPD, and CEF there was no significant increase in IFN-γ secretion in AHF from baseline (TTag 0.75 vs 9.4, PPD 0.75 vs 0, CEF 0.75 vs 0, p=NS). IFN-γ production following TTag exposure was significantly reduced in AHF when compared to that seen in pathological (9.4 vs 155, p<0.001) and healthy controls (9.4 vs 300, p<0.001). Conclusion We have identified an endotoxin tolerant monocyte phenotype in patients with acute hepatic failure, characterised by an anti-inflammatory cytokine secretion profile in response to microbial stimuli. This is also associated with severe impairment in mounting a Th1 response against tetanus toxoid, a reflection of defective antigen presentation. These findings strongly support monocyte dysfunction as a pathogenic event in the immunoparesis of acute hepatic failure.

Disclosures: The following people have nothing to disclose: Charalambos G. Antoniades, Philip A. Berry, Ivana Carey, Astrid Scalori, Julia Wendon, Diego Vergani
853 EVALUATION OF THE BIOMARKER, DIMETHYLARGININE SCORE-ISCHEMIA MODIFIED ALBUMIN RATIO (DASIMAR) TO IDENTIFY RISK OF PROGRESSION TO ACUTE ON CHRONIC LIVER FAILURE AND MORTALITY (ACLF) IN PATIENTS WITH DECOMPENSATED CIRRHOsis

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With increasing recognition of the disease entity acute on chronic liver failure (ACLF), and limited management options available when patients evolve to multi-organ failure, there is a clinical need for early biomarkers identifying patients with decompensated cirrhosis at risk of progressing to ACLF and mortality. Recently we described the prognostic value of dimethylarginines and a dimethylarginine score (DAS) in decompensated liver disease, and its association with hepatic inflammation. This study aimed to assess the additional utility of ischaemia modified albumin ratio- IMAR (reflecting oxidative injury corrected for albumin variability in liver disease) combined with DAS in defining outcome in decompensated alcholic cirrhosis. Plasma acquired 48 hours after admission from 52 decompensated alcoholic cirrhotic patients (bilirubin >85μmol/L; increasing ascites and creatinine <150μmol/l) managed with supportive therapy, was analyzed for asymmetric and symmetric dimethylarginines (fragmentation specific stable isotope dilution electrospray tandem mass spectrometry) and IMAR (modified albumin-cobalt binding assay with correction for albumin concentration). Patients were characterized by histology and inflammatory indices and studied prospectively to determine development of organ failure (defined by need for organ support, ACLF). 28 patients developed ACLF. 13/28 died (9 from renal failure and 4 from sepsis, with progression to multi-organ failure). DASIMAR was not only significantly greater in ACLF patients vs. non-ACLF (table below) but also in non-surviving ACLF patients compared to survivors: 2.7±0.1 vs. 4.1±0.4, p<0.001. Whilst DAS correlated with inflammation and renal function as previously described, IMAR correlated with severity of liver disease (r =0.54, p<0.0001) and portal pressure (r =0.44, p<0.01). The DASIMAR score had a significant predictive utility for outcome in ACLF patients: AUROC of 0.92±0.05 and 95% confidence intervals 0.82-0.99; sensitivity of 92% and specificity of 80%. In conclusion, addition of IMAR to a previously described DAS score is a good marker of outcome in patients with decompensated liver disease. DAS and IMAR encompass different elements key to the development of ACLF (liver failure, renal dysfunction, inflammation and severity of portal hypertension). Further validation of DASIMAR as a biomarker may be useful in guiding intervention in ACLF.

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<td>ACLF</td>
<td>1.96±0.2</td>
<td>1.28±0.1</td>
<td>3.26±0.2</td>
</tr>
<tr>
<td>Non-ACLF</td>
<td>0.97±0.04</td>
<td>0.95±0.06</td>
<td>1.91±0.1</td>
</tr>
<tr>
<td>ACLF vs Non ACLF</td>
<td>p&lt;0.0001</td>
<td>p&lt;0.01</td>
<td>p&lt;0.0001</td>
</tr>
</tbody>
</table>

Disclosures:
The following people have nothing to disclose: Rajeshwar P. Mookerjee, Nathan A. Davies, Neil Dalton, Rajiv Jalan

854 BLOOD LACTATE AS A PREDICTOR OF POOR PROGNOSIS IN ACUTE LIVER FAILURE IN ACETAMINOPHEN OVERDOSE

Caroline M. Bates, Narendra Kochar, Janice S. Davidson, Peter C. Hayes, Kenneth Simpson; Scottish Liver Transplant Unit, Royal Infirmary of Edinburgh, Edinburgh, United Kingdom

Introduction Hyperlactataemia is considered an indicator of poor prognosis in acute liver failure due to acetaminophen overdose. The Kings College Hospital (KCH) criteria have been modified in the United Kingdom to incorporate admission and post resuscitation lactate levels in this aetiology. Patients with a lactate greater than 3.5mmol/l 24 hours post overdose or greater than 3.0mmmol/l following fluid resuscitation fulfil a separate criterion for poor prognosis and can be listed for super-urgent orthotopic liver transplant (OLT). Aim To assess the sensitivity and specificity of the lactate criteria when applied to patients admitted to the Scottish Liver Transplant Unit (SLTU). Methods We retrospectively analysed all admissions to SLTU between 1st June 2004 and 31st May 2007 with a diagnosis of acetaminophen overdose. The initial lactate level on admission and a second sample following fluid resuscitation were recorded. We analysed the sensitivity and specificity of these lactate levels in relation to poor prognosis compared with KCH criteria. Results 96 patients were admitted within this 36 month time period with a diagnosis of acetaminophen overdose. The initial lactate level on admission in the remaining 69 patients was 3.8 mmol/l (range 1.1-19.53mmol/l). 37 patients had a lactate >3.5 mmol/L, 22 patients survived (2 fulfilled KCH criteria) and 15 died (13 fulfilled KCH criteria). The sensitivity of initial lactate for predicting death from FHF was 88%, but specificity was only 58%. KCH criteria were 88% sensitive and 94% specific for predicting death. 3 patients within the lactate <3.5mmol/l group subsequently fulfilled KCH criteria, 2 died. 32 patients had a second lactate value, post admission following fluid resuscitation (median time 12.2 (4.4– 27) hours). Within this group 20 patients had a post resuscitation lactate > 3.0mmol/l, 8 of these patients survived (median lactate 3.72 (3.03- 14.61) mmol/l). 2 of the 8 fulfilled KCH criteria. 12 patients died (med lactate 7.07 (3.03–16.86) mmol/l, 11 of these patients fulfilled KCH criteria. Post resuscitation lactate >3.0mmol/l proved 100% sensitive but only 60% specific for predicting death in predicting mortality in acetaminophen overdose. Conclusion The incorporation of lactate into poor prognostic criteria may reduce the time to listing for OLT. In our experience lactate lacks the specificity to be the sole criterion for listing for liver transplantation in acetaminophen overdose. KCH criteria remain sensitive and specific in predicting poor prognosis.

Disclosures:
The following people have nothing to disclose: Caroline M. Bates, Narendra Kochar, Janice S. Davidson, Peter C. Hayes, Kenneth Simpson

855 ELAD® CELLULAR AND SYSTEM PERFORMANCE IMPROVEMENTS

John Brotherton2, Dar He2, Shapour Asslani2, Michael Millis1; 1Dept of Sugery, University of Chicago, Chicago, IL; 2Vital Therapies, Inc, San Diego, CA

One of the concerns with immortalized human hepatocytes has been the robustness of the metabolic pathways. As evidence mounted that the pathways expressed were dependant on the conditions the cells were exposed to, effort was placed on opti-
mizing the metabolic function, by modifying the materials and conditions. METHODS: C3A cells were inoculated into improved polysulfone hollow fiber cartridges with 0.2 micron pores and allowed to grow to confluence. Oxygen delivery and culture media were optimized, with pH and temperature maintained at physiologic conditions. Standard metabolic assessments were performed and compared to the cartridges utilized in the Phase 1 and 2 clinical trials. RESULTS: See Table below CONCLUSIONS: The metabolic growth and maintenance conditions that the C3A cells are exposed to and the physical characteristics of the hollow fiber cartridge utilized significantly affect the metabolic repertoire of the cells. The improved metabolic characteristics may have significant affect on the level of metabolic support provided by the ELAD System. The new system with improved metabolic characteristics is currently in a randomized, multi-center clinical trial assessing the safety and efficacy of the ELAD System for acute decompensation of chronic liver disease

**Metabolic Activity of ELAD Cartridges**

<table>
<thead>
<tr>
<th>Factor V (mg/dl/Cart)</th>
<th>ELAD</th>
<th>Phase 1 and 2 Cartridges</th>
<th>New Cartridges and Conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose Consumption (mg/dl/cart)</td>
<td>13.0 ± 1.8 (n=64)</td>
<td>17.6 ± 2.3 (n=111)</td>
<td></td>
</tr>
<tr>
<td>Lactate Prod to Glu Consumption Ratio</td>
<td>0.59 ± 0.04 (n=25)</td>
<td>0.38 ± 0.13 (n=111)</td>
<td></td>
</tr>
<tr>
<td>Urea Production (mg/dl/cart)</td>
<td>NA</td>
<td>88.5 ± 45.8 (n=89)</td>
<td></td>
</tr>
<tr>
<td>Albumin Production (mg/dl/cart)</td>
<td>264.6 ± 85.3 (n=145)</td>
<td>365.2 ± 87.1 (n=109)</td>
<td></td>
</tr>
<tr>
<td>Glucose Consumption (mg/dl/h/cart)</td>
<td>28.7 ± 5.1 (n=34)</td>
<td>41.6 ± 9.8 (n=70)</td>
<td></td>
</tr>
<tr>
<td>TGF-α Production (mg/dl/cart)</td>
<td>50.3 ± 18.1 (n = 24)</td>
<td>485.7 ± 407.9 (n=16)</td>
<td></td>
</tr>
</tbody>
</table>

All Differences are statistically significant to the 0.001 level.

Disclosures:
John Brotherton - Employee: Other
Dar He - Employee: Other
Shapoor Assiani - Employee: Other
Michael Millis - Consultant/Adviser: Other

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**856**

**ELEVATED SERUM α-NH₂-BUTYRATE:LEUCINE RATIO MAY PREDICT SPONTANEOUS SURVIVAL IN PEDIATRIC ACUTE LIVER FAILURE**

David Rudnick1, Dennis Dietzen1, Ross Shepherd1, Yumirle P. Turmelle1, Song Zhang2, Robert Squires2, Pediatric Acute Liver Failure Study Group The 2; 1Washington University School of Medicine, St. Louis, MO; 2University of Pittsburgh Medical Center, Pittsburgh, PA

Up to half of pediatric acute liver failure (PALF) patients do not survive without liver transplantation; however, early identification of those unlikely to recover spontaneously is challenging. The establishment of the PALF Study Group offers opportunities to test novel strategies that could improve prediction of clinical outcomes in this serious, often fatal condition. Clinical experience suggests that recovery from ALF depends on the ability of the liver to regenerate. Based on this, we hypothesize that molecular markers characteristic of regeneration in the rodent partial hepatectomy (PH) model might have utility as markers of liver regeneration in PALF patients, and thus permit more reliable identification of patients likely to recover spontaneously.

**Methods:** Results of comprehensive amino acid analysis on liver and plasma recovered from mice 6 hours after PH were compared to those from non- and sham-operated mice (3-6 mice per group). Amino acids that were elevated in mice subjected to PH were measured in sera from PALF patients and compared to clinical outcomes. For this analysis, the initially-collected serum samples from 20 randomly selected patients in each of two outcomes groups were analyzed. Outcomes groups included spontaneous survival and death or transplantation. There were no significant differences in age, gender, initial encephalopathy grade, diagnosis, or number of positive King’s College criteria between groups. **Results:** α-NH₂-adipic acid (AAA) and α-NH₂-butyrate (AAB) were identified as elevated in liver and plasma from mice subjected to PH compared to control animals (Table). Analyses of PALF sera showed greater AAB in patients who survived without transplantation (29.2±5.9 µmol/l) compared to those who were transplanted or died (13.9±3.2, p=0.03). The AAB to leucine ratio (AAB:LEU) was also higher in spontaneously surviving PALF patients (0.29±0.03) than in the other group (0.14±0.03, p=0.01). 80% (12 of 15) of patients with AAB:LEU>0.21 survived without transplantation compared to only 32% (8/25) with a ratio<0.21 (p=0.008). **Conclusion:** These data show that the serum AAB:LEU ratio may predict outcomes in PALF patients. Further large scale, prospective studies should be done to assess the utility of this novel candidate diagnostic tool in the management of acute liver failure.

**857**

**ADMISSION LEVELS AND EARLY CHANGES IN SERUM IL-10 ARE ASSOCIATED WITH POOR OUTCOME IN ACUTE LIVER FAILURE AND ACUTE-ON-CHRONIC LIVER FAILURE**

Philip A. Berry, Charalambos G. Antoniades, William Bernal, Munther Hussain, George Auzinger, Elizabeth Sizer, Diego Vergani, Julia Wendon; Institute of Liver Studies, Kings College Hospital, London, United Kingdom

Background: The degree of immunoparesis contributes to prognosis in acute liver failure (ALF) and acute-on-chronic liver failure (AOCLF). The anti-inflammatory cytokine IL-10 is thought to mediate this phenomenon. Although levels of the pro-inflammatory cytokines IL-6 and TNF-α have been examined, the significance of IL-10 levels has not been studied. Aim: We investigated the prognostic value of admission IL-10 levels and their evolution during the early phase of treatment in intensive care, in comparison to the pro-inflammatory cytokines IL-6 and TNF-α. Method: Cytokine levels (IL-10, -6 and TNF-α) were measured within 48 hours of admission in 51 ALF and 39 AOCLF patients admitted to an intensive care environment. Follow-up measurements were obtained a median of 2 days later, in 35 patients, 15 of whom died within 30 days or required liver transplantation. Results: Levels of all measured cytokine were significantly increased in patients with a poor outcome. IL-10 outperformed TNF-α and IL-6 in the prediction of poor outcome in the whole cohort (AUROC 0.73 vs 0.66 and 0.72). IL-10 performed optimally in the subgroups with ALF (0.80 vs 0.63 and 0.70) and acetaminophen induced ALF (0.92 vs...
0.67 and 0.81). IL-6 performed best in the cirrhosis subgroup. Levels of all cytokines rose significantly in the 15 non-surviving or transplanted patients at subsequent measurement; IL-10 by a factor of 2, TNF-α by 2.6 and IL-6 by 1.13. No significant changes were seen in the surviving patients. In the ALF subgroup with poor prognosis only TNF-α changed significantly (x 2.4), and in the cirrhosis group only IL-6 changed significantly (x 1.6), although a ten-fold rise in IL-10 was seen. Conclusion: The magnitude of the anti-inflammatory response at admission, and its evolution during the early phase of treatment, predicts outcome as well as the pro-inflammatory response in acute hepatic syndromes, and supports a vital role for this immunological phenomenon in the outcome of these patients.

**AUROC with respect to poor prognosis for admission levels of IL-6, TNF-α and IL-10**

<table>
<thead>
<tr>
<th></th>
<th>All n=90</th>
<th>ALF n=51</th>
<th>A-ALF n=33</th>
<th>AnCLF n=39</th>
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</thead>
<tbody>
<tr>
<td>IL-6</td>
<td>0.72</td>
<td>0.70</td>
<td>0.81</td>
<td>0.8</td>
</tr>
<tr>
<td>TNF-α</td>
<td>0.66</td>
<td>0.63</td>
<td>0.67</td>
<td>0.78</td>
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<tr>
<td>IL-10</td>
<td>0.73</td>
<td>0.80</td>
<td>0.92</td>
<td>0.72</td>
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</table>

Disclosures:
The following people have nothing to disclose: Philip A. Berry, Charalambos G. Antoniades, William Bernal, Munther Hussain, George Auizinger, Elizabeth Sizer, Diego Vergani, Julia Wendon

**858 FOLLOW-UP OF PATIENTS WITH SEVERE ACUTE LIVER DISEASE USING AN ON-LINE 13C METHACETIN BREATH TEST: A NEW TOOL FOR DECISION MAKING IN CLINICAL HEPATOLOGY**

Gadi Lalazar, Tomer Adar, Yaron Ilan; Liver Unit, Hadassah Hebrew University Medical Center, Jerusalem, Israel

Decision making in the treatment of patients with severe acute liver disease is based on several clinical and laboratory parameters. Currently used parameters, including clinical assessment, serum liver enzymes, synthetic function and serum ammonia levels, lack the ability to accurately assess liver function. Frequent determination of liver function is of importance in the follow-up of patients with severe acute liver disease, including those with fulminant hepatic failure. The BreathID® continuous online 13C methacetin breath test (MBT) enables accurate assessment of the metabolic function of the liver. Aim: To assess the role of the MBT for follow-up in patients with severe acute liver disease. Methods: 15 patients (9 males and 6 females, ages 14-60) with severe acute liver disease, diagnosed with autoimmune hepatitis (5), acute HIV (1), acute HBV (1), drug induced liver injury (4, 2 due to paracetamol) and neoplastic disease (1), were followed-up with 13C MBT during the acute phase of their illness. MBT was performed after an 8 hour fast and ingestion of 75mg methacetin. The correlation between standard follow-up parameters, including clinical assessment, serum liver enzymes, synthetic function, serum ammonia levels; and breath test scores including the PDR peak (percentage 13C dose recovered), CPDR30 and CPDR60 (cumulative PDR at 30 and 60 minutes, respectively) was assessed. Results: Clinical improvement and normalization of biochemical parameters were detected by progressive improvement of 13C MBT scores. In ten patients improvement was detected using MBT 1–3 days earlier than any of the conventional parameters. This was demonstrated by an early positive trend (slope) in breath test values. A model of recovery based on exponential function (1-const.*exp(time_from_baseline/time_constant) for MBT returning to normal metabolic function; and (1+const.*exp(time_from_baseline/time_constant) for blood test value decrease and return to normality, was used to extract the time to recovery. CPDR60 reached normal values within an average 4.5 days compared with 22.8, 12.8, 8.9 and 40.3 for ALT, AST, INR and Bilirubin, respectively. Conclusions: Assessment of liver function by the BreathID® 13C MBT is a rapid, non-invasive follow-up tool in acute liver disease of diverse etiologies. This data suggests that, while correlating with standard parameters, the MBT serves as a more sensitive decision making tool for follow-up of these patients in the setting of severe acute liver disease.

Disclosures:
Yaron Ilan - Consultant/Adviser: Other
The following people have nothing to disclose: Gadi Lalazar, Tomer Adar

**859 EARLY PREDICTION OF OUTCOME OF ACUTE LIVER FAILURE USING BEDSIDE MEASUREMENT OF INTERLEUKIN-6**

William Bernal, Georg Auzinger, Elizabeth Sizer, Julia Wendon; Liver Intensive Therapy Unit, Institute of Liver Studies, London, United Kingdom

Background. Identification of patients with acute liver failure (ALF) with a poor prognosis who would benefit from liver transplantation remains sub-optimal. Current selection criteria are limited by low sensitivity and by late identification of LT candidates; additional prognostic markers are needed to improve prediction of outcome. Circulating levels of Interleukin-6 (IL-6) show highly significant differences between survivors and non-survivors of ALF and may now be rapidly and accurately measured at the bedside using point-of-care (POC) testing. In a cohort of patients with ALF and using POC testing we prospectively examined the clinical correlates of IL-6 and evaluated its potential for use in the early and rapid prediction of outcome. Patients and Methods. 56 patients with ALF admitted to a transplantation ICU were studied. 40 had ALF from acetaminophen-induced hepatotoxicity and 16 from non-acetaminophen causes. Median age was 36 years (inter-quartile range 27-44), encephalopathy grade 3 (1-4), INR 4 (2.9-6), Bilirubin 75 µmol/l (53-127), arterial lactate 4 mMol/l (2.2-8.7) and creatinine 169 µmol/l (94-262). IL-6 was measured within 24 hours of admission in whole arterial blood using a bedside semi-quantitative lateral flow immunoassay (Milenia Quickline IL-6). Statistical testing utilised non-parametric testing and receiver-operating characteristic (ROC) techniques. In assessment of prognostic value, analysis was performed without the inclusion of patients who underwent LT as ‘non-survivors’. Results. 11 patients died, 19 underwent LT and 26 survived with medical management alone. Median IL-6 was 100 to 300 pg/ml (100 to 300-200 to 1000) and correlated with arterial lactate (Spearman r=0.431, p<0.001) and requirement for vasopressors (r=0.528, p<0.001). IL-6 was higher in non-survivors (>300 pg/ml) than medical survivors (100-300 pg/ml). Excluding LT patients, area under the ROC was 0.83 (95%CI 0.66-1); 0.884 (0.74-1) in acetaminophen and non-significant in non-acetaminophen patients. The optimal threshold value for identification of non-survivors was 300 pg/ml. Applied to the whole cohort, excluding LT patients, 8/9 (89%) with >300 pg/ml and 3/28 (11%) <300 pg/ml died (p<0.0001). In acetaminophen patients 6/7 (86%) >300 pg/ml and 2/24 (8%) <300 pg/ml died (p<0.0005); sensitivity was 0.75, specificity 0.96, accuracy 0.9, negative predictive value (NPV) 0.91 and positive PV 0.86. Conclusion. Bedside testing of IL-6 provided a rapid and apparently accurate early assessment of prognosis in patients with ALF. Its predictive accuracy was greatest in patients with acetaminophen-induced disease in whom further study is warranted.
860 PATIENTS WITH SEVERE HEPATIC ENCEPHALOPATHY HAVE DIFFERENT PATTERNS OF RECOVERY OF NEUROCognitive DYSFUNCTIONS

Deanna Oliver1, Fatma Barakat1, Meghan Carlson1, William Perry1, Jan Stange2,1, Tarek Hassanein1; 1University of California, San Diego, San Diego, CA; 2University of Rostock, Rostock, Germany

Patients with severe Hepatic Encephalopathy (HE) who regain consciousness after correcting the precipitating event are judged as improved based on level of consciousness. Information regarding the degree of recovery of Neurocognitive functions from the presenting HE is unknown. We monitored neurocognitive recovery of patients presenting with severe HE grades 3 & 4 by evaluating attention-concentration, visuosconstruction, simple and complex computations, and verbal memory. The aim was to determine if patients with HE grades 3 & 4 recover in a similar path from their hepatic encephalopathy.

Methods: A prospective trial of MARS & SMT for patients with HE grades 3 and 4 was conducted. Data of subjects who responded and did not receive a liver transplant were analyzed. Patients were assessed using the Hepatic Encephalopathy Scoring Algorithm (HESA) for the first 5 days. HESA is an adaptation of the West Haven Criteria. Results: 14 subjects (10 MARS, 4 SMT) had sustained improvement in their HE by at least two grades over 5-day study period without liver tx. Mean age was 50 ± 13 years. 7 patients had HE Grade 3. All patients began the study with 100% impairment. Grade 3 and 4 patients responded similarly, but poorly in testing for Amnesia of recent events. However, in testing for Slow Responses, Shortened Attention Span and Complex Computations, Grade 4 patients had no recovery of functioning while Grade 3 patients did (Table 1). Grade 3 patients had more recovery of their Construction Ability and Simple Computations compared to Grade 4 patients. Both groups had the greatest recovery in their hepatic encephalopathy.

Disclosures:
The following people have nothing to disclose: William Bernal, Georg Auzinger, Elizabeth Sizer, Julia Wenden

861 β-CATENIN ACTIVATION IS CRITICAL FOR HEPATIC REGENERATION AFTER ACUTE LIVER FAILURE

Udayan Apte1, Gang Zeng1, Benjamin Cieply1, Klaus Kaestner2, Satdarshan P. Monga1; 1Department of Pathology, University of Pittsburgh School of Medicine, Pittsburgh, PA; 2Genetics, University of Pennsylvania School of Medicine, Philadelphia, PA

Acute liver failure (ALF) remains a disease with limited treatment options. Improved prognostic in this otherwise fatal condition has been associated with spontaneous liver regeneration indicating therapeutic relevance of ‘regenerative’ therapies. Here, we report activation of the Wnt/β-catenin pathway to be essential for liver regeneration following ALF. ALF was induced in the mice by acetaminophen overdose, which is also the leading cause of liver failure in the Western countries. A non-lethal dose of acetaminophen in mice, which induces both liver injury and regeneration, led to rapid activation of β-catenin secondary to GSK-3β down-regulation and dissociation of the metastable complex. Administration of lethal dose of acetaminophen, which is associated with lack of regeneration, led to failure of β-catenin activation. β-Catenin conditional knockout mice that lack CYP2E1, when treated with acetaminophen following CYP2E1 induction, failed to exhibit liver regeneration. Retrospective examination of biopsies from ALF patients from acetaminophen overdose identified high correlation between nuclear/cytoplasmic β-catenin and spontaneous liver regeneration and vice versa. Further analysis suggested that β-catenin immunohistochemistry on patient biopsies could predict spontaneous regeneration with a positive predictive value and specificity of 100% and sensitivity of 55%. These data affirm the role of β-catenin in liver regeneration after ALF and suggest therapeutic and prognostic significance of this observation.

Disclosures:
The following people have nothing to disclose: Udayan Apte, Gang Zeng, Benjamin Cieply, Klaus Kaestner, Satdarshan P. Monga

862 CEREBRAL BLOOD FLOW AUTOREGULATION IN EXPERIMENTAL LIVER FAILURE

Thomas J. Dethloff1, Gitte M. Knudsen2, Finn Stolze Larsen1; 1Dept Hepatology, afd. 2.12.1, Copenhagen University Hospital Rigshospitalet, Denmark, Copenhagen Ø, Denmark; 2The Neurobiology Research Unit, Copenhagen University Hospital Rigshospitalet, Denmark, Copenhagen, Denmark

In acute liver failure (ALF) alterations in cerebral blood flow are common features, which increase the patients’ risk for neuronal damage due to hypotension. It is well-known that the cerebral blood flow (CBF) autoregulation is impaired in ALF, but the pathophysiological mechanism remains obscure. The purpose of this study was to determine, which of the components of liver failure accounts for this impairment of CBF autoregulation. Four different rat models were chosen, each representing different aspects of ALF: galactosamine (GIN) intoxication versus 90% surgical hepatectomy (PHx90) was applied to examine the effects of liver necrosis versus shear reduction in functional liver mass. A model of porta-caval shunting (PCA) assessed the effects of shunting of portal blood (and hence toxins) into the systemic circulation. Combining the PCA model with ammonia infusion provided information about the additional effects of hyperammonemia. The CBF autoregulation was impaired in both the GIN and PHx90 groups (p<0.0001 compared to control group). By contrast, despite high arterial ammonia, high cerebral glutamine concentration, and significantly increased CBF and ICP, autoregulation was intact in the PCA and PCA+NH3 groups (vs. control: p= 0.16 and p=0.91).

Disclosures:
The following people have nothing to disclose: Deanna Oliver, Fatma Barakat, Meghan Carlson, William Perry, Jan Stange, Tarek Hassanein
Increased water content of the brainstem (PCA vs. control \( p=0.041 \); PCA+NH3 vs. control \( p=0.002 \)) or cerebellum (PCA+NH3 vs. control \( p<0.001 \)) did not affect CBF autoregulation. In conclusion, liver necrosis, porta-caval shunting of blood and hyperammonemia do not impair CBF autoregulation, whereas reduced functional liver mass is associated with loss of CBF autoregulation.

**Impaired autoregulation (autoregulatory plateau phase) in experimental ALF.** In healthy animals CBF is independent of CPP (horizontal plateau phase) while a sloped plateau reveals an impaired autoregulation.

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**AN HLA-DR EXPRESSING MONOCYTE INFILTRATE AND AN ABUNDANCE OF IL-10 TRANSCRIPTS CHARACTERIZE THE LIVER OF ACUTE LIVER FAILURE**

Charalambos G. Antoniades, Philip A. Berry, Munther Hussain, Maria Serena Longhi, Alberto Quaglia, Julia Wendon, Diego Vergani; King's College Hospital, Institute of Liver Studies, London, United Kingdom

Introduction Central to the immunoparalysis in acute liver failure (ALF) is monocyte deactivation, characterised by a reduction in the percentage and absolute number of monocytes expressing the HLA-DR molecule. This reduction and concomitant elevations of circulating levels of both pro-(TNF-α) and anti-(IL-10) inflammatory cytokines, are strongly associated with severity of acute hepatic injury and predict poor outcome. It is not known whether the profound depletion of immunocompetent, HLA-DR expressing monocytes is confined to the periphery or reflects intra-hepatic events. Aim: To compare monocyte/macrophage HLA-DR expression, pro- and anti-inflammatory cytokines in the liver and peripheral blood of patients with ALF. Methods: The macrophage specific CD68 marker, HLA-DR, TNF-α and IL-10 were detected by single epitope immunohistochemistry in serial paraffin-embedded explanted liver sections from 11 ALF patients. Peripheral total monocyte count was determined using Coulter counter and peripheral blood monocyte HLA-DR (mHLA-DR) was determined, 24 hours before transplantation in all 11 ALF and compared to 59 pathological controls with compensated cirrhosis (CLD group) by flow cytometry after staining 50µl fresh blood labelled with monoclonal antibodies for HLA-DR and CD14. mHLA-DR expression is given as % and total number of HLA-DR positive monocytes. Hepatic TNF-α and IL-10 gene expression was determined in 7 out of 11 explanted liver frozen tissue samples using specific primers real-time (rt) PCR. Results All 11 ALF explant specimens were characterised by a large infiltrate of CD68 positive macrophages concentrated in areas of centrilobular necrosis, the majority of these cells strongly expressing of HLA-DR (figure). This contrasted with the findings in the periphery where a profound reduction in median mHLA-DR % (11.6% vs 71%, p<0.001) and total number of HLA-DR expressing monocytes (0.06 x 10⁹/L vs 0.36 x 10⁹/L, p<0.001) was found when compared to pathological controls. Hepatic gene expression of TNF-α and IL-10 was concentrated in areas of necrosis with that of IL-10 (18±2.5) being significantly higher than of TNF-α (3.6±2.0, p=0.021). Conclusion Our data show that in the liver of ALF there is an abundance of HLA-DR expressing intra-hepatic macrophages in striking contrast to their paucity in the periphery; illustrating the dichotomy between local and systemic immune responses during systemic inflammation. The high hepatic IL-10 expression suggests a concomitant anti-inflammatory cytokine profile that could partly explain the IL-10 induced systemic immunoparalysis observed in ALF.

Disclosures: The following people have nothing to disclose: Charalambos G. Antoniades, Philip A. Berry, Munther Hussain, Maria Serena Longhi, Alberto Quaglia, Julia Wendon, Diego Vergani
tality in animals and rescued 90-100% mice, p<0.001. Conclusions: Acute liver failure associated with toxic injury may be the result of profound perturbations in the ATM signaling pathway. Despite extensive liver injury in this situation, hepatic support through transplantation of suitable cells in an appropriate extrahepatic location, such as the peritoneal cavity, will have therapeutic potential. This animal model will have multiple uses in defining the cell therapy potential of various cell populations, including stem cells, as well as other potential interventions for acute liver failure.

Disclosures:
The following people have nothing to disclose: Ekaterine Berishvili, Brigid Joseph, Sriman Bandi, Rabih Srigiang, Yao-Ming Wu, Sanjeev Gupta

865 PRECLINICAL ASSESSMENT OF HEPAHOPE BIOARTIFICIAL LIVER SYSTEM

Sung-Soo Park1, Yoon-Kyong Yang1, Young-Im Lee1, Jaeho Jung1, Delai Zhao1, Young Park1, Hyoun Yoon1, Jun-Seok Park1, Jennifer Tam1, Chintya Ganda1, Hyung-Taek Lee1, Sunny Yang1, Charles Grupper1, Robert G. Gish2, Brendan McGuire3, Angela Panoskaltsis-Mortari4, Dong Jin Suh2, Han Chu Lee3, James Ferguson5; 1HepaHope, Irvine, CA; 2California Pacific Medical Center, San Francisco, CA; 3University of Minnesota, Minneapolis, MN; 4University of Ulsan, Seoul, South Korea; 5ISIS services, Berkeley, CA

Acute liver failure (ALF) is one of major cause of death worldwide. HepaPheresisTM System (HepaHope BiArtificial Liver System containing 2 bioreactors each with ~75g porcine liver slices) was designed to treat such liver failure patients as a potential life-saving alternative. Designated pathogen free, transgenic hCD46 swine from Mayo Clinic were utilized as liver sources. In vitro and in vivo studies were conducted to demonstrate the safety and efficacy of the HepaPheresisTM System for human clinical trial. In vitro studies underwent to evaluate the safety and performance of the HepaPheresisTM System. Human plasma was used to mimic clinical condition. The liver slices in HepaPheresisTM System were proved to be functioning by clearing lidocaine and diazepam and accumulating their metabolites (Dimethylxylidine, temazepam and nordiazepam). Up to 60% of ammonia was cleared with conversion to blood urea nitrogen. HepaPheresisTM System did not activate porcine endogenous retrovirus (PERV) in liver slices. GLP long term (3 weeks) and short term (3 days) safety studies were conducted to test the safety of HepaPheresisTM System by using canines (16 dogs) which were grouped into treatment (with liver slices) and control (without liver slices). All animals showed no significant clinical complications. Biomarkers, physiologic, coagulation and immunologic parameters were in normal range in both control and treatment groups. PERV was not detected in treated canine serum by RTqPCR. In vivo efficacy study was performed by using combined surgical (two stage de-vascularization) and ammonia induced canine ALF model. 10 dogs were arranged to treatment, control and sham group. ALF Scoring system was applied to evaluate reliable liver failure status (liver damage, hepatic encephalopathy and coagulopathy). Based on survival time measured from the end of the HepaPheresisTM System treatment to the death of animal, the treatment group animals survived 3 to 6 times longer than control and sham animals. Other biomarkers especially ammonia were lower in treatment group than sham and control groups. Treatment animals showed decreased intracranial pressure during the BAL treatment and delayed onset of increased intracranial pressure after treatment. The series of studies showed the safety of HepaPheresisTM System. There was no PERV activation during in vitro study and no significant complication in canine treatment. Efficacy of HepaPheresisTM System was demonstrated by ammonia and drug metabolism in vitro study and survival benefit in canine ALF model.

Disclosures:
The following people have nothing to disclose: Ekaterine Berishvili, Brigid Joseph, Sriman Bandi, Rabih Srigiang, Yao-Ming Wu, Sanjeev Gupta

866 ACTIN FREE GC GLOBULIN: A RELIABLE MARKER OF OUTCOME IN ACUTE AND ACUTE ON CHRONIC LIVER FAILURE

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Introduction Gc globulin, a component of the actin scavenger system has previously been shown to be a useful marker of prognosis in acute (ALF) and acute on chronic liver failure (AoCLF) in a retrospective cohort from this institution. We measured actin free (and therefore biologically available) Gc Globulin (Af-Gc), and prospectively assessed its use in ALF and AoCLF patients admitted to a liver intensive therapy unit. Methods Af-Gc was measured in 40 consecutive patients with ALF and 28 with AoCLD within twenty four hours of admission. ALF patients were subdivided into true acute liver failure (tALF; ie with encephalopathy, n=22) or acute hepatic dysfunction alone (AHD; n=18). Af-Gc was measured using sandwich ELISA (AntibodyShop®). Biochemical, physiological and outcome variables were recorded. Results are expressed as median and interquartile range (IQR). Transplantation was considered equivalent to death. Results In ALF patients, Af-Gc values were significantly lower in patients with tALF as opposed to those with AHD (15.1 (2.1-38.9) vs 53.2(27.9-87.7); p<0.001) and also in patients who were listed or fulfilled transplant criteria compared to those that did not (10.4 (2-34.4) vs 53.2 (27.9-87.7); p<0.001; sensitivity 82%; specificity 78%). Af-Gc was significantly higher in those with AoCLF when compared to ALF (59.4mg/L (39.1-105.1) vs 27mg/L (5.3-57.7); p<0.001). In AoCLF patients, Af-Gc correlated with MELD score (r=0.65; p<0.001). Af-Gc was significantly lower in patients requiring filtration (45.6mg/L (21.1-62.1) vs 68.8 (56.3-110.4); p<0.02) and also in those did not survive to 30 days (40.2 (21.1-61.3) vs 68.6 (58.6-68.6); p<0.005). Af-Gc values < 55.6mg/L (AUC
867 INTEGRATED METABONOMIC INVESTIGATION IN THE MODEL OF LIVER INJURY AND REGENERATION INDUCED BY CCl4 IN RATS

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The Metabonomic approach in toxicological studies is used to detect and quantify changes in the levels of metabolites as a response of living systems to toxicity. In the present study NMR based metabonomics, combined with pattern recognition techniques, was applied in order to study metabolic alterations in liver tissue extracts and plasma of rats intoxicated by CCl4. Acute liver injury was induced in adult male Wistar rats by intraperitoneal administration of CCl4 (1 ml/kg BW). The animals were sacrificed at 0 (controls) and at 12, 24, 36, 48, 60 and 72 h after CCl4 administration. Liver tissue and blood samples were collected from all animals for 1H-NMR spectroscopic analysis, estimation of biochemical parameters and histopathological examination. NMR spectra were segmented in 0.04 ppm regions and integrated. The data were analyzed using Principal Component Analysis (PCA) to maximize information recovery. Spectroscopic and biochemical data correlate with histopathological analysis verifying that the maximum of the toxic insult occurred at 24 h post-CCl4 administration, followed by a regenerative process later on. Differences in the levels of glucose, glycogen, lactate, alanine and choline were observed in the aqueous soluble liver extract between controls and CCl4 treated subjects. The lipid soluble liver extract revealed an overall increase in the lipids during the toxic phase compared to controls, gradually regaining the control levels at 72h. Alterations in the levels of glucose, lipoproteins, lactate and formic acid in plasma were also noted. PCA of liver tissue extracts and plasma samples revealed a distinct separation between animals under toxic insult and those at the regenerative state and the control group was totally separated from the CCl4-treated animals. These results suggest that 1H-NMR based metabonomics when applied in a rat model of acute liver injury can distinguish the toxic insult from the regenerative state. Alterations in the levels of metabolites in both liver and plasma reflect changes in energy usage and in metabolic pathways.

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868 ANATOMICAL LOCATION OF ACUTE LIVER INJURY DETERMINES THE BONE MARROW RESPONSE TO LIVER INJURY

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It has been demonstrated by many groups, including our own, that haematopoietic stem cells are mobilised into the peripheral blood (PB) in liver injury. The chemokine SDF-1 has a role in this. However, there are reports in which mobilisation is not seen. We postulated that anatomical location of liver injury may be a factor. We compared two models of acute liver injury in NOD/SCID mice. 4 mice were injured with carbon tetrachloride (CCl4) (0.3 ml/kg) and sacrificed at 24 hours. 4 mice were treated with 70mg/kg phenobarbital daily for 4 days and then with a single dose of 50mg/kg cocaine. They were sacrificed at 24 hours. 4 control animals were used. PB, bone marrow (BM) and liver were collected from each animal. CD34+ cells were counted in both PB and BM. The gradient hypothesis suggests that haematopoietic stem cells migrate along an SDF-1 concentration gradient. We measured SDF-1 concentrations in BM supernatant, plasma and liver homogenate from these animals by ELISA. Similar degrees of injury were obtained in both injury groups, as determined by ALT (>6000iu/µl). CCl4 produced a peri-central injury; cocaine a peri-portal injury. There was an increase in PB CD34+ cells in the cocaine group compared to control (mean 1.32% vs. 0.206% monocytes). In addition, there was an increase in BM CD34+ cells seen in the cocaine group (mean 7.097% vs. 4.72% monocytes). These increases were not seen in the CCl4 group. In control animals the BM/plasma SDF-1 ratio was 0.13ng/mg total protein/0.01ng/mg = 13, whilst in cocaine injury it was 0.31ng/mg/0.26ng/mg = 1.2. This represents a near reversal of the BM/plasma SDF-1 gradient and may account for the increase in PB CD34+ cells seen above. A smaller gradient reversal was seen in CCl4 (0.1/0.06=1.7), but in the absence of an increase in BM CD34+ cells (4.93%), there was no increase in PB CD34+ cells (0.05%). There was a decrease in liver SDF-1 concentrations in cocaine injury (0.0098 ng/mg protein, control 0.037 ng/mg), borne out by diminished staining intensity for SDF-1 by immunohistochemistry. There was no change in the CCl4 group. We postulated that peri-portal injury in the cocaine injured group might cause bile duct injury, resulting in decreased liver SDF-1, but Feulgen staining did not confirm excess apoptosis. Conclusions 1) Anatomical location of liver injury (peri-portal vs. peri-central) determines bone marrow response in NOD/SCID models of acute liver injury. 2) Reversal of the BM/ PB SDF-1 gradient with an increase in plasma SDF-1 is associated with stem cell mobilisation in our model of peri-portal injury. 3) Intra-hepatic SDF-1 plays a minor role in stem cell recruitment in our model.

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Renal Failure in Acute and Acute on Chronic Liver Failure: A Role for Novel Renal Biomarkers?

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Introduction Renal failure is common in acute (ALF) and acute on chronic liver failure (AoCLF). Novel renal biomarkers have been pioneered but have not yet been examined in this patient cohort. We report our experiences of two such markers, Neutrophil Gelatinase Associated Lipocalin (NGAL) and Cystatin C (Cys C), in acute and acute on chronic liver failure. Methods Blood and urine samples were collected within 24 hours of admission to a specialist liver intensive care unit. Novel biomarkers were compared with standard markers of renal function. Serum Cys C was measured by immunonephelometry and NGAL by sandwich ELISA (urine (uNGAL) and plasma (pNGAL), AntibodyShop®). Biochemical and physiological data was collected prospectively over the first 5 days of admission. Renal dysfunction was defined as the need for renal replacement therapy (RRT) or by reduced estimated glomerular filtration rate (eGFR<60). Results are expressed as median and interquartile range. Results In both ALF (n=40) and AoCLF (n=28) patients, pNGAL correlated significantly with day 1 serum creatinine (sCr) r=0.54 (p<0.001), day 1 urine output r=-0.53, (p<0.001), day 3 urine output (r=-0.54, p<0.001). Cys C had similar correlations. uNGAL had strong correlations with sCr (r=0.72, p<0.001) and with eGFR (r=0.77, p<0.001) on day 3. Day 3 values of both pNGAL and uNGAL were significantly higher in patients requiring RRT, as were day 5 pNGAL values in those needing RRT. No significant differences were observed for Cys C. Receiver operator characteristic curves (ROC) showed pNGAL predicted the need for RRT on days 3 and 5 (Day 3: pNGAL AUC 0.84 [0.73-0.94], p<0.001 vs sCr AUC 0.77 [0.64-0.90], p<0.001. Day 5: pNGAL AUC 0.72 [0.57-0.88], p=0.02 vs sCr /Cys C/eGFR not significant). For ALF patients, uNGAL correlated very strongly with day 3 need for RRT (r=0.70, p=0.02), urine output (r=0.85, p<0.001) and eGFR (r=0.83, p<0.001). Both uNGAL and pNGAL were significantly higher in acetaminophen induced ALF than non acetaminophen ALF (uNGAL 374ng/ml (155->500) vs 42ng/ml (5-205) respectively, p=0.02; pNGAL 296ng/ml (199-458) vs 158ng/ml (60-338) respectively, p=0.03). This reflected the higher incidence of renal impairment in the acetaminophen group (95% vs 40%, p=0.02). Admission uNGAL and pNGAL were associated with the need for RRT on day 3 of admission (uNGAL AUC 0.9 [0.71-1.1], p=0.05; pNGAL AUC 0.83[0.69-0.98], p=0.002). Conclusions Plasma and urine values of NGAL represent promising markers of early renal impairment in liver disease. Further investigation may clarify the pathological processes by which renal failure occurs in liver disease.

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Staged Algorithm for Activating Living Donor Liver Transplantation for Acute Liver Failure in Adults

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Background and Aims: Organ scarcity is a major problem worldwide. Facing a patient with acute liver failure (ALF), waiting time is a devastating factor which correlates with high mortality rate. In this report we present an algorithm used to activate a living donation (LD) for a patients with ALF. Patients and Methods: Data of all patients admitted to ICU between 1/2002 and 3/2007 that were diagnosed with ALF and fulfilled King’s criteria for liver transplantation was collected. For patients who were listed as status one, a simultaneous search for LD was started among close family members, aged 18-50, who express their wish to donate. Potential donors underwent medical, psycho-social and imaging evaluation. Upon completion the most appropriate potential donor was presented for final approval by an ethic committee. Even though LD was found and approved, the patient was still waiting for a cadaveric donation (CD). Only when grade III encephalopathy occurred and the patient was sedated, ventilated and no CD was found, LD was performed within 12-24 hours. Waiting time was calculated from listing to transplantation or death. Critical waiting time was referred to the period patient was intubated. Results: Twenty six patients [17 women], aged 1.5-58 years (median 30) with ALF, fulfilled King’s criteria for liver transplantation with an average MELD score of 43 (range 17-58). Sixteen families were informed of a possible need for LD, and 65 potential donors from 13 families started an active evaluation. Nineteen had completed evaluation, 5 of them eventually were declined. Nine potential donors were approved for 9 patients. Eleven patients recovered without transplantation (42%). Twelve underwent liver transplantation (46%); 7 had LD and 5 received CD (2 had an approved potential LD, 2 were transplanted during evaluation of LD and one with no potential LD). Three patients died while waiting (12%): none of them had a potential LD. Critical waiting time was longer for patients who died without transplantation compared with patients transplanted with LD or CD (8.3 vs 2.1 (p<0.001) vs 1.6 (p=0.005) days, respectively). All living donors had an excellent recovery with no major complications. Three months patients and graft survival were 100% for recipients of LD, and 80% for recipients of CD (over median follow up of 42 months (3.5-55 months). Conclusions: Ethical debate of LD for adult’s ALF exists. Nevertheless, the use of a cautious algorithm which provides a safety net of LD may minimize the impact of luck in finding an organ when critically needed. It may reduce waiting-time mortality rate and potentially improves patient’s outcome.

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THE RATIO OF COMPUTED TOMOGRAPHY-DERIVED LIVER VOLUME / STANDARDIZED LIVER VOLUME IS A USEFUL PROGNOSTIC FACTOR OF ADULT ACUTE LIVER FAILURE

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[Background and Aim] Acute liver failure (ALF) is still one of fetal diseases. Since cadaverous liver transplantation is rarely performed by religious reasons in Japan, living donor liver transplantation (LDLT) is usually done for progressive ALF cases. Because a living donor is always a healthy volunteer, prognostic markers are needed to decide whether the patient really needs transplantation. King's College Hospital (KCH) criteria and The Model for End-Stage Liver Disease (MELD) score are famous and useful. Liver atrophy not included in these criteria seems to be one of poor prognostic factors. The aim of this study was to determine whether liver atrophy is a useful poor prognostic marker for ALF. [Methods] Computed tomography-derived liver volume (CTLV) of 30 adult ALF patients treated in our institution was calculated at the time of diagnosis. Their standardized liver volume (SLV) was also calculated using the formula shown in the previous report (Urata, et al. Hepatology 1995). Patients were divided into two groups; group A, 13 patients recovered from ALF without surgical procedure; group B, 17 patients died due to liver failure or underwent LDLT. The CTLV/SLV ratios of two groups were compared. Actual liver weight (ALW) was measured in 14 cases when LDLT or autopsy was performed and compared to their latest CTLV. [Results] The CTLV/SLV ratio was significantly correlated with the ALW/SLV ratio (p<0.001, r²=0.959). The mean CTLV/SLV ratio of group A and B was 1.019±0.182 and 0.757±0.240, respectively (p<0.01). Receiver operating characteristic curve analysis of this showed a good diagnostic potential [area under curve=0.86, p<0.05]. The statistical difference between group A and B was mostly significant (p=0.0002) at the probability cut-off point 0.80 of the CTLV/SLV ratio with 76.5% sensitivity and 92.3% specificity. In the factors of KCH criteria, jaundice duration prior to hepatic encephalopathy (p<0.05) and serum total bilirubin levels (p<0.01) were significantly different between the groups. No significant difference was noted in etiology, age, PT-INR. There were significantly more cases having more than three factors of KCH criteria in group B (p<0.05). There was not the significant difference about MELD score. Multiple logistic regression analysis identified that the CTLV/SLV ratio was an only independent predictor (p<0.05, OR=22.1, 95%CI=1.83-234). [Conclusion] The CTLV/SLV ratio is very useful procedures to objectively estimate liver atrophy and to predict the prognosis of adult ALF cases. A patient with the CTLV/SLV ratio under 0.80 is predicted to have poor prognosis, and transplantation should be considered as soon as possible.

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A NEW GENOTYPE OF HEPATITIS C VIRUS ORIGINATING FROM CENTRAL AFRICA

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The characterization of hepatitis C virus (HCV) variants from different parts of the world has shown that they can be classified into 6 genotypes. Their distributions differ geographically. Genotypes 1, 2 and 4 display significant genetic diversity in Africa while genotypes 3 and 6 show high genetic diversity in southern Asia. On the other hand, genotype 5 is comprised of only one subtype and its origin remains to be established. We have identified three variants (QC69, CS101285 and CS101300) that cluster together but do not classify within the 6 genotypes of HCV. In order to better characterize these variants and to study their evolutionary relationships with the other genotypes, the entire coding region sequence of QC69 and partial coding region sequences of CS101285 and CS101300 were determined. QC69 was isolated from a patient residing in Canada while CS101285 and CS101300 were obtained from patients residing in Belgium. However, all three patients originated from the Democratic Republic of Congo where they are believed to have been infected. QC69 contained an open reading frame of 3013 amino acids. It showed 33.4% to 35.5% nucleotide and 28.1% to 31.0% amino acid divergence with genotypes 1a-H77, 2a-HCJ6, 3a-NZL1, 4a-ED43, 5a-EUH1480 and 6a-EUHK2. Phylogenetic analysis based on complete coding region sequences (Figure) shows that QC69 forms a branch distinct from other genotypes confirming that it constitutes a novel HCV genotype. Additional analysis performed on individual gene regions indicates a closer relationship between genotype 6c and genotype 2 variants analogous to the relationship observed between genotype 1 and 4 variants. This analysis also showed that QC69 was not produced as a result of recombination events between known genotypes. Further epidemiological studies based on coding region sequences are required to determine the prevalence and spread of this genotype in Africa. The finding of a new genotype in Central Africa will contribute in elucidating the origin of the worldwide HCV epidemic. We propose that these three isolates be classified as genotype 7 of HCV.

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OUTBREAK OF A NEW HEPATITIS C VIRUS SUBTYPE (SUBTYPE 1M) IN A HEMODIALYSIS UNIT

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Nosocomial transmission, especially in the hemodialysis setting, is one of the most frequent causes of hepatitis C virus (HCV) infection worldwide. We describe an outbreak of a new HCV genotype 1 subtype in a hemodialysis unit, illustrating how new HCV genotypes may spread in the developing world and further.

METHODS: The 64 patients undergoing regular renal dialysis in the hemodialysis unit of Hubert Kutuku Maga Hospital in Cotonou, Benin (Africa) have been tested for the presence of anti-HCV antibodies and HCV RNA. Two HCV genomic regions, located within the non-structural 5B and the core-E1 coding regions, have been PCR-amplified and sequenced in all cases at the French National Reference Center for Viral Hepatitis B, C, and delta. Genetic and phylogenetic analyses have been performed to assess the genetic relatedness between the strains, in order to evaluate the frequency of between-patient transmissions in the context of hemodialysis.

RESULTS: 19 hemodialysis patients out of 64 (29.7%) were HCV-infected; 17 of them were infected with HCV genotype 1, one with genotype 2 and one with genotype 3. Among the 17 patients infected with genotype 1, 4 (23.5%) were infected with subtype 1i, one with subtype 1b, and 12 (70.6%) could not be subtyped. The 4 patients infected with genotype 1i clustered together and distinctly from other reference genotype 1 strains, suggesting between-patient transmissions in the hemodialysis unit. In the 12 patients that could not be subtyped, the sequences were analyzed by a variety of distance-based, parsimony, and maximum-likelihood methods and evidence was sought for consistent phylogenetic grouping together and distinctness from other subtypes. All studied HCV strains were closely related together and segregated into one monophyletic cluster with high bootstrapping values, suggesting nosocomial transmission in the hemodialysis unit. Furthermore, these strains differed from all of the known HCV genotype 1 subtype nucleotide sequences (1a to 1l) by 16-26% in the NS5B region and 24-33% in the core-E1 region. Since the cluster members diverged from subtypes 1a to 1l by more than 15% of their nucleotide sequence, they could be assigned a new, provisional subtype designation: subtype 1m.

CONCLUSION: We report a high prevalence of nosocomial transmission of HCV in a hemodialysis unit in Benin, Africa. We identified a new subtype of HCV genotype 1, provisionally designated as subtype 1m, that was heavily transmitted among the regular hemodialysis patients. This study witnesses how new subtypes of HCV genotypes may be transmitted though nosocomial routes in the developing world before further spreading.

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HEPATITIS C VIRUS RNA IS DETECTED IN CERVICOVAGINAL FLUIDS OF MENSTRUATING WOMEN, IN A MENOPAUSAL WOMAN WITH BLOODY CERVICOVAGINAL FLUID, BUT NOT IN WOMEN WHO HAVE HAD Hysterectomy

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OBJECTIVE: Sexual transmission of hepatitis C (HCV) accounts for approximately 20% of new infections. Nevertheless, the presence of HCV RNA in the female genital tract has not been extensively examined; in 3 published cross-sectional studies, 19-25% of women of childbearing age had detectable HCV RNA. We sought to define the prevalence of female genital tract HCV RNA shedding in a prospective study of pre- and post-menopausal women.

METHODS: Women with untreated chronic HCV and detectable serum HCV RNA were enrolled. Menstrual status and history of hysterectomy were recorded. Women inserted two Dacron swabs into their vaginas every morning as they would a tampon, placed the swabs into dry cryovials, and stored them immediately in their home freezers for 18-56 consecutive days. Menstruating women indicated menstruation dates. HCV RNA and hemoglobin were detected in cervicovaginal fluids using real-time RT PCR and spectrophotometry, respectively. Statistical analyses were performed using nonparametric tests and generalized estimating equations taking into account repeated measures.

RESULTS: 16 women were enrolled; median age was 50 (range 27-59). Seven women were menstruating, 6 were menopausal, and 3 had had hysterectomies. Baseline serum VL was 6.43 (range 5.17-7.36 log10 IU/ml) and did not differ by menstruation or hystereCTomy status. Menstruating women were more likely than menopausal women to have HCV RNA detected in cervicovaginal fluids on at least one day (86% versus 16%, p=.02). For menstruating women, detection of HCV RNA was more likely during menstruation (OR= 56.4, 95% CI (23.0,138.3)) or when hemoglobin was detected in cervicovaginal fluids, even when menstruation was not occurring (OR= 35.4, 95% CI (12.2,102.6)). The median quantity of HCV RNA detected in cervicovaginal fluids in menstruating women was 4,631 (range 231-69,580 IU/ml). Only 1 menopausal woman had HCV RNA detected in cervicovaginal fluids; she also had hemoglobin detected on 8 days during the 56 day sample collection period.

CONCLUSIONS: In women with chronic hepatitis C, HCV RNA detection in cervicovaginal fluids is associated with menstruation or the detection of hemoglobin on non-menstruation days. HCV RNA was not detected in women who had had hysterectomies, suggesting that the presence of the cervix plays a role in HCV RNA genital tract shedding in women. The quantity of HCV RNA detected was low, supporting the epidemiologic finding that HCV is not efficiently transmitted sexually. These findings highlight the fact that HCV is primarily a blood-borne virus, and may have implications for menstruating women with chronic hepatitis C.

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HEPATITIS C TESTING, CARE AND TREATMENT: EVOLUTION OVER TEN YEARS IN FRANCE

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With a prevalence of Hepatitis C virus antibodies (anti-HCV) of about 1% in the population in 1994, hepatitis C was considered as a serious public health issue by French health authorities that consequently implemented a national prevention and control program in 1999. To assess its impact we set up two surveillance systems to 1) monitor trends in anti-HCV screening (Rena-VHC) and 2) to follow up epidemiological-clinical characteristics of HCV patients newly referred for care and treatment in hepatology centres (Pôles-de-Référence) and conducted a population based prevalence survey in 2004. We present the change over time of several key indicators. We used four data sources. Two were national cross sectional population-based serosurveys of French adult metropolitan residents that collected risk factors and awareness of HCV infection. Serum samples were tested for anti-HCV and HCV RNA. The third was Rena-VHC, based on 159 laboratories spread all over France that report number of performed anti-HCV tests, number of positive tests, age and sex of positive diagnosed persons. The forth was Pôles-de-Référence which relies on 26 hepatitis C reference centres and describes clinical and epidemiological characteristics of newly referred patients, including circumstances of anti-HCV testing, risk exposure for HCV transmission, HCV RNA serum status and severe liver disease (cirrhosis, hepatocarcinoma).

From 1994 to 2004, the awareness of an anti-HCV seropositive status increased from 24% to 57% (95%CI: 43-71). In 2004, awareness was 93% (95% CI:77-98) for persons with history of injection drug use and, 67% (95% CI: 41-85) in case of transfusion <1992. From 2000 to 2005, Rena-VHC showed a 45% increase of overall anti-HCV performed tests and a significant decrease in the proportion of positive tests (p<0.0001, X2 linear trend). From 2001 to 2005 in Pôles-de-Référence, routine medical check-up was increasingly reported as a reason for anti-HCV screening (41% vs 52%) whereas screening in case of risk factors declined (25% vs 17%). The proportion of patients at first referral in the year of screening decreased slightly (49% vs 43%) and proportion of patients with cirrhosis (Metavir score F4) remained stable (11%). Our analysis of four sources of information indicates convergent results which suggests that the hepatitis C program was effective, particularly in increasing the proportion of HCV patients screened. However the decrease of positive tests with an increase of overall tests suggests an excess of screening of persons at low risk. The screening strategy needs to evolve to better and more timely identify those at risk of HCV infection for earlier intervention.

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INJECTION DRUG USE, SOCIAL NETWORKS AND HEPATITIS C: UNDERSTANDING THE BEHAVIOURAL AND IMMUNOVIROLOGICAL FACTORS LEADING TO TRANSMISSION - THE NETWORKS II STUDY

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Hepatitis C virus (HCV) infection affects an estimated 180 million people worldwide, and causes 50–76% of liver cancers and two thirds of liver transplants in the developed world. The burden of disease and costs attributable to HCV-related chronic liver disease will grow due to complications of cirrhosis and hepatocellular carcinoma in ageing chronically-infected cohorts and as new HCV infections arise. It is imperative that research efforts focus on ways to prevent new HCV infections to reduce the global burden of disease. In Networks II study we established a longitudinal IDU cohort study; 251 IDUs are followed for two years. Networks II enables us to study HCV transmission (naïve, recurrent and superinfection) and behavioural and immunological determinants of this and HCV clearance using a social network approach where the relationship between the IDUs is studied. Recruitment commenced in Melbourne, Australia in July 2005. Every three months participants completed a questionnaire about sexual, injecting and other risk behaviour, network data, and provided a blood samples. Blood tests included HCV antibody, HCV RNA, genotype and molecular sequencing. Samples were also HLA typed and then tested for HCV specific CD8+ T cell response using minimal T cell epitopes. We describe the results for the first year of the study. The traditional (naïve) incidence in our cohort to date is 29.1% per annum (95% CI 17.5, 48.3) and 79.2% (95% CI 23.5, 245.4) in IDUs injecting for less than one year. The incidence of reinfection was 50.6% (33.3, 76.9) and of superinfection 20.8% (12.9, 33.4). A key driver of HCV infection was having all members of your network HCV-infected whereas the number of injecting partners and the level of needle-sharing were less important when measuring disease transmission. We identified 39 persistently HCV RNA negative people who injected with >=1 RNA-positive partner, and six who recently injected with a needle used by >=1 RNA-positive partner; this suggests they possess innate resistance or develop protective immunity. Our immunological data suggest that IDUs who clear HCV maintain functionally potent memory CD8+ T cells that are highly cytotoxic and produce antiviral cytokines (IFNg and TNFa); conversely, CD8+ T cells from IDUs with persistent HCV infection are functionally downregulated with compromised cytotoxic and cytokine secreting capacity. The results of Networks II thus far have advance understanding of the dynamics and immunovirology of HCV infection transmission. It is our expectation that the study will provide further insights that will contribute to HCV prevention intervention strategies and vaccine development.

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The following people have nothing to disclose: Margaret E. Hellard, Campbell Aitken, Mandvi Bharadwaj, Scott D. Bowden
Purpose: People with chronic hepatitis C (HCV) may face barriers to accessing health services. The aims of this study were to determine the extent to which perceived barriers may complicate HCV care and to examine the relationship between barriers and socioeconomic (SES) factors. Methods: One hundred and twenty eight (n=128) patients referred for HCV at two hepatology centers completed instruments on a laptop computer during one of their first two clinic visits. The Barriers to Care Scale (BACS), a 12-item scale with 4 subscales developed for HIV patients, was modified for this study. Items were rated on a 4-point scale, ranging from 1 (No problem) to 4 (Major Problem) to indicate the extent to which each barrier made it difficult to access HCV care. Nonparametric tests were used to explore associations between SES and BACS subscales.

Results: The majority of participants were male (55%), Caucasian (81%), married (53%) with a mean age of 46 years. SES characteristics were as follows: Working full or part-time (70%); Household income of < 40K (56%); No insurance (11%); Traveled over 30 minutes to clinic (54%); and Lack of self-transportation (21%). The greatest barriers rated by participants were lack of personal financial resources, lack of HCV knowledge in their community, and feeling stigmatized for having HCV. Long distance to a hepatitis facility and lack of professionals competent in HCV care were also highly rated as barriers to HCV care (See Table 1). The following associations were found between BACS subscales and SES factors: 1) Distance was a barrier for unemployed patients (p=.04) and patients without self-transportation (p=.006); 2) Community stigma was greater for women compared to men (p=.04); and 3) Personal resources were a barrier for unmarried patients (p=.001), patients without private insurance (p=.007), and patients without self-transportation (p=.000). Conclusions: HCV patients perceive numerous barriers to accessing HCV care, which are related in part to SES factors. These data must be considered when designing interventions to increase access to care for HCV patients.

<table>
<thead>
<tr>
<th>Table 1: Barriers to Care (1= No problem; 4=Major problem)</th>
<th>Means(Std)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Geographical/Distance - Long distance to facility - Lack of transportation</td>
<td>1.9 (1.0) 1.4 (1.78)</td>
</tr>
<tr>
<td>2. Medical/Psychosocial - Medical professionals who do not provide care for HCV - Lack of professionals who are trained and competent in HCV - Lack of mental health professionals - Lack of psychosupport groups for HCV patients</td>
<td>1.6 (1.0) 1.9 (1.0) 1.4 (1.73) 1.6 (1.83)</td>
</tr>
<tr>
<td>3. Community Stigma - Level of knowledge about HCV in community - Community stigma against persons with HCV</td>
<td>2.3 (1.08) 2.1 (1.8)</td>
</tr>
<tr>
<td>4. Personal Resources - Lack of employment opportunities for people with HCV - Lack of understanding work environment for people with HCV - Personal financial resources - Lack of adequate and affordable housing</td>
<td>1.8 (1.07) 1.8 (1.03) 2.4 (1.10) 1.7 (1.98)</td>
</tr>
</tbody>
</table>

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878 BARRIERS TO HEALTHCARE ACCESS AMONG CHRONIC HEPATITIS C PATIENTS

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BACKGROUND: Although hepatitis C (HCV) is common in the United States, the majority of patients are ineligible for clinical trials due to rigorous enrollment criteria and do not undergo standard treatment due to comorbid medical conditions and adherence failure. The majority of trials available in the U.S. are targeted at treatment naive genotype 1 HCV infected patients. AIM: To determine the candidacy of treatment naive HCV infected patients for HCV clinical trials referred to a tertiary referral center. METHODS: Using a hepatitis C registry database, we reviewed 230 consecutive new patients referred with a positive HCV antibody by ELISA to The University of Chicago Center for Liver Diseases between April 1, 06 and March 31, 2007. HCV infection was confirmed with PCR for HCV RNA and genotype status was identified. A retrospective chart review of treatment naive genotype 1 HCV infected patients using computerized records was performed to determine: patients’ demographic data, presence of advanced or concomitant liver disease and comorbidities. Both eventual standard of care HCV treatment and reasons for HCV study exclusion were recorded. RESULTS: Of the 230 patients, 128 (55.7%) were treatment naive genotype 1 HCV infected patients. The other 102 patients were: previously treated (n=68), other genotypes (n=22), unknown genotypes (n=3) or were HCV PCR negative (n=9). Of the 128 naive genotype 1 patients, 9 (7.0%) were enrolled in clinical trials and 17 (13.3%) were started on standard Peg-IFN based HCV therapy. Reasons for trial exclusion include: declined trial (n=26, 25.5%), decompensated or concomitant liver disease (n=18, 17.6%), malignancy in the last 5 years (n=14, 13.7%) renal insufficiency (n=13, 12.7%), ongoing alcohol abuse in the last 6 months (n=11, 10.8%), illicit substance use in the last 12 months (n=8, 7.8%), psychiatric illness (n=8, 7.8%), HIV (n=7, 6.9%), lost to follow up (n=6, 5.9%), cardiopulmonary issues (n=6, 5.9%) and uncontrolled diabetes (n=3, 2.9%). Seventeen (16.7%) had more than one criteria for study exclusion. Of the 17 on standard treatment, 12 (70.1%) had exclusion criteria for clinical trials. CONCLUSIONS: 1) Rigorous enrollment criteria of current clinical trials precludes most HCV infected patients referred to tertiary care referral centers. 2) The most common reasons for trial exclusion from treatment naive genotype 1 studies include: treatment refusal, decompensated or concomitant liver disease, malignancy and renal insufficiency, respectively. 3) Further studies should be done to determine feasibility of expanding enrollment criteria of future clinical trials.

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The following people have nothing to disclose: Rohit Satoskar, Amanda DeVoss, Katherine Wherity
879 ROLE OF CYP2D6*4 AS A GENETIC MARKER FOR FAST FIBROSIS PROGRESSION RATE IN CHRONIC HEPATITIS C: VALIDATION OF A PILOT STUDY IN LARGE COHORT

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BACKGROUND: Fifteen to twenty percent of chronic hepatitis C (HCV) patients progress during their life time to end stage liver disease and it crucial to identify them preemptively in order to give them a suitable treatment. However, markers of fibrosis progression rate are not available, yet. A recent pilot study has demonstrated an association between CYP2D6 polymorphism and fibrosis rate AIM: The aim of our study was to verify our preliminary data in a larger cohort and to determine whether CYP2D6*4, the poor metabolizer allele can predict fibrosis progression rate. METHODS: One hundred fifty six caucasian patients with chronic HCV and were recruited. All the patients had a liver biopsy assessed by the META VIR scoring system. No patient had received interferon treatment before biopsy sample was obtained. They were divided into “fast fibrosers” and “slow fibrosers” according to Poynard’s fibrosis progression curves, based on age of exposure and duration of infection. We assessed the effect of the following factors on fibrosis progression rate: estimated duration of infection; age; age at infection; hepatitis C virus C (HCV) genotype; HCV viremia; CYP2D6 polymorphism; and histological activity grade. DNA was extracted from peripheral blood and CYP2D6*4 was tested by polymerase chain reaction (PCR) method, using fluorescent hybridization probes in a LightCycler instrument. RESULTS Thirty four patients were classified as ‘fast fibrosers’ and 109 patients as ‘slow fibrosers’. The mean rate of fibrosis progression per year was 0.094 fibrosis unit (95% CI 0.0796-0.108). Using stepwise logistic regression we found that CYP2D6 polymorphism and liver biopsy activity were independent factors associated with fast rate of fibrosis progression. There was no association between fibrosis progression rate and age of infection or HCV genotype. The OR for CYP2D6 polymorphism was 2.02 (95% CI 1.02-3.95). Means that carrying one CYP2D6*4 allele doubles the risk for fast progression to fibrosis, and homozygous state increases the risk by four folds. CONCLUSIONS: This study indicates that CYP2D6 genotyping is a useful tool to predict liver fibrosis progression rate in HCV patients. Prospective studies are needed to further validate the data that bear significant therapeutic implication.

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880 IMPACT OF HEPATITIS B CORE ANTIBODY POSITIVITY ON CHRONIC HEPATITIS C PATIENTS

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AIM: To investigate whether HBc-Ab positivity is a risk factor of hepatocarcinogenesis in chronic hepatitis C patients. METHODS: We retrospectively analyzed the incidence of hepatocellular carcinoma (HCC) among patients with HCV infection followed up at our institution. Between 1994 and 2004, a total of 1431 patients who were positive for HCV, negative for HBs-Ag, and without HCC visited our out patient clinic and were followed up. We enrolled 995 of them whose status of HBc-Ab at visit could be determined. They were divided into two groups according to HBc-Ab; 574 patients were positive for HBc-Ab and 421 patients were negative for HBc-Ab. Characteristics of those two groups were compared by Student T-test and chi-square test. The cumulative incidence of HCC was assessed by Kaplan-Meier method and the difference between the groups was assessed by the log-rank test. The effect of positivity of HBc-Ab on hepatocarcinogenesis was assessed by multivariate Cox proportional hazard regression adjusted for other risk factors such as age, sex, alcohol intake, comorbidity with diabetes mellitus, presence of obesity and liver function. RESULTS: There were 514 male and 481 female patients with the median age of 60.1 year (range, 52.4 - 66.9). There were significantly more men (51.7% vs. 48.3%, P < 0.001) and elderly patients (mean age, 61.0 vs. 57.0, P < 0.001) in those with HBc-Ab than those without. HCC developed in 302 patients during mean follow-up period of 6.3 years, showing cumulative incidence rates at 3, 5 and 10 years of 15.8%, 29.9%, and 49.5% in those with HBc-Ab and 11.8%, 20.8% and 39.4% in those without, respectively (P = 0.022 by the log-rank test). Adjusted by other significant factors including age, gender, alcohol intake, diabetes mellitus, obesity, albumin, aspartate aminotransferase, platelet counts and prothrombin activity, HBc-Ab positivity was not a significant risk factor (hazard ratio, 0.996; 95%CI, 0.766 - 1.218; P = 0.772). The apparent difference of HCC incidence between HBc-Ab positive and negative groups was confined by differences in gender and age. CONCLUSIONS: HBc-Ab positivity was not a significant independent risk factor of hepatocarcinogenesis among chronic hepatitis C patients.

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881 CLARIFICATION OF INTERSPOUSAL HCV INFECTION IN ACUTE AND CHRONIC HEPATITIS C PATIENTS BY PHYLLOGENETIC ANALYSES OF HCV E1 AND NS5B REGION

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AIM: The aim of this study was to clarify the source of HCV infection in acute and chronic hepatitis C patients using phylogenetic analyses of nucleotide sequences of HCV E1 and NS5B region. Patients and Methods: Four acute hepatitis C patients and one chronic hepatitis C patient, whose spouses were HCV-positive, were hospitalized. Three of four acute hepatitis C patients were male and the other was female. One chronic hepatitis patient was female. The ages of acute hepatitis C patient ranged from 33 to 73 years old. And the age of chronic hepatitis patient, whose serum had been revealed to be HCV-positive
14 years before hospitalization, was 62 years old. No patient was intravenous drug user. No blood transfusion was performed in any patient. The diagnosis of acute hepatitis C was based on medical records, laboratory tests including HCV markers, and ultrasound and sonography. Additional tests on HCV viral load and HCV genotype were carried out. Then phylogenetic analysis of nucleotide sequences of partial HCV E1 region (427 nucleotides) of the acute hepatitis C patients and their spouses were performed. In addition, phylogenetic analysis of nucleotide sequences of partial HCV E1 region and partial NS5B region (336 nucleotides) of chronic hepatitis patient and her spouse were performed. Informed consent was obtained from all patients. Results: The peak values of serum ALT in 4 acute hepatitis patients ranged from 1054 U/l to 4500 U/l and those of serum total bilirubin ranged from 3.6 mg/dl to 23.8 mg/dl. HCV antibody in the serum changed from negative to positive in the course of hospitalization and HCV RNA could be detected in every patient. Therefore, they were diagnosed as acute hepatitis caused by HCV infection. Three of four couples of acute hepatitis C had HCV RNA of the same genotype. The genotypes were 1b in two couples and 1a in one couple. Homogeneity of nucleotide sequences of HCV partial E1 region ranged from 99% to 100%. The results of phylogenetic analyses suggested that interspousal HCV infection occurred in three couples. The couple of chronic hepatitis C patients had HCV RNA of the same genotype. Homogeneity of nucleotide sequences of HCV partial NS5B region and E1 region was 97% and 92% respectively. The rate of nucleotide substitution of HCV during marriage was speculated to be 0.5–1.1x10⁻³ /site/year. These results of phylogenetic analysis implied interspousal HCV infection in this couple. Conclusion: Interspousal infection may be an important source of acute and chronic HCV infection. The usefulness of phylogenetic analysis of nucleotide sequences of HCV partial E1 and NS5B region for clarifying interspousal HCV infection was validated.

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**883 INDIRECT SERUM MARKERS OF FIBROSIS PREDICT MORTALITY IN WOMEN WITH HIV AND HCV INFECTIONS**

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Co-infection with HCV is a major cause of morbidity and mortality in persons with HIV, with liver disease the leading cause of non-AIDS related death. Predictors of mortality are poorly defined and men predominate in most studies. We evaluated whether two indirect markers of hepatic fibrosis, the APRI and FIB-4 scores, predict mortality in the Women’s Interagency HIV Study (WIHS). Methods: WIHS is a prospective, multicenter, cohort study of women with or at risk for HIV. HCV status was assessed at enrollment; lab and clinical data were measured semi-annually. Associations between fibrosis scores and all-cause mortality were assessed using time-dependent Cox regression analyses. Results: 3168 women (160 HCV+HIV-, 831 HCV+HIV+, 1523 HIV+HCV, and 654 HIV-HCV) were followed up to 12 yrs (median 6 yrs); median age 35 yrs. Compared to HCV-, HCV+ women had higher median APRI (0.5 vs 0.2) and FIB-4 (1.2 vs 0.6) at baseline, (p-value <0.0001 for both). At baseline, 88% of HIV+HCV+ and 63% of HIV+HCV- women had minimal fibrosis, based on fibrosis scores, while 1% of HIV+HCV- and 6% of HIV+HCV+ women had severe fibrosis. 539 women with HIV died. The table shows baseline and last available APRI among the deceased. In multivariable models, APRI predicted all-cause mortality in WIHS. Moderate (0.5–1.5) or severe (>1.5) elevations at baseline conferred hazard ratios (HR) of 1.5 [95%CI,1.2–1.8] and 2.7, [95%CI,2.0–3.7], respectively, after controlling for HIV, HR 4.0 [CI,2.7–5.7]; HCV, HR 1.8 [CI,1.4–2.1]; HBV, HR 1.7 [CI,1.1–2.7]; HAART, HR 0.8 [CI,0.7–1.0]; heavy alcohol, HR 1.0 [CI,0.8–1.4]; age (10 year increments), HR 1.4 [CI,1.3–1.6]; body weight, HR 0.8 [CI,0.6–0.9]. FIB-4 results were similar. Conclusion: HCV+ women had higher baseline APRI and FIB-4 scores compared to HCV- women. Among HIV+/HCV+ women, those who died had evidence of more fibrosis, based on fibrosis scores, compared to HIV+/HCV-, at baseline and at last follow-up. Women with moderate or severe fibrosis had increased mortality risk (1.5 and 2.7-fold, respectively), compared to women with low fibrosis scores, after controlling for HCV, HIV, HAART, alcohol and weight. APRI and FIB-4 may have clinical prognostic utility among women with HIV and HCV.

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**882 AUTO-ANTIBODIES IN CHRONIC HEPATITIS C INFECION: HOW FREQUENT ARE THEY AND WHAT DO THEY MEAN IN A COHORT OF 963 PATIENTS?**

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Background: Chronic hepatitis C infection is associated with an increased prevalence of non-organ specific autoantibodies. Previous small studies have given widely varying estimates of prevalence and conflicting results with regard to the clinical implications for disease activity and response to treatment. Aim: To define the prevalence of anti-nuclear (ANA) and anti-smooth muscle (ASM) antibodies within a large cohort of treatment-naive chronic hepatitis C patients, and assess for differences in patient demographics, biochemical and histological markers of disease activity, and subsequent response to anti-viral treatment. Results: We studied 963 patients. 172 (17.9%) had at least one autoantibody, of which 118 (12.3%) were ASM and 68 (7.1%) were ANA. Autoantibody-positive patients were older (43 vs 39 years, p 0.001) but there were no significant differences in gender, BMI, alcohol intake, ethnicity or viral genotype. The presence of autoantibodies was associated with an increase in interface hepatitis score (1.1 vs 0.8, p 0.005) but no difference in other necroinflammatory measures, liver function tests or immunoglobulins. Fibrosis stage was not increased in patients with autoantibodies (2.0 vs 1.9, p 0.8), nor was the rate of fibrosis progression on later biopsies (50% progression vs 34%, p 0.1). There was no difference in response to anti-viral treatment (sustained virological response 52% in both groups, p 1.0). Conclusions: Autoantibodies are frequent in chronic hepatitis C infection, occurring in an older population and in association with increased interface hepatitis. However, they do not carry increased risk of fibrosis or failure of therapy.

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POPLATION BASED HEPATITIES A, B AND C SERO-
SURVEY AND GENOTYPING IN BRAZIL IN CAPITALS
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Objectives: To estimate the seroprevalence of hepatities A (HAV), B (HBV) and C (HCV) and genotypes for HBV and HCV in three macroregions of Brazil (Northeast-NE, Central-West-CW and Federal-District-DF regions). Methods: A population-based survey was carried out in these regions (2004/2005), as part of a national survey, which is being conducted in all Brazilian regions. The sample was representative of the ensemble of state capitals in each region and for each age group considered. The sample was stratified by capital with a uniform sampling fraction. In each capital census tracts and blocks were drawn with probability proportional to size; then a systematic sample of households was drawn and their residents selected. The study was approved by regional and national ethical committees. Serology for HAV, HBV and HCV (ELISA) and genotyping for HBV and HCV were performed. Results: A total of 10,525 individuals were investigated. Seroprevalence of AntiHAV IgG in NE region: 41.50% (95%CI 35.80-47.36) in the age group 5-9 and 56.60% (95%CI 51.15-61.92) in the age group 10-19; in CW: 32.3 % (95%CI 27.1-37.8) in the age group 5-9 and 55.9% (95%CI 50.2-60.9) in the age group 10-19; and in DF: 33.8% (95%CI 27.1-37.8) and 65.1% (95%CI 59.1-70.7%) for age 5-9 and 10-19 age groups, respectively. Seroprevalence of AntiHBC in NE region ranged from 2.12% (95%CI 1.51-2.88) in the age group 10 to 19 to 11.62 (95%CI 10.20-13.16) in the age group 20-69; in CW region: 1.3% (95%CI 0.8-1.9) in the aged group 10 to 19 and 12.7% (95%CI 11.2-14.2) in the age group 20-69, and in DF: 1.1% (95%CI 0.6-2.1) in group aged 10-19 and 8.3% (95%CI 6.6-10.1) in the age group 20-69. Seroprevalence of AntiHBe in NE region ranged between 2.12% (95%CI 1.51-2.88) in the age group 10 to 19 to 11.62 (95%CI 10.20-13.16) in the age group 20-69; in CW region: 1.3% (95%CI 0.8-1.9) in the aged group 10 to 19 and 12.7% (95%CI 11.2-14.2) in the age group 20-69, and in DF: 1.1% (95%CI 0.6-2.1) in group aged 10-19 and 8.3% (95%CI 6.6-10.1) in the age group 20-69. Seroprevalence of HBSAg in NE: 0.11% (95%CI 0.01-0.39) in the age group 10-19 and 0.48 (95%CI 0.22-0.91) in the age group 20-69; in CW: ranged from 0.2% to 0.7% and in DF ranged from 0.2% to 0.4% according to age groups. HBV-DNA genotypes identified D, A and F in all regions. Anti-HCV in NE: 0.4 (95%CI 0.55-1.50) in the age group 10-19 and 1.5% (95%CI 1.30-2.61) in the age group 20 to 69; in CW: 1.1% (95%CI 0.6-1.6) in the aged group 10-19 and 1.9% (95%CI 1.42-2.7) in the age group 20-69 and in DF 1.2% (95%CI 0.5-2.0) in the age group 10-19 and 1.7% (95%CI 1.0-2.7) in age group 20-69. HCV-RNA genotype 1, 1b, and 3a were identified in all three regions. Conclusion: This population based survey detected high prevalence of HAV infection among children with evidence of early viral exposure. The HBV endemicity was low probably due to HBV vaccination offered by the national routine immunization program. High prevalence of anti-HCV was detected in all regions and different genotypes were found at Brazilian population.

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sustained-response (64.8% vs. 52.3%, p=0.003). Stratification for HCV genotypes showed that this effect was restricted to HCV genotype-1 (66.3% vs. 51.0%, p=0.001). Prevalence of LDLR 3’UTR 2728-GG declined from non-response to relapsed-response to sustained-response (66.3% vs. 55.7% vs. 48.7%, p=0.001). Multivariate analysis confirmed the LDLR 3’UTR 2728-A/G SNP as an independent predictive factor for treatment response. There was no obvious association between LDLR SNPs and histological severity of liver fibrosis and inflammation in chronically HCV-infected patients. CONCLUSION: LDLR polymorphisms appear to be associated with spontaneous and treatment-induced recovery from HCV genotype-1 infection. Common SNPs affecting the host lipoprotein metabolism might contribute to genetic susceptibility to chronic HCV infection and response to therapy.

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886 CORRELATES OF INCIDENT HCV INFECTION AND SERONEGATIVE-IMMUNE STATUS IN HIGH RISK, IDU PRISONERS
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Introduction: High rates of hepatitis C (HCV) infection are reported amongst prison inmates, primarily linked to injecting drug use (IDU). HCV-specific cellular immunity is found in a small subset of high risk, seronegative subjects and may protect against chronic infection. Both factors are likely to influence incident infection rates and outcomes. The aim of this study is to determine HCV incidence in seronegative IDU prison inmates, the prevalence of HCV-specific immunity, and associated risk behaviours. Subjects and methods: Adult inmates have been recruited into a prospective cohort – the Hepatitis C Incidence and Transmission Study-II (HITS-II) study provided they are: greater than 17 years of age; have a lifetime history of IDU; and imprisonment and documented negative HCV antibody status within 12 months. Subjects complete a detailed interview to record demographics and risk behaviours. Blood is collected to screen for: HCV antibodies by ELISA (Innogenetics); HCV viraemia (Bayer); interferon-gamma production by enzyme-linked immunospot (ELISpot) and multiplex in vitro cytokine production following stimulation with HCV peptides. Thresholds for positive responses were based on data in low risk, seronegative subjects (n=15). The frequencies of demographic and behavioural risk factors were compared (SPSS for Windows v.13.0) Results: 242 inmates have been enrolled, and 184 tested for HCV antibodies and viraemia. Five had antibody indeterminate status and were therefore excluded from the analyses. Of the 179 subjects, 39 (22%) had seroconverted. Female gender (p=0.047), an increased number of incarcerations (p=0.013), tattooing (p=0.021), and methadone treatment (p<0.0001) were associated with seroconversion. Of the 179 subjects, 102 have been tested for HCV-specific immunity, with 22/78 seronegatives (28%) and 18/24 (75%) seroconverters having positive responses in the interferon gamma ELISpot. Seronegative-immune status was positively associated with specific patterns of IDU, and negatively with a recent break from IDU (all p<0.01). The specificity of the ELISpot responses differed in that anti-Core, NS2 and NS4a responses were not found in seronegative-immune subjects. The multiplex assay revealed that seronegative-immune subjects also had generally low levels of interleukin-10 and high levels of tumour necrosis factor-alpha production in response to NS4-5 stimulation. Conclusions: Both incident infection and seronegative-immune status are common in high risk IDU prisoners. Further follow-up of the subjects in the HITS cohort will evaluate the details of the predictive risk behaviours and protective efficacy of the immune responses.

Disclosures:
The following people have nothing to disclose: Paul S. Haber, Barbara Cameron, Lisa Elliott, Suzy Teutsch, Rose French, Kate Dolan, Bill Rawlinson, Michael Levy, John M. Kaldor, Andrew R. Lloyd

887 ANALYSIS OF HCV IN SOUTH EAST ASIA
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Aim: We performed a detailed evolutionary analysis of HCV genotype-6 across S.E. Asia. Methods: Plasma was obtained from 31 patients admitted to Mahosot hospital, Vientiane, Laos with jaundice and/or hepatitis and who were HCV sero-positive. HCV viraemia was determined by RTPCR of the 5’UTR. Amplification of Core (464 bp) and NS5B (377bp) was performed in viremic patients by nested PCR. These sequences were concatenated and aligned with all genotype-6 sequences available from the HCV database that spanned both regions (n=105). The country of origin of all sequences were determined. Maximum likelihood phylogenies were estimated using RAUP under the HKY+G model with gamma-distributed among-site rate heterogeneity. Molecular clock and coalescent analyses were performed using BEAST. Results: 21/31 Laos HCV seropositive patients were viremic. Core regions were successfully amplified in 16 patients and these were all found to be HCV genotype-6. In 14/16 patients NS5B was amplified. Phylogenetic analysis of core/NS5B sequences in conjunction with 105 database genotype-6 sequences showed that: (a) genotype-6 was restricted to S.E. Asia or immigrants from S.E Asia. (b) Strikingly high genotype-6 diversity was observed within several countries. (c) There was close genetic proximity to strains from different countries (d) HCV genotype-6 has been evolving in S.E Asia for an estimated 1,200 years. Conclusions: HCV subtypes of genotype-6 are diversely spread throughout S.E. Asia, suggesting a history of virus movement and spread over many centuries.

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THE PRACTICE OF HEPATITIS VACCINATION IN PATIENTS WITH HEPATITIS C AND THE VALIDITY OF PROCEDURE AND DRUG CODES FOR HEPATITIS A AND B VACCINATIONS IN VA ADMINISTRATIVE DATABASES

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Background: Hepatitis A and B vaccination is recommended in patients with chronic liver disease. We conducted a cross-sectional study to examine this practice in HCV-infected patients at a large VA hospital and to validate the CPT codes and drug codes for hepatitis A and B vaccinations in VA administrative databases. Methods: We reviewed charts of 243 HCV-infected patients seen at the Michael E. DeBakey VA Medical Center in Houston with (n=84) and without cirrhosis (n=159) who were randomly sampled from national VA administrative databases. We calculated rates of hepatitis A and B vaccination and serologic evaluation. We also calculated positive (PPV) and negative (NPV) predictive values for CPT and drug codes, as well as percent agreement (and kappa scores) between medical records and administrative data. Results: Approximately 7% of HCV-infected patients without cirrhosis and approximately 20% of patients with HCV and cirrhosis received hepatitis A or B vaccinations with a vaccine series completion rate between 65% and 72%. Of patients who did not receive both hepatitis A and B vaccinations, 11 and 20 patients had negative hepatitis A and B serology, respectively. Therefore, 23% and 43% had neither vaccination nor serology for hepatitis A and B, respectively. In both groups, the proportions were 36% for patients with cirrhosis. The presence of CPT or VA drug codes for hepatitis B vaccine yielded a PPV and NPV of 98.0% and 94.0%, respectively with 94.2% agreement (kappa = 0.67). The presence of codes for hepatitis A vaccine yielded a PPV and NPV of 93.2% and 94.0%, respectively with 93.9% agreement (kappa = 0.64). PPV and NPV for hepatitis A and B vaccinations were also high in the subset of patients with HCV and cirrhosis. Conclusions: Hepatitis vaccination rates in HCV-infected patients with and without cirrhosis are low, and over 25% of patients do not receive a full vaccination series. Presence of codes in VA administrative data for hepatitis A and B vaccinations in HCV-infected patients are highly predictive of the presence of vaccinations in medical records, and therefore can be reliably used for research purposes. Future studies to identify patient, provider, and facility determinants of hepatitis vaccination need to be performed.

COMMUNITY TRENDS IN DIAGNOSIS AND TREATMENT OF HEPATITIS C

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Introduction: National data shows decreasing rates of hepatitis C but little is known about community based rates of recognition and treatment. Methods: The Olmsted County, MN hepatitis C registry collects data on all people diagnosed with hepatitis C residing within the county. The registry is populated with demographic data, dates of diagnosis, risk factors, contraindications to treatment and treatment information. We present data on trends for rates of diagnosis, rates of treatment and treatment contra-indications from our registry. Results: The registry includes data on 558 people. We found no changes in the age (average 36 years) and gender distribution (41%) of newly diagnosed cases from 1991 to the present. The incidence rate increased in the 1990’s (highest 1996 to 1999 at 39/100000) but is down to 13/100000 women and 19/100000 men (p for trend over time in both genders <0.0001) in the most recent years. There has been no change in genotype distribution over the period. Rates of treatment of newly identified cases have risen especially since 2000 (p for trend 0.001). Conclusion: New cases of hepatitis C continue to decline in the community population but many of those with hepatitis C are still not receiving therapy. Improved access to insurance and multi-disciplinary support for treating of non-hepatic co-morbidities, especially mental health problems might increase the rate of treatment in diagnosed cases.
Does Gender Matter? A Prospective Evaluation of Risk Factors for HCV Infection and Treatment Candidacy in Women

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Background: Historically, women have comprised a small percentage of the veteran population. However, they are now the fastest growing group of veterans. Because veterans have a higher overall prevalence of HCV than non-veterans, a better understanding of gender issues and HCV treatment candidacy may be especially important. Aim: To compare risk factors for HCV infection and treatment candidacy between women and men veterans. Methods: Data were prospectively collected in HCV RNA positive veterans from 24 Veterans Affairs Medical Centers. Risk factors for HCV were assessed by questionnaire and treatment candidacy was determined by standardized criteria. Patients were excluded from treatment if at least one of thirteen standardized criteria were met. Results: Of 4,269 patients, 118 (2.8%) were women. Univariate analyses showed that women were younger, more educated, and less likely to have a history of injection drug use, incarceration, heavy alcohol use or snorting cocaine, but were more likely to have had surgery, a needle stick injury or a blood transfusion. Despite these differences, baseline laboratory results and treatment candidacy (43% vs 40%, p = ns) were similar in men and women. Reasons for exclusion from therapy were similar in men vs women, such as ongoing substance use (20% vs 13%, p = 0.05), mental health issues (18% vs 19%, p = ns) and advanced liver disease (14% vs 13%, p = ns), but as table one shows, men were more likely to have more than contraindications to therapy (p<0.05). Men were also almost twice as likely as women to have 3 or more reasons for exclusion from therapy (OR=1.69, 95% CI=1.07 - 4.26). Conclusions: Women veterans with HCV have fewer high risk behaviors than men, yet their overall treatment candidacy rates are similar. These seemingly contradictory results may be due to patients’ exclusion from therapy based on only on a single contraindication and that women were much more likely than men to have only one reason for exclusion. These results suggest the need for gender-specific HCV risk factor screening and counseling, as well as an opportunity to improve treatment candidacy through substance use and mental health interventions, especially in women veterans who have fewer contraindications to HCV treatment.

Table 1. Exclusion criteria met for HCV treatment based on gender (maximum of 13 yes responses) N = 2,450

<table>
<thead>
<tr>
<th>Exclusion Criteria</th>
<th>Women (n=61)</th>
<th>Men (n=2,386)</th>
</tr>
</thead>
<tbody>
<tr>
<td>One exclusion criteria met</td>
<td>51 (79.6%)</td>
<td>1,665 (69.7%)</td>
</tr>
<tr>
<td>Two exclusion criteria met</td>
<td>52 (83.6%)</td>
<td>1,757 (21.7%)</td>
</tr>
<tr>
<td>Three or more exclusion criteria met</td>
<td>3 (4.9%)</td>
<td>204 (8.6%)</td>
</tr>
</tbody>
</table>

Prevalence and Predictors of Obesity Among Individuals Testing Positive for Hepatitis C Antibody in a Multicultural, Urban, Tertiary Care Referral Clinic

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Background: Obesity is considered an important factor contributing to hepatic steatosis and poor response to anti-viral therapy in patients with chronic hepatitis C virus (HCV) infection. Objective: To determine the prevalence of obesity and explore the possible factors associated with obesity in a HCV antibody positive, multicultural tertiary referral clinic population. Methods: Patient records describing visits to a downtown tertiary care liver clinic in Toronto, Canada between 1990 and 2006 were reviewed. We extracted data describing demography, lifestyle, and mode of HCV transmission, as well as weight and height. The one sample proportion test was used to compare the prevalence of obesity in the clinic population testing positive for HCV antibody and the Canadian general population. Regression analyses and propensity score analysis (based on the propensity to test positive for HCV antibody) were applied to explore possible factors associated with obesity in this population. Results: The 1,118 out of 3,505 HCV antibody positive patients met our inclusion criteria. The included patients had a significantly higher prevalence of obesity (28.8% vs. 14.9%, P<0.001) than the Canadian general population. After stratification by age and gender, the clinic population remained with a significantly higher prevalence of obesity than the Canadian general population in every age/gender category. In the univariate analyses age was positively associated with BMI (coefficients 0.073; P<0.001) and obesity (OR 1.020; 95% CI 1.003 to 1.038); Asian origin was negatively associated with BMI (coefficients -0.187; P<0.001); serum testing for positive HCV RNA was significantly associated with obesity (OR 1.731; 95% CI 1.075 to 2.788). In the multivariate regression analyses, age (OR 1.019; 95% CI 1.002 to 1.037) and serum positive HCV RNA (OR 1.798; 95% CI 1.109 to 2.917) were independent predictors for obesity. A total of 112 pairs of subjects qualified for matching by propensity score. The matched propensity score analysis demonstrated that the subjects with a positive serum HCV RNA test had significantly more obesity (32.1% vs. 18.8%; p<0.021) than those with a negative HCV RNA test. Conclusion: Age and serum HCV RNA [indicating chronic HCV infection] contribute to the high prevalence of obesity observed in a clinic population exposed to HCV.

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The following people have nothing to disclose: Wendong Chen, Terrence Wong, George Tomlinson, Murray D. Krahn, Jenny Heathcote
COMPARTMENTALIZATION OF HEPATITIS C VIRUS QUASISPECIES FOLLOWING LIVER TRANSPLANTATION
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Introduction. Hepatitis C virus (HCV)-related cirrhosis is the main indication for liver transplantation (LT). HCV infects the liver graft in almost all HCV-infected patients following LT. HCV compartmentalization is defined as nonrandom distribution of viral variants among the different sites of replication, and potentially reflects the presence of different HCV reservoirs including non-hepatic cells during HCV infection. Aiming to address the impact of compartmentalization for outcome of HCV infection following LT, we analyzed the chronological evolution of HCV compartmentalization between plasma, a likely representation of hepatotropic variants, and PBMCs, presumably representing lymphotropic strains. Patients and methods. Two patients undergoing LT for HCV-related cirrhosis were included in the study presented here; plasma and PBMC samples were taken before LT, 7 days, 1 month and 12 months after LT. Clinical data were collected along with biochemical, virological and histological parameters. Plasma and PBMC quasispecies were studied by cloning and sequencing in the HVR1 region of the E2 glycoprotein. The compartmentalization was analyzed using a phylogenetical approach focusing on specific amino-acid signatures. Results. The compartmentalization persisted during LT: both patients exhibited the presence of specific viral variants in each compartment. For the first patient, bootstrapped phylogenetic trees suggested a compartmental distribution of viral variants before transplantation, still maintained at day 7, month 1 and month 12 following LT (clusters of cellular variants with bootstrap values >60% at each time); interestingly, no positive selection pressure was observed in the PBMC fraction as shown by the Nei and Gojobori method. Two amino-acid signatures were observed for this patient: a lysine mutation to a serine in position 397 of HVR1 and an aspartic acid mutation to an asparagin in position 404, present in plasma at month 1, were absent in PBMCs. Compartmentalization following LT has been shown for the second patient, and absent selective pressure in the PBMC fraction was also observed. Conclusions. Immuno-suppressive status of liver-transplanted patients sustains HCV compartmentalization with potential selection of hepatotropic but not lymphotropic variants following LT. Further studies are underway to analyze the impact of compartmentalization for mechanisms of HCV reinfection.

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STEATOHEPATITIS: RISK FACTORS AND IMPACT ON DISEASE SEVERITY IN HIV-HCV COINFECTION
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Hepatic steatosis (HS) has been reported in those with HIV-HCV coinfection and associated with use of antiretroviral therapy (ART) and insulin resistance (IR). However, the presence of features of steatohepatitis (SH) including cytoplasmic ballooning (CB), its risk factors, and impact on disease severity in such patients are unknown. AIM: To prospectively define the prevalence and severity of the features of SH, its risk factors, and impact on the severity of liver disease in HIV-HCV coinfection. METHODS: Consecutive subjects with HIV-HCV coinfection underwent liver biopsy. SH was defined by the presence of HS and CB with or without Mallory hyaline or pericellular fibrosis (PF). Its individual parameters were graded according to the NASH CRN criteria (Kleiner et al, Hepatol 2005). Inflammation and fibrosis were also graded by the Ishak score; Ishak 3-6 fibrosis represented advanced fibrosis (AF). IR was measured by HOMA-IR and metabolic syndrome (MS) was defined by ATP III criteria. RESULTS: A total of 222 subjects (74% male, mean age 45, 78% African American) were studied. The mean BMI was 26 and 18% were obese (BMI > 30). Median[IQR] AST, ALT, and ALP were 62[43-102], 68[43-101], and 112[85-104], respectively. All patients were HCV RNA positive and 90% were genotype 1. Mean CD4 count was 535, 10% had a CD4 < 200, and HIV was < 400 copies in 45%. The prevalence of risk factors for HS were as follows: diabetes (31%), hypertension (15%), dyslipidemia (8%), MS (9%), and alcohol abuse (21%). ART therapy included a nucleoside reverse transcriptase inhibitor (NRTI) in 93%, non-nucleoside reverse transcriptase inhibitor (NNRTI) in 30% and protease inhibitor (PI) in 47%. HS was present in 23% and SH was present in 17%. The steatosis was absent in 77%, mild (5-33%) in 19%, and moderate-severe (>33%) in 4% of affected subjects. CB and PF were present in 30% and 13% respectively. The mean(SD) Ishak score was 6.9(4.6) and 33% had AF. Both HS and CB were associated with BMI, MS, HOMA and presence of either was strongly associated with AF [p<.0001]. MS and absence of PI use were independent predictors of HS by multiple logistic regression; alcohol use and HOMA-IR was associated with CB; NRTI use were also associated with SH, and use of d4T with AF. CONCLUSIONS: SH is commonly present in HIV-HCV coinfection. Alcohol consumption, IR and specific components of ART contribute to steatosis and SH. Both steatosis and SH increase the risk of having advanced fibrosis. These data raise the possibility that specific types of ART (NRTI, PI, and specifically d4T) may contribute to the progression of fibrosis in HIV-HCV coinfection.

Disclosures:
The following people have nothing to disclose: Richard K. Sterling, Melissa J. Contos, Richard T. Stravitz, Velimir A. Luketic, Michael Fuchs, Mitchell L. Shiffman, Arun J. Sanyal
ACUTE HEPATITIS C IN HIV-INFECTED MEN WHO HAVE SEX WITH MEN IN FRANCE IN 2006 AND 2007

Christine Larsen1, Laurent Alric2, Isabelle Aupérin3, Marie-Laure Chaix4, Elisabeth Delarocque-Astagneau1, Stéphanie Dominguez2, Xavier Duval5, Anne Gervais5, Jade Ghosn5, François Linard6, Yann Le Strat7, Jean-Yves Le Talec8, Lionel Piroth9, Stanislas Pol10, Annie Velter1

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BACKGROUND
Recent European reports suggest that hepatitis C virus (HCV) in specific circumstances may be sexually transmitted among HIV-infected (HIV+) men who have sex with men (MSM). A prospective study is conducted during 2006 and 2007 in France to describe clinical and epidemiological characteristics of HCV-infected MSM and to evaluate the incidence of HCV among this population. Preliminary results on descriptive analysis are presented here. METHODS A random and proportional probability sample of 84 medical wards was drawn according to the number of HIV and AIDS cases in MSM reported since 2003 to the National HIV surveillance system. Acute HCV was defined by a positive anti-HCV antibody (Ab) or HCV polymerase chain reaction (PCR) within one year of a documented negative anti-HCV test. Information was collected on socio-demographics, clinical and biological status of acute HCV, HBV and HIV infections and on HCV risk factors. Two types of questionnaires were completed: one by physicians on clinical data and HCV risk factors and one (self-administered) by patients on sexual behavior, HCV risk factors and exposures. RESULTS Between January 2006 and May 2007, 43 out of the 49 patients meeting the case definition of acute HCV were included after consent, mainly (72%) in Paris. Median age at HCV diagnosis was 38 years [min: 26; max: 58]. Median duration of HCV infection was 6.5 years [min: 0; max: 22]. HCV was suspected because of elevated ALT in 72% of the patients (unknown: 23%). HCV infection was asymptomatic for 66% (unknown: 16%). HCV viral load was undetectable for 16 of the 22 treated patients. 66% of the patients with available HCV genotyping (21/32) were infected with genotype 4 whereas this genotype represents only 10% of the prevalent cases in France. Self-administered questionnaires were available for 27 patients: in the 6-month period prior to HCV, the median number of sexual partners was 20 [min: 0; max: 170]; unprotected anal sex was reported in 81%, having had sexually transmitted infections (STI) in 74% and bleeding during anal sex in 41%. Recreational drug use before sex (such as ecstasy, cocaine,...) was specified by 63% of the patients. CONCLUSIONS In France, HIV+ MSM with frequent unprotected anal sex together with STI and mucosal trauma may be at risk of sexual HCV transmission. Therefore, it is necessary to reinforce prevention strategies targeted to this population.

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The following people have nothing to disclose: Christine Larsen, Laurent Alric, Isabelle Aupérin, Marie-Laure Chaix, Elisabeth Delarocque-Astagneau, Stéphanie Dominguez, Xavier Duval, Anne Gervais, Jade Ghosn, François Linard, Yann Le Strat, Jean-Yves Le Talec, Lionel Piroth, Stanislas Pol, Annie Velter

FACTORS ASSOCIATED WITH SEVERE LIVER DISEASE IN NEWLY REFERRED HEPATITIS C VIRUS-INFECTED FRENCH DRUG USERS: A MULTICENTER STUDY OF 4373 PATIENTS, 2001-2004

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AIMS To analyze factors associated with severe liver disease in drug users infected with hepatitis C virus (HCV) at first referral to a hepatology reference center in France between 2001 and 2004. METHODS A national surveillance system was set up in 2000 to describe changes in epidemiological and clinical characteristics of HCV-infected patients at first referral in 26 hepatology reference centers. Data on risk factors, suspected year of infection, past or current alcohol consumption, HCV RNA status, ALT level, HBV and HIV status were routinely collected. The severity of the liver disease is assessed either by a "clinical" evaluation based on biochemical tests and morphological examination (mainly ultrasonography) or by a histological evaluation (Metavir scores at liver biopsy). Participants included in this analysis are those recorded in the system from 2001 to 2004 and reporting past or current injecting or nasal drug use. Severe liver disease (SLD) was defined as the presence of cirrhosis or primary liver cancer. A log-Poisson model was used to estimate prevalence ratios. RESULTS Among the 4 373 drug users, median duration of HCV infection was 18 years, 7% were HCV-infected (HIV+) and 38% reported past excessive alcohol consumption. Of these, 3 153 were HCV RNA+ with available clinical evaluation of SLD at first referral. Predominant reported HCV genotypes were genotype 3a (24%), genotype 1a (23%) and genotype 1b (14%). SLD at "clinical" evaluation was diagnosed in 7% and was independently associated with genotype 3 [prevalence ratio (PR) = 1.5; 95% confidence interval (CI): 1.1-2.0], HIV+ status [PR = 2.3; 95% CI: 1.1-4.6], past excessive alcohol consumption [PR = 2.5; 95% CI: 1.7-3.6], duration of HCV infection ≥ 18 years [PR = 3.7; 95% CI: 2.2-6.1] and age > 40 years [PR = 4.1; 95% CI: 2.2-7.7]. CONCLUSIONS Our findings based on surveillance data suggest that HCV genotype 3 may be associated with severe liver disease in drug users. They also underscore the role played by excessive alcohol consumption and HIV infection in the severity of HCV infection in this population. These results highlight the need for targeted management of HCV-infected drug users, especially on alcohol consumption and HIV co-infection. Moreover, the role of genotype 3 in severe liver disease needs to be further explored.

Disclosures:
The following people have nothing to disclose: Christine Larsen, Jean-Pierre Bronowicki, Patrice Couzigou, Elisabeth Delarocque-Astagneau, Odile Goria, Dominique Guyader, Patrick Hillon, Patrick Marcellin, Corinne Pioche, Françoise Roudot-Thoraval, Christine Sylvain, Jean-Pierre Zarski
Apolipoprotein E4 (APOE4) allele protects from chronic hepatitis C virus (HCV) infection

Tobias Mueller1, Reinhard Gessner2, Christoph Sarrazin2, Eckart Koettgen2, Juliane Halang1, Eckart Schott2, Alexandra Bergk2, Viola Weich2, Beate Schlosser1, Bertram Wiedenmann2, Thomas Berg2

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BACKGROUND/AIMS: Host genetic single nucleotide polymorphisms (SNPs) are likely to influence the outcome of HCV infection. Infectious HCV particles have been shown to bind to host lipoproteins and low-density lipoprotein receptors (LDLR) have been termed candidate receptors for the hepatocellular internalization of these “lipo-viro particles” (LVP). Recent studies suggested that common SNPs in the gene encoding apolipoprotein E (apoE) - a major structural LDL component and natural ligand of LDLR and well-characterized risk factor for elevated plasma cholesterol levels - might be associated with spontaneous resolution of HCV infection, severity of histological liver damage and response to therapy in chronic HCV infection. The aim of the present study was to further investigate the role of apoE polymorphisms in a large group of well-characterized patients with chronic hepatitis C.

METHODS: We determined the prevalence of apoE genotypes and apoE alleles in 701 chronically HCV-infected patients, 523 healthy controls and 283 individuals with non-HCV associated chronic liver disease by PCR and RFLP analysis. We went on to assess whether apoE SNPs might be associated with the severity of histological inflammation and fibrosis of 555 liver biopsies from chronic HCV carriers. RESULTS: The comparison of apoE allele proportions in chronically HCV-infected patients revealed significant differences in comparison to healthy controls (p=0.002). This was primarily due to a substantial under-representation of the apoE4 allele (10.5% vs. 13.8% in healthy controls; p=0.011) and apoE4 allele positive genotypes (20% vs. 27% in healthy controls; p=0.002) in the group of patients with chronic HCV infection. The frequency of apoE4 alleles was increased in the group of patients with histological mild liver disease but very similar for severe hepatic fibrosis and inflammation. Patients with non-HCV associated chronic liver disease presented with an apoE allele and genotype distribution very similar to healthy controls and differed also significantly from HCV patients CONCLUSION: The significant under-representation of the apoE4 allele in chronically HCV-infected patients suggests that apoE4 allele carriers might be protected from chronic HCV infection. Common apoE SNPs affecting the host lipoprotein metabolism might therefore contribute to the genetic susceptibility to chronic HCV infection.

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Background/Aim: The role of serum HBV-DNA levels in the natural history and management of chronic HBV infection is currently under investigation. We evaluated the fluctuating course of serum HBV-DNA levels during the natural history of chronic HBV infection in the general population of Northern-Eastern Greece, in association with liver disease progression. Patients/Methods: 257 adults (34±12.3yrs old) with chronic HBV infection, recruited and prospectively followed-up for a period up to 12 yrs (1992/94-2006). Viral markers/liver biochemistry/physical examination performed every 6m. Liver biopsy/abdominal ultrasound were performed at entry and every 2.4yrs. Serum HBV-DNA levels measured by Amplicor HBV Monitor. Results: 14/257 (5.5%) patients were HBeAg(+) with mean HBV-DNA levels 2.5±10(7)copies/ml (6.2±10(6)-7.1±10(7)). At entry, 195/257 (76%) were HBeAg/anti-HBe(+), with a history of normal ALT for at least 12 months: a) 97/195 (50%) individuals had undetectable serum HBV-DNA levels at entry although 31/97 (32%) had detectable in at least one occasion, always below 10(4)copies/ml, had no liver disease at entry and at follow-up period by imaging evaluation, b) 94/195 (48%) had HBV-DNA levels <10(4)copies/ml, normal liver histology (97%) at entry and at follow-up period and, c) 4/195 (2%) had HBV-DNA >10(4)copies/ml, nearly normal liver histology of whom 2 exhibited abnormal ALT levels after 3yrs. At entry, 48/257 (19%) patients were HBeAg(1/anti-HBe(+), with a history of elevated ALT levels for >6m; a) 12/48 (25%) had an erratic ALT pattern, mean HBV-DNA levels 3.5±10(5)copies/ml (2.1±10(3)-1.5±10(8)) of whom 5/12 (42%) had HBV-DNA levels <10(4)copies/ml for at least one occasion and, b) 36/48 (75%) had persistent elevated ALT>1.5xULN, mean HBV-DNA levels 1.2±10(6)copies/ml (6.7±10(3)-8.7±10(8)) of whom 8/36 (22%) had HBV-DNA >10(4)copies/ml for at least one occasion. In HBeAg(-) patients with active disease, 22/48 (46%) had moderate/severe histology at entry and 5/48 (10%) developed liver disease progression during the follow-up period. The multivariate-adjusted OR (95% CI) of fibrosis was 604.865 (67.233-999.999) for serum HBV-DNA levels >10(4) vs <10(4)copies/ml; 0.002 (0.001-0.015) for serum ALT level at study entry >40IU vs <40IU; 0.057 (0.006-0.519) for age >40 vs <40 years old. Conclusions: a) 27% of patients with chronic HBeAg(+) hepatitis could be misclassified as inactive carriers if testing was performed on one occasion and not serially, b) serum HBV DNA levels >10(4)copies/ml were significantly associated with liver disease activity and c) close monitoring of serum HBV-DNA is a useful clinical tool in the management of chronic HBeAg(+) patients.

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Background and Aim: Seroclearance of hepatitis B surface antigen (HBsAg) is a rare event in chronic hepatitis B patients. We conducted a large scale longitudinal study investigating the virological, histological and clinical aspects, including the risk of development of hepatocellular carcinoma (HCC) in patients with HBsAg seroclearance. Patients and Methods: Two hundred and ninety-eight patients (211 male and 87 female; median age on presentation: 43.1 years) with HBsAg seroclearance were recruited and followed up every 3 – 6 months for clinical assessment. Intrahepatic HBV DNA and covalently closed circular DNA (cccDNA) were measured by real-time PCR. Serum HBV DNA was measured by the Artus HBV RG Test (Qiagen, Germany). Liver stiffness was assessed by FibroScan (Echosens, France). Results: The median age of HBsAg seroclearance was 49.6 years. The median follow-up duration was 108.9 months and the median follow-up duration after HBsAg seroclearance was 36.4 months. Liver biopsies were performed on 29 patients (median time of biopsy: 48.6 months after HBsAg seroclearance). All had detectable intrahepatic HBV DNA (median 1.68 copies/cell), and cccDNA were detectable in 23 patients (79.3%, median: 0.03 copies/cell). Of the 29 patients with liver biopsy, 9 and 16 patients had sera available within 1 year and between 5 – 10 years after HBsAg seroclearance, respectively, for HBV DNA analysis. All 9 patients had undetectable HBV DNA (<1.1 IU/mL) within 1 year of HBsAg seroclearance, and 4/16 patients had detectable HBV DNA levels between 5 – 10 years of HBsAg seroclearance (median: 2.37 IU/mL). Of the 26 patients with adequate liver tissues for histological examination, 4 had mild fibrosis (F1) and 5 had minimal necroinflammation. FibroScan was performed on 76 and 78 patients who had HBsAg seroclearance at age <50 and ≥50 years respectively. Significant fibrosis (liver stiffness > 8.1 kPa) was observed only in 7.9% (6/76) patients with HBsAg seroclearance at age <50 compared to 29.5% (23/78) patients with HBsAg seroclearance at age ≥50 (p = 0.001). Seven patients developed HCC (median age: 69.3). Kaplan-Meier analysis showed that the chance of HCC development in patients with HBsAg seroclearance at age <50 was significantly less than those with HBsAg seroclearance at age ≥50 (p = 0.004). Conclusion: Although serum HBV DNA was detectable in only a small proportion of patients with HBsAg seroclearance, intrahepatic HBV DNA was still present in all patients. Nevertheless, patients who cleared HBsAg at age <50 had significantly less fibrosis and lower chance of HCC development than those with HBsAg seroclearance at ≥50 years.

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The following people have nothing to disclose: Danny Wong, Ching Lung Lai, James Fung, David But, Ivan Hung, John Yuen, Frederic Fung, John Young, Man-Fung Yuen
902 REDUCED HBV REPLICATION IN HBEAG-NEGATIVE PATIENTS IS DUE TO LOWER COVALENTLY CLOSED CIRCULAR DNA CONTENT AND IMPAIRED VIRION PRODUCTIVITY

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Background&Aims: Knowledge of factors regulating transcriptional activity of hepatitis B virus (HBV) covalently closed circular DNA (cccDNA) may help understanding mechanisms of viral decay and how these processes are thwarted in chronically HBV-infected patients. Methods: Liver biopsies from 119 treatment naïve chronically infected patients [42 HBeAg-positive and 77 HBeAg-negative] were determined for transcriptional and replicative activity of HBV. Results: Significantly lower median serum HBV-DNA (-4log) and intrahepatic HBV-DNA (-2log) and cccDNA (1log) amounts were measured in HBeAg-negative vs. HBeAg-positive patients. Despite a good correlation found between intrahepatic amounts of pregeny virions and serum HBV-DNA titres in all patients, cccDNA levels did not correlate with serum titres in HBeAg-negative individuals. Analysis of HBV-RNA transcripts demonstrated that impaired virion productivity in HBeAg-negative individuals was due to lower steady-state levels of pregenomic RNA produced per cccDNA molecule. Interestingly, preS/S RNAs levels and serum HBsAg concentrations did not differ between HBeAg-positive and HBeAg-negative patients when normalized for cccDNA contents, demonstrating that subviral particle production was not impaired in HBeAg-negative patients and correlated with cccDNA levels. Though the majority of HBeAg-negative individuals harboured cccDNA with common precore and/or basal core promoter (PC/BCP) mutations, occurrence of these variants did not responsible for reduced viral replication. Instead, replacement of wild type cccDNA with populations of BCP mutations re-established high virion productivity. Conclusions: Lower viremia in HBeAg-negative individuals is not only due to lower cccDNA content but also to impaired virion productivity, which can arise without emergence of HBeAg variants and without affecting HBsAg production.

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903 HIGH VIRAL LOAD AND HEPATITIS B VIRUS (HBV) SUBGENOTYPE CE HAVE INCREASED RISK OF HEPATOCELLULAR CARCINOMA (HCC) – A 7-YEAR PROSPECTIVE FOLLOW-UP STUDY

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Background: The determination of risk factors for HCC is important to design cancer surveillance strategy. Genotype C HBV is associated with a higher risk of HCC than genotype B HBV, but the effect of HBV subgenotypes on HCC is uncertain. We aimed to investigate the impact HBV DNA levels and HBV genotypes/subgenotypes on the risk of HCC. Methods: A prospective cohort of chronic HBV infected patients recruited in a surveillance program for hepatocellular carcinoma (HCC) since 1997 was studied. Ultrasound and alpha-fetoprotein were regularly performed for HCC surveillance. HBV DNA at recruitment was measured by Taqman real-time polymerase chain reaction assay. HBV subgenotyping was determined by direct sequencing of the S gene. Results: Among 1006 patients (68% male) aged 48 ± 7 years, 86 (9%) patients developed HCC at a median follow-up of 7.7 years. 377 (38%) patients had liver cirrhosis. 146 (15%) patients had low serum albumin. 851 (85%) patients had ALT levels below 1.5 x upper limit of normal and the mean log HBV DNA was 4.72 ± 1.84 copies/ml. 152 (15%) patients had received anti-viral treatment during the follow-up period. The hazard ratio of each log step increase in HBV DNA was 1.38 (95% CI 1.23, 1.55; p<0.0001). With referent to the low HBV DNA stratum (log HBV DNA ≤ 4.5 copies/ml), the hazard ratio for HCC of the intermediate HBV DNA stratum (log HBV DNA > 4.5 – 6.5 copies/ml) was 1.62 (95% CI 1.05, 2.48; p=0.027) and that of the high HBV DNA stratum (log HBV DNA > 6.5 copies/ml) was 2.73 (95% CI 1.76, 4.25; p<0.001). Among 769 patients with genotyping results, 330 patients had genotype B HBV (all subgenotype Ba) and 439 patients had genotype C HBV (94 subgenotype Ce and 345 subgenotype Cs). With referent to genotype B HBV, HBV subgenotype Ce has the highest risk of HCC (hazard ratio 2.75; 95% CI 1.66, 4.56; p<0.001) and HBV subgenotype Cs has intermediate risk (hazard ratio 1.70; 95% CI 1.09, 2.64; p=0.02). On multivariate analysis, HBV DNA (hazard ratio 1.79; 95% CI 1.27, 2.52; p<0.001) and HBV subgenotypes (hazard ratio 1.53; 95% CI 1.17, 2.01; p=0.002) were independent risk factors of HCC. Liver cirrhosis, male gender, older age and low serum albumin were also risk factors of HCC whereas serum bilirubin, ALT level, ascites and use of anti-viral treatment were not. Using genotype B HBV and low HBV DNA stratum as referent, the hazard ratio of HCC for HBV subgenotype Cs was 2.85 (95% CI 1.68, 4.84; p<0.001) and that for HBV subgenotype Ce was 4.00 (95% CI 2.08, 7.69; p<0.001). Conclusions: High HBV DNA level and HBV genotype C, particularly subgenotype Ce, are independent risk factors of HCC in chronic hepatitis B.

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The following people have nothing to disclose: Chi-Hang Tse, Frankie Ma, Jane Koh, Vincent W. Wong, Grace LH Wong, Joseph J. Sung, Tony SK Mok

904 ANALYSIS OF HBV MUTATIONS IN PATIENTS WITH EPIDEMIC FULMINANT HEPATITIS

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Background and aims: It is thought that both host and viral factors contribute to the development of fulminant hepatitis B. Especially, mutations in the core promoter region (A1762T/G1764A) and/or the precore region (G1896A) have been reported to be associated with fulminant hepatitis. We experienced five patients who died of fulminant hepatitis B from the same infectious source. The HBV isolates obtained from them were 99.8-100% identical to each other, and the genotype was revealed to belong to B2, which is known to cause fulminant hepatitis less frequently. This strain had mutations including A1762T/G1764A and G1896A. Additionally, it had a mutation of G1862T in the precore region. We focused on the high pathogenicity of this strain, and analyzed mutations that are associated with fulminant hepatitis. Methods: The full-length genome of HBV that was divided into two overlapping
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regions was amplified with polymerase chain reaction (PCR). A plasmid carrying 1.3-fold the HBV genome obtained from a fulminant hepatitis patient (pBFH2) was constructed with the PCR products. Using site-directed mutagenesis, the mutations at nt 1762/1764, 1896 and 1862 in pBFH2 were converted to the wild type nucleotides with comprehensive combinations. HepG2 cells were transfected with these plasmids, and HBV-related particles that were released into the culture medium 3 days after transfection were assayed with real-time PCR (HBV DNA) and enzyme-linked immunosorbent assay (HBsAg, HBeAg). Intracellular HBV DNA in the harvested HepG2 cells was measured with southern blot analysis and real-time PCR.

Results: In the analysis of HBV DNA in the culture medium, the largest single factor contributing to the replication efficiency was A1762T/G1764A. The presence of G1862T alone reduced the extracellular HBV DNA, and the production was recovered with coexisting A1762T/G1764A. When intracellular single-stranded (ss) HBV DNA was assayed by real-time PCR, it was revealed that G1862T accumulated the ss HBV DNA in cells, and coexisting A1762T/G1764A increased the accumulation further. Conclusions: Although G1862T was reported to reduce the replication of HBV, the fact that the mutation was found in this fulminant strain prompted us to investigate in more detail information about this mutation. In this study, it was suggested that the presence of G1862T impaired the release of HBV virion and the replicative intermediates were accumulated in hepatocytes. When A1762T/G1764A, which is known to enhance the viral replication, was present with G1862T, the replicative intermediate in cells was further increased, which might be associated with fulminant hepatitis.

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The following people have nothing to disclose: Jun Wu, Mengji Lu, Zhongji Meng, Martin Trippler, Ruth Broering, Agnes Szczechonek, Ulf Dittmer, Michael Roggen, Guido Gerken, Joerg F. Schlaak

905
HBV SUPPRESSES TLR-MEDIATED INNATE IMMUNE RESPONSES IN PARENCHYMAL AND NON-PARENCHYMAL LIVER CELLS

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Background and Aims: It has been suggested that the genes reflecting the innate immune response are not detectable in the initial phase of HBV infection. Recent studies indicate that HBV may have developed strategies to suppress the initial antiviral response of the host including TLR pathways. We have previously shown that non-parenchymal liver cells (NPC; Kupffer cells (KC), liver sinusoidal endothelial cells (LSEC)) can be activated by TLR 3 and -4 to produce IFN-β and other mediators which can potently suppress HBV replication. Therefore, the aim of our study was to investigate whether HBV has the ability to suppress TLR-induced antiviral responses in parenchymal and non-parenchymal liver cells. Methods: We have isolated murine KC by counterflow elutriation as well as murine LSEC by anti-LSEC antibodies which correlated with suppressed ISG induction by poly I:C and LPS on the transcriptional level. TLR 1-9 stimulation had no direct antiviral effect on differentiated HBV-Met cells in contrast to primary hepatocytes and undifferentiated cells. Conclusions: Our data indicate that HBV can suppress the antiviral activity of NPC induced by TLR 3 and -4 stimulation. Integrated HBV can downregulate the innate immune response of hepatocytes towards TLR stimulation. This is of particular relevance for the regulation of the local innate immune response against HBV in the liver and may explain why HBV appears to behave as "stealth virus" initially after infection.

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The following people have nothing to disclose: Jun Wu, Mengji Lu, Zhongji Meng, Martin Trippler, Ruth Broering, Agnes Szczechonek, Ulf Dittmer, Michael Roggen, Guido Gerken, Joerg F. Schlaak

906
CHANGES IN SERUM HBV DNA LEVEL USING A TRAJECTORY MODEL PREDICT THE RISK OF HCC IN CHRONIC HEPATITIS B PATIENTS: THE R.E.V.E.A.L.-HBV STUDY

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Introduction: Elevated serum HBV DNA level is a strong risk predictor of developing hepatocellular carcinoma (HCC) in chronic hepatitis B (CHB) patients. Our aims were to evaluate the relationship between HBV DNA viral load changes over time and HCC risk, and the baseline factors associated with HBV DNA level changes over time. Methods: A sub-group of the R.E.V.E.A.L.-HBV cohort of HBsAg-seropositive and anti-HCV-seronegative patients, without liver cirrhosis at entry was included in this analysis. There were 1,289 participants with HBV DNA ≥10^4 copies/mL at entry with ≥2 HBV DNA measurements included, and 2,020 participants with HBV DNA <10^4 copies/mL at entry as the reference group. Low, medium, high and very high serum HBV DNA levels were defined as <300, 300—<10^5, 10^5—<10^7, and ≥10^7 copies/mL, respectively. Low, medium, and high serum ALT levels were defined as <15, 15—44, and ≥45 U/L, respectively. A semi-parametric group-based modeling was used to determine major trajectory classes of HBV DNA and ALT. Factors associated with HBV DNA trajectory were examined using polytomous logistic regression. Cox’s proportional hazards modeling was used to analyze associations between HBV DNA trajectory classes and HCC risk. Results: There were 89 new HCC cases among 3,309 participants during 40,024 person-years of follow-up. Among those with HBV DNA ≥10^4 copies/mL, four HBV DNA trajectory classes were identified: class I, medium-to-low (n=148); class II, medium-to-medium (n=380); class III, high-to-high (n=542); and class IV, persistently very high (n=219). Four ALT trajectory classes were identified: class A, low-to-medium (n=1,056); class B, medium-to-medium (n=1,507); class C,
high-to-medium (n=187); and class D, medium-to-high (n=174).

Gender, HBsAg status, and ALT level at entry were significantly associated with the HBV DNA trajectory. Compared with HBV DNA <10^4 copies/mL at study entry as the reference group, the multivariate-adjusted hazard ratio HRadj (95%CI) of developing HCC by HBV DNA trajectory class was 0.7 (0.1—5.1) for class I; 2.6 (1.1—6.0) for class II; 8.5 (4.6—15.8) for class III; and 10.0 (4.7—20.7) for class IV after adjustment for gender, age, cigarette smoking, alcohol consumption, and ALT trajectory. The HRadj (95%CI) of developing HCC by ALT trajectory class was 1.4 (0.8—2.7) for class B, 3.2 (1.5—6.9) for class C, and 2.3 (1.0—5.2) for class D compared with class A as the reference group. Conclusions: Serum HBV DNA level over time is a strong predictor of HCC independent of gender, age, cigarette smoking, alcohol consumption, and ALT trajectory.

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907
HBV VIRAL LOAD LESS THAN 10^4 COPIES/mL IS ASSOCIATED WITH SIGNIFICANT RISK OF HEPATOCELLULAR CARCINOMA IN CHRONIC HEPATITIS B PATIENTS: AN UPDATE FROM THE R.E.V.E.A.L.-HBV STUDY

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Introduction: Major international treatment guidelines classify CHB patients with low-level viremia (<10^4 copies/mL) as having inactive disease with little or no risk of disease progression. Risk of disease progression in these subjects has not been properly evaluated. Methods: All HBsAg-negative subjects without HCV infection (anti-HCV negative) and the subset of the R.E.V.E.A.L.-HBV study cohort without HCV infection or liver cirrhosis at baseline were included in this analysis. Ascertainment of HCC was by data linkage with computerized profiles of the National Cancer Registry and Death Certification System in Taiwan, and all HCC cases were confirmed using established criteria. The risk of HCC progression by baseline and follow-up viral load was determined (in comparison to HBsAg-negative persons). The risk of HCC associated with even the lowest detectable level of HBV DNA was 3-fold greater and highly significant. During follow-up, an HBV DNA level maintained between 300—9,999 copies/mL was associated with a significantly higher risk of HCC when compared to those achieving a level <300 copies/mL.

HRadj (95%CI) 2.7 (1.1—6.6). Conclusion: Results show that CHB patients with an HBV viral load of less than 10^4 copies/mL are at a significantly higher risk of developing HCC than HBsAg-seronegative persons. The risk of HCC associated with HBsAg-seronegative persons, the HRadj (95%CI) of developing HCC by ALT trajectory class was 1.4 (0.8—2.7) for class B, 3.2 (1.5—6.9) for class C, and 2.3 (1.0—5.2) for class D compared with class A as the reference group. Conclusions: Serum HBV DNA level over time is a strong predictor of HCC independent of gender, age, cigarette smoking, alcohol consumption, and ALT trajectory.

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- Uchenna Iloeje - Employee: Bristol-Myers Squibb
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908
HEPATITIS B VIRUS VACCINE ESCAPE MUTANTS HAVE IMPAIRED VIRION SECRETION EFFICIENCY IN CELL CULTURE

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Background/Aims: Vaccine failure and graft reinfection despite immunoglobulin are attributed to escape mutants with amino acid substitutions in the alpha determinant of viral envelope proteins. Recent studies revealed virion secretion defect of the prototype G145R mutant. In this study we systemically analyzed virion secretion capacity of 20 potential immune escape mutants. Methods: Huh7 cells were transiently transfected with a 1.5mer replication construct that cannot express envelope proteins and a 0.7mer expression construct of envelope proteins. Virus particles were immunoprecipitated from culture supernatant by an anti-preS2 antibody before Southern blot analysis. Results: All the mutants expressed similar levels of envelope proteins based on Western blot analysis of cell lysate. However, secreted HBsAg as measured by the Abbott kit was reduced moderately or completely for mutants affecting several cysteines or residues near G145. Those mutants showed total loss or marked reduction in virion secretion. In addition, several mutants with no impairment in HBsAg detection also displayed moderate or severe reduction in virion secretion. Co-expression of wild-type envelope proteins at 1:1 but not 1:5 (wt : mutant) ratio markedly improved virion secretion. Introduction of an M133T second-site mutation, which creates a novel N-linked glycosylation site, also restored virion secretion. Conclusions: Structural changes around the alpha determinant impair virion secretion. The detection of immune escape mutants in patient blood may result from rescue by the co-infecting wild-type virus. Immunogenic escape mutants will not replace wild-type virus unless under conditions of anti-HBs immune pressure, or if they acquire a compensatory mutation.

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infection.
cDNA histones are hypo-acetylated and methylated in occult patients with inactive HBV and occult HBV carriers (6) HBVccc-HBV synthesis, HBVcccDNA levels are similar in HDV intracellular cccDNA levels (5) Despite the persistence of replication (4) Serum HBsAg amount does not correlate with patients indicating different grade of HDV interference on HBV HBV DNA levels may be largely different among HDV infected parable between HDV positive and negative individuals (3) according to their HDV status whereas cccDNA levels are com-

cation, serum and total intrahepatic HBV DNA levels differ individual (2) In anti-HBe positive patients with active HBV repli-
cation (hfgl2) in response to HBV proteins. Method: HBc, HBs or HBx transcription factor(s) and the upstream signal transduction pathway involved in transcription of the human fgl2 gene (hfgl2) in response to HBV proteins. Method: Hbc, HBs or HBx expression plasmids were cotransfected with a hfgl2 promoter luciferase report construct into CHO cells and HepG2 cells respectively. Peripheral blood mononuclear cells (PBMC) were isolated from 10 patients with severe acute on chronic (AOC) hepatitis B, 10 patients with minimal chronic hepatitis B, 8 HBsAg carriers, and 6 health volunteers as controls. A polyclonal antibody against c-Ets-2 was used to detect the expres-
dion of c-Ets-2 in response to HBc protein, while JNK inhibitor SP600125 abolished the upregulated expression of c-Ets-2 in response to HBc protein by flow cytometry, Western blotting and confocal microscopy. Phosphorylation of ERK, JNK and p38 MAPK in PBMC was detected by Western blotting. Results: Luciferase assay showed both Hbc and HBx protein, but not HBs protein enhanced hfgl2 transcription in both CHO cells and HepG2 cells. A strong regulatory region from -712 to -568 was demonstrated to play an important role in hfgl2 gene transcription in response to both HBc and HBx proteins.

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### 910 HEPATITIS B VIRUS PROTEINS INDUCE ACTIVATION OF HFGL2 TRANSCRIPTION THROUGH C-ETS-2 TRANSCRIPTION FACTOR AND MAPK SIGNAL PATHWAY

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Objective: Fibrinogen–like protein 2 (fgl2)/ fibroleukin is piv-
otal in the pathogenesis of both experimental and human ful-
minant viral hepatitis. This work was to characterize the transcription factor(s) and the upstream signal transduction pathway involved in transcription of the human fgl2 gene (hfgl2) in response to HBV proteins. Method: Hbc, HBs or HBx expression plasmids were cotransfected with a hfgl2 promoter luciferase report construct into CHO cells and HepG2 cells respectively. Peripheral blood mononuclear cells (PBMC) were isolated from 10 patients with severe acute on chronic (AOC) hepatitis B, 10 patients with minimal chronic hepatitis B, 8 HBsAg carriers, and 6 health volunteers as controls. A polyclonal antibody against c-Ets-2 was used to detect the expres-
dion of c-Ets-2 in response to HBc protein, while JNK inhibitor SP600125 abolished the upregulated expression of c-Ets-2 in response to HBc protein by flow cytometry, Western blotting and confocal microscopy. Phosphorylation of ERK, JNK and p38 MAPK in PBMC was detected by Western blotting. Results: Luciferase assay showed both Hbc and HBx protein, but not HBs protein enhanced hfgl2 transcription in both CHO cells and HepG2 cells. A strong regulatory region from -712 to -568 was demonstrated to play an important role in hfgl2 gene transcription in response to HBc or HBx proteins by a series of promoter deletion assay. By site-directed mutagenesis, EMSA and ChIP, binding of transcription factor c-Ets-2 and its cognate cis-element in the region -712/-
568 was demonstrated to play an important role in hfgl2 gene transcription in response to both Hbc and HBx proteins. shRNA interference of the c-Ets-2 expression was able to abolish hfgl2 gene transcription by 64.8% and 60.0% in response to HBc and HBx proteins respectively. Peripheral blood mononuclear cells (PBMC) were isolated from 10 patients with severe acute on chronic (AOC) hepatitis B, 10 patients with minimal chronic hepatitis B, 8 HBsAg carriers, and 6 health volunteers as controls. A polyclonal antibody against c-Ets-2 was used to detect the expres-
dion of c-Ets-2 in response to HBc protein, while JNK inhibitor SP600125 abolished the upregulated expression of c-Ets-2 in response to HBc protein by flow cytometry, Western blotting and confocal microscopy. Phosphorylation of ERK, JNK and p38 MAPK in PBMC was detected by Western blotting. Results: Luciferase assay showed both Hbc and HBx protein, but not HBs protein enhanced hfgl2 transcription in both CHO cells and HepG2 cells. A strong regulatory region from -712 to -568 was demonstrated to play an important role in hfgl2 gene transcription in response to HBc or HBx proteins by a series of promoter deletion assay. By site-directed mutagenesis, EMSA and ChIP, binding of transcription factor c-Ets-2 and its cognate cis-element in the region -712/-
568 was demonstrated to play an important role in hfgl2 gene transcription in response to both Hbc and HBx proteins. shRNA interference of the c-Ets-2 expression was able to abolish hfgl2 gene transcription by 64.8% and 60.0% in response to HBc and HBx respectively. In human, c-Ets-2 protein was highly expressed in PBMC isolated from patients with severe AOC hepatitis B when compared with health controls. Increased phosphorylation activation of ERK, JNK and p38 MAPK were found in PBMC isolated from patients with severe AOC hepatitis B when compared with health controls. Treatment with ERK inhibitor PD098059 abolished the upregulated expression of c-Ets-2 in response to Hbc protein, while JNK inhibitor SP600125 abolished the upregulated expression of c-Ets-2 in response to HBx protein. Conclusion: Hbc and HBx initiated the transcription of hfgl2 gene through c-Ets-2 transcription factor, which was dependent on the activation of ERK and JNK signal path-
way in corresponding to either Hbc or HBx proteins. This work
911 EFFICIENT CROSSTALK BETWEEN HBV-SPECIFIC T HELPER AND CYTOTOXIC T CELLS CHARACTERIZES SUCCESSFUL CONTROL OF HEPATITIS B VIRUS INFECTION

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Strong T helper 1 (Th1) HBV-specific immune response leads to cytotoxic T lymphocyte (CTL) activation and control of viral replication. In patients with chronic hepatitis B infection, inefficient viral control may derive from impaired memory/effector CTL maturation caused by inadequate crosstalk between HBV-specific Th1 cells and CTL. Programmed cell death (PD-1) receptor, linked to functional suppression of HBV-specific T cells, may be involved in this process. Aims: To evaluate T helper and CTL HBV-specific responses in four stages of HBV infection and establish their impact on memory/effector cell maturation. Patients: 34 HLA-A2+ve children (16 males, median age 12.8 y) were divided into 4 groups according to HBcAg/HBsAg status and ALT activity (Table 1); 5 HCV infected children (3 males, median age 13.2 y) and 5 healthy individuals (1 male, median age 31.5 y) served as controls. Methods: HBV-specific Th immune responses were determined by interferon (IFN)-γ, IL2 and IL-10 Elispot after stimulation of peripheral blood mononuclear cells (PBMC) with HBV antigens (HBcAg, HBsAg and HBsAg). CD8+ reactivity after incubation with the HLA-A2 restricted immunodominant HBV-core (a,b) pentamer was determined by INF-γ, granzyme B and IL-2 Elispot and staining with HLA-A2 HBV-core (a,b) pentamer. Phenotype of HBV-specific cells was determined by flow-cytometry with anti-CD27, CD28 , CD45RA, CD62L and PD-1 antibodies. HBV DNA was quantified by real-time PCR (log10 copies/ml). Results: HBV-specific production of Th1 cytokines was higher in group D than groups A, B and C. (IFN-γ HBV core: 479±145 vs. 12.6±3±4.4, 62.6±21.5 and 64.3±23.1 specific spot forming cells per 1 million PBMC (spSFC/10^6 PBMC), p=0.03). Levels of proinflammatory cytokines produced by Th cells andCTL after antigen-specific stimulation correlated with the number of HBV-core (a,b) pentamer+ve cells (r=0.569, p=0.001) and mature memory/effector CTL (r=0.413, p=0.02). The number of PD-1+ve HBV-specific CTL was lower in group D than in groups A, B and C (76±4.8 vs 91.3±2.3, 81.6±7 and 81.8±5.4%, p=0.02). HBV DNA viral load correlated inversely with HBV-specific Th1 (r=-0.36, p=0.046) and CTL responses (r=-0.59, p=0.001) and number of pentamer+ve cells (r=-0.37, p=0.03). Conclusion: Efficient crosstalk between HBV-specific T helper and cytotoxic T cells leads to maturation of memory/effector T cells and successful control of viral replication.

912 EVOLUTION OF VIRAL QUASISPECIES IN THE POLYMERASE GENE OF HEPATITIS B VIRUS DURING ANTIVIRAL TREATMENT: FROM NAIVE TO VIRAL BREAKTHROUGH

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Background/Aim: Drug resistance is emerging as the single most significant factor in treatment failure. The presence of different viral genomes in a patient (viral quasispecies), including preexisting mutations in the polymerase gene of hepatitis B virus (HBV), might be a mechanism of virus survival under selective antiviral drug pressure. We thus investigated viral quasispecies evolution and preexisting drug resistance mutations. Patients and Methods: Five chronic hepatitis B patients with lamivudine (LAM) failure were enrolled in this study. Patient sera were obtained before the treatment and at the time of viral breakthrough during LAM treatment. The polymerase chain reaction (PCR) product of the HBV polymerase gene (rt1-rt280) was cloned into pOE7T Easy Vector. After transformation, 100 clones were picked up at each time point. Cloned HBV DNA was confirmed using XbaI and PstI digestion and sequenced. The reference gene of HBV genotype C was NCBI GenBank No.AF286594, which was considered the wild type. Results: At baseline, the wild-type was present at 0–11% in five patients. Several substitutions in the HBV DNA polymerase region were observed, including reported antiviral resistant sites. Patients had 31–81 mutations of various types. Before treatment (naive), rtL80I (1%), V84F (5%), rtT128I (1%), rtV173L (2%), rtV173A (2%), rtI181V (1%), rtI181L (8%), S202G (2%), rtV173L (2%), rtL180M (1%), rtA181T (1%), rtA181I (1%), rtS202G (2%), rtL180M (1%), rtL180P (1%), V84F (5%), rtT128I (1%), rtV173L (2%), rtV173A (2%), rtI181V (1%), rtI181L (8%), S202G (2%), rtV173L (2%), rtL180M (1%), rtA181T (1%), rtA181I (1%), rtS202G (2%), rtL180M (1%), rtL180P (1%), V84F (5%), rtT128I (1%), rtV173L (2%), rtV173A (2%), rtI181V (1%), rtI181L (8%), S202G (2%), rtV173L (2%), rtL180M (1%), rtA181T (1%), rtA181I (1%), rtS202G (2%), rtL180M (1%), rtL180P (1%), V84F (5%), rtT128I (1%), rtV173L (2%), rtV173A (2%), rtI181V (1%), rtI181L (8%), S202G (2%), rtV173L (2%), rtL180M (1%), rtA181T (1%), rtA181I (1%), rtS202G (2%), rtL180M (1%), rtL180P (1%), V84F (5%), rtT128I (1%), rtV173L (2%), rtV173A (2%), rtI181V (1%), rtI181L (8%), S202G (2%), rtV173L (2%), rtL180M (1%), rtA181T (1%), rtA181I (1%), rtS202G (2%), rtL180M (1%), rtL180P (1%), V84F (5%), rtT128I (1%), rtV173L (2%), rtV173A (2%), rtI181V (1%), rtI181L (8%), S202G (2%), rtV173L (2%) were identified, while rtT54S, rtH55R, rtS106C, rtY124N, rtD134G, rtP143Q, rtS223A, rtL229V, and rtP237H mutations became prevalent. Conclusion: We demonstrated the evolution of mutations in the polymerase gene of HBV during antiviral treatment. Our study may help clarify the drug resistance pathway and emphasize the early detection of drug-resistant mutants before or during...
913 FACTORS ASSOCIATED WITH HBsAg SEROCLEARANCE IN ASYMPTOMATIC CARRIERS OF ENDEMIC AREAS DURING A LONG FOLLOW-UP PERIOD OF UP TO 17 YEARS

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Background: The seroclearance of hepatitis B s antigen (HBsAg) is a rare event and usually confers favorable outcome if there is no preexisting cirrhosis or viral superinfection, though adverse complications still can occur. However, the factors associated with HBsAg seroclearance are still under investigation. Aim: The aim of this long-term follow-up study was to reexamine the rates of HBsAg seroclearance and possible factors associated with HBsAg seroclearance. Patients/Methods: There were 1139 HBsAg-positive asymptomatic adult carriers, 31.9±23.3 years old, men (59%) and women enrolled in Eastern-Northern Greece between 1990 and 1999 in this analysis prospectively followed-up for a period up to 17 years (1990-2007). Viral markers/liver biochemistry/physical examination performed at entry and every 6-24 months. Abdominal ultrasound was done at entry and every 3/4yrs. Serum HBV-DNA levels measured by Amplicor HBV Monitor kit. The HBsAg status in last follow-up samples was also tested. Logistic regression analysis was used to derive multivariate-adjusted odds ratio (OR) and 95% confidence interval (CI) for factors associated with HBsAg seroclearance. Results: Serum HBsAg cleared in 137 patients (12%) at the mean age of 41±15.3 years after the entry. The cumulative probability of HBsAg seroclearance after 17 years was only 5.3% (varied from 1.9% in low endemic areas to 21% in high endemic areas) for carriers with age at entry <20 years but around 27% for those with age at entry >20 years (varied form 11% in low to 45% in high endemic areas). Hepatitis relapse in 182 patients (16%) 0.5 to 16 years after the entry as depicted by ALT levels elevated >1.5 UNL and detectable serum HBV-DNA levels. During the follow-up period, age at entry and sustained remission of hepatitis were significantly associated with HBsAg seroclearance (p<0.05). The multivariate-adjusted OR (95% CI) of HBsAg seroclearance was 1.21 (1.32-2.43) for serum ALT level at study entry >40 IU versus <40 IU; 6.26 (1.87-23.47) for age <20 years versus >20 years; and 2.18 (7.25-23.91) for serum HBV DNA level <10(3) copies/mL versus >10(3) copies/mL. Conclusions: The cumulative rate of HBsAg seroclearance was 30% in asymptomatic carriers after 17 years of follow-up. Longer follow-up period and sustained remission were associated with a significantly increased HBsAg seroclearance rate.

Disclosures: The following people have nothing to disclose: Stamatia Kotsiou, George Zacharakis, John Koskinas, Fevernia Tzara, Nikolaos Vafeiadis, John Messaritakis, Athanasios Archimandritis, Eustratios Maltezos, Kostantinos Papoutsellis.

914 HEPATITIS B VIRUS QUASISPECIES EVOLUTION IN HBEAG SEROCONVERTERS

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Background: We have recently demonstrated that hepatitis B virus (HBV) quasispecies diversity is higher in patients in HBsAg serocarriers (Gastroenterology, in press), while non-serocarriers have very low viral diversity. However, it is unclear how HBV viral genetic diversity transitions from a low diversity state to a high diversity state. Aim: To evaluate the long term evolution pattern of HBV quasispecies in spontaneous seroconverters that transition from low to high viral diversity state. Methods: Eight chronic hepatitis B HBeAg seroconverters, with long term stored serum and clinical follow-up of 6-15 years before seroconversion were studied. Two chronic hepatitis B patients without HBeAg seroconversion with matching length of follow-up and stored serum were included as controls. Serum samples from 7-12 time-points were used for nested PCR, cloning and sequencing of the precore/core gene (20 clones/sample). In total, 1685 sequences were analysed. Only genotype B patients were involved in this study. Sequences were aligned using ClustalX, then SPLITMA phylogenetic trees were constructed using Pebble 1.0 following which maximum likelihood estimates of pairwise distances under a GTR+I+G model was assessed. Viral diversity and substitution rates were then estimated. Results: The genetic diversity in HBeAg seroconverters was very low at baseline and similar to controls but gradually increased from 2.7±1.0exps-3 substitutions/site to 1.1±1.0exps-2 substitutions/site prior to seroconversion (Spearman's correlation analysis, R=0.67 and p<0.0001) followed by a striking increase post-seroconversion. The evolutionary rates in these patients showed a more complex pattern over time. Conclusions: There is a gradual transition of HBV quasispecies from a low diversity to high diversity status just prior to HBeAg seroconversion. High viral diversity appears to be a pre-requisite for HBeAg seroconversion.

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915 TLR9 EXPRESSION OF PLASMACYTOID DENDRITIC CELLS IN PATIENTS WITH CHRONIC HEPATITIS B INFECTION

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Background and Aims: Chronic HBV infection is the result of a complex interaction between a replicating noncytopathic virus and a down-regulated antiviral immune response. Immature plasmacytid dendritic cell (pDC) is the cell type responsible for the majority of the IFN-I production, which is the most important part of the native immunity against virus and mature plasmacytid dendritic cell is the antigen-representing cell, which may prime naive CD4+ T cells and develop Th1 response. TLR9 functions as a pattern recognition receptors (PRRs) by interacting with CpG motif of virus. High level of cytokines such as interferon (IFN)-α, IFN-β are expressed by CpG stimulation. Previously, we have shown that TLR9 low expression and functional impairment of pDCs in patients with chronic hepatitis B. This antiviral treatment to prevent antiviral therapy failure. Key Words: Hepatitis B virus, Mutations, Antivirals, Resistance, Quasispecies

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study aims to evaluate the frequency and function of circulating pDCs in patients at various stages of HBV infection, and to explore the role of TLR9 and pDCs function in the disease progression after HBV infection. Methods: Peripheral blood was collected from 69 hepatitis B (HB) virus-infected persons, including 44 cases of asymptomatic HBV infection, 30 cases of chronic HB, 25 cases of liver cirrhosis, and 21 healthy blood donors used as controls. Flow cytometry was used to analyse the frequency of circulating pDCs and the mean fluorescence intensity (MFI) of TLR9. Fresh peripheral blood mononuclear cells (PBMCs) were stimulated in vitro using CpG 2216. The supernatants were measured for IFN-α production using ELISA method. Results: Compared with healthy controls, the MFI of TLR9 from patients was significantly reduced (p < 0.0001). In addition, the MFI of TLR9 in patients with asymptomatic HBV infection and liver cirrhosis was significantly decreased compared with that of chronic HB (p < 0.05). The peripheral pDCs frequency in patients with chronic HB and liver cirrhosis were significantly more lower compared to the control group (p < 0.05). Moreover, pDCs frequency inversely correlate with ALT levels (r = -0.348, p < 0.05). The ability of PBMCs to secrete IFN-α also decreased significantly in patients with chronic HBV infection. Conclusions: Our results suggest that patients with chronic HBV infection have a significantly lower TLR9 expression of circulating pDC, as well as decreased number and impaired function of circulating pDC, which may be partially related to HBV disease progression in these patients.

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916 HEPATITIS B VIRUS X PROTEIN (HBX) TRIGGERS HEPATIC STELLATE CELLS (HSCS) ACTIVATION THROUGH TRANSFORMING GROWTH FACTOR BETA (TGF-β) SIGNALLING PATHWAY

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Activated hepatic stellate cells are the principle cell type responsible of liver fibrosis due to their great capacity to synthesize different extracellular matrix fibrillar proteins like collagen type I, and profibrotic growth factors like connective tissue growth factor (CTGF), and TGF-β. Hepatitis B virus is the main cause of hepatocarcinoma being HBx closely implicated in its development. However, HBx contribution to liver fibrosis has not been investigated. Our aim was to study if HSCs activation could be mediated by HBx and through which signalling pathways. METHODS Primary human HSCs were incubated with conditioned media from the following hepatic cell lines: CMX that bears two head to tail copies of the HBV genome. CHL and HepG2 were the control parental cell lines. To study HSCs which bears two head to tail copies of the HBV genome. CHL and HepG2 were the control parental cell lines. To study HSCs activation markers expression were reduced. Neutralization assays showed that HBx influence is mediated by TGF-β signalling pathway. In conclusion, these results strongly suggest that HBx could be a promoting agent of liver fibrosis being capable of inducing the secretion of soluble mediators that result in the paracrine activation of HSCs at least in a TGF-β manner.

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917 INFLUENCE OF SERUM HBV DNA LEVEL ON A RECURRENCE OF HEPATOCELLULAR CARCINOMA AFTER CURATIVE TREATMENT WITH RADIO-FREQUENCY ABLATION

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Background: Elevated serum HBV DNA level is a strong risk predictor of hepatocellular carcinoma (HCC) independent of HBsAg, serum alanine aminotransferase level, and liver cirrhosis (JAMA 2006). However, there are few studies investigating influence of serum HBV DNA level on recurrence of HCC. Aim: The purpose of this study was to evaluate whether serum HBV DNA level was associated with the recurrence rate after curative treatment of HBV related-HCC (HBsAg positive and HCV-Ab negative) with radio-frequency ablation (RFA) retrospectively. Methods: Consecutive patients with HBV related-HCC who were curatively treated with RFA at their first hospital admission from February 1999 to September 2006 were analysed. Concentration of HBV DNA is measured by transcription-mediated amplification (TMA) or polymerase chain reaction (PCR). Age, gender, albumin, alanine aminotransferase, platelet count, AFP, L3(%), DCP, tumor size, and the tumor number were also obtained for analysis. Risk factors for recurrence after RFA were evaluated using univariate and multivariate analyses. Results: A total of 66 patients (47 males, 19 females, mean age 66.4 ± 8.5 years) with HBV related-HCC were curatively treated with RFA during the study period. These patients were followed up for a mean of 3.2 years. recurrence of HCC occurred in 38 patients. 1-, 3- and 5-year recurrence rates were 30.0%, 60.4%, and 74.3%, respectively. By univariate analysis, platelet count (<15 x 10^4/mm³), the tumor number (multiple) and HBV DNA (≥ 4.0 logcopies/ml) were associated with the recurrence of HCC. A stepwise, multivariate Cox regression analysis revealed that platelet count (risk ratio, 3.74; 95% CI, 1.56-8.97, p = 0.003) and concentration of HBV DNA (risk ratio, 2.87; 95% CI, 1.16-7.09, p = 0.023) were independent risk factors for recurrence of HCC.
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HEPATITIS B VIRUS (HBV) X PROTEIN (HBX) PROMOTES HUMAN HEPATIC STELLATE CELLS ACTIVATION AND PROLIFERATION MODULATED BY MATRIX METALLOPROTEINASE 2 (MMP-2)

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Human Hepatic stellate cells (HHSCs) activation is crucial for liver fibrosis development. This event is characterized by α-smooth muscle actin (α-SMA) synthesis, an increased production of profibrotic factors like collagen type 1 (COLI), MMP-2, connective tissue growth factor (CTGF) and a successive enhanced proliferation that define the later perpetuation phase.

HBx is a multifunctional regulator that modulates gene transcription and several signalling pathways. Also it has been shown to be essential for hepatocellular carcinoma (HCC) development; however its role in fibrosis is still unknown.

OBJECTIVE: The aim of this study is to analyze the effects of HBx protein over HHSCs activation and perpetuation steps.

METHODS: Primary HHSCs were incubated with conditioned media from hepatic cell lines: CMX which expresses HBx in a dexamethasone (DX)-inducible manner; 2.2.15, which bears two copies of the HBV genome. Parental cell lines CHL and HepG2 were used as controls. Human HHSCs were incubated during 24 hours with different conditioned media. Fibrosis markers expression α-SMA, COLI, CTGF and MMP-2, was analyzed by immunofluorescence, Western Blot (WB) and zymography. HHSC proliferation was spectrophotometric analyzed using a cell growth determination kit. RESULTS: HHSCs activation was demonstrated by the “de novo” expression of α-SMA as showed in immunofluorescence. WB and zymography from HHSCs exposed to conditioned media from HBx expressing cells, displayed an increased expression COLI, CTGF and MMP-2 as compared to human HHSCs incubated with control media. In proliferation assays, conditioned media from CMX and 2.2.15 cells had a positive effect on HHSCs growth, compared to those exposed to CHL or HepG2 media. Furthermore, HHSCs incubated with presence of conditioned medium from dexamethasone-stimulated CMX cells showed a higher proliferation rate than those conditioned with unstimulated CMX or CHL media. CONCLUSION: Our results reveal that HBx could trigger HHSCs activation as demonstrated by the enhanced expression of COLI, CTGF and α-SMA in HHSCs exposed to HBx viral protein. Additionally, an increased proliferation has been shown in HHSCs grown in the presence of conditioned medium from HBx-expressing cells. Furthermore, since a direct link between MMP-2 expression and HHSCs proliferation has been reported (Olaso E et al., 2001; Ikeda K et al., 1999), direct and paracrine induction of MMP-2 by HBx may contribute to this enhanced proliferation too. Taken together our results confirm the modulating role of HBx over HHSCs not only in the activation process but also the following perpetuation step modulated at least by the induction of MMP-2.

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EXPRESSION LEVELS OF HNF4A LINK EFFICIENT HBV REPLICATION TO HEPATOCYTE DIFFERENTIATION STATE

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The efficiency of HBV replication is discussed to depend on hepatocyte differentiation. Cellular factors that link HBV replication to hepatocyte differentiation, however, are not known so far. We hypothesized that expression levels of liver specific transcription factors determine hepatocyte differentiation as well as efficiency of HBV replication. Methods: We correlated expression levels of hepatocyte differentiation markers and transcription factors to efficiency of HBV replication following adenovirus-mediated transfer of 1.3 HBV genomes at equal levels into freshly isolated primary human hepatocytes (PHH), HepG2, HepaRG, HuH7 (hepatoma) and Pop10 (hepatocyte) cell lines. We quantified expression of albumin, ferritin, liver specific antigen (LSA), organic anion transporting polypeptide - C (OATP-C), bile salt exporting pump (BSEP), CYP1A2, 2′,3′-triphosphodiysterogenase (TDO), steroid regulatory element binding protein-2, apolipoprotein B100 and hepatocyte nuclear factors (HNF) HNF1α, HNF4α, HNF3α/β/γ, C/EBPα/β and LRH-1 by real-time RT-PCR and Western Blot. To monitor HBV replication, we quantified HBV pregenomic RNA (pgRNA), HBV core and large (L) envelope proteins, replicative intermediates and progeny virus. Results: The efficiency of HBV replication was 13.6-, 14.4- and 15.7-fold higher in PHH than in HepG2, HepaRG or HuH7 cells, respectively, and increased in HepaRG cells 3.3-fold upon differentiation. No HBV replication was detected in Pop10 cells. In all cells, expression levels of pgRNA determined replication efficiency and correlated closely with expression of LSA, TDO, OATP-C and BSEP as well as HNF1α, HNF3α and HNF4α. When maintaining HBV replicating cell lines HepG2.H1.3 and HepG2.2.15 under differentiating conditions, pgRNA increased 8.6- and 8.1-, HBV core 14- and 10-, Lproteins 8.8- and 7.1- and progeny HBV 715- and 100-fold, respectively. Again, this correlated with increased levels of HNF1α, HNF3α and HNF4α and mRNA for OATP-C and BSEP. Knock down of HNF4α and HNF1α using specific siRNAs decreased pgRNA by 77% and 57%, expression of HBV core by 80% and 45% and Lproteins by 30% and 75%, HBV replicative intermediates by 50% each and HBV progeny release by 88% and 74%, respectively. Decrease in HBV replication was confirmed at the single-cell level after knock down of HNF4α. Levels of HNF4α closely correlated with HBV core protein and pgRNA in all analyzed tumor-peritumor samples of patients with HBV related hepatocellular carcinoma. Conclusion: High expression levels of HNF4α, maintaining a high level of hepatocyte differentiation and polarization, are required for efficient HBV replication.

Disclosures:

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Introduction: Previous publications from the R.E.V.E.A.L.-HBV study have demonstrated that HBV DNA at the time of cohort entry strongly predicts future risk of hepatocellular carcinoma (HCC). However, the impact of chronically elevated HBV DNA over time on the risk of HCC accounting for changes in serum ALT level has not been evaluated. The aim of this study was to assess HCC risk associated with multiple observations of serum HBV DNA and ALT levels over time. Methods: A subset of the R.E.V.E.A.L. study cohort with baseline HBV DNA ≥10^4 copies/mL, no evidence of liver cirrhosis at entry, anti-HCV negative and ≥2 frozen serum samples available for HBV DNA testing were analyzed. HCC was ascertained through data linkage with computerized profiles of the National Cancer Registry and Death Certification System in Taiwan. All HCC cases were confirmed using established criteria. Multivariable adjusted hazards ratios (HR_adj) were derived using time-dependent Cox proportional hazard models where both HBV DNA and serum ALT were time dependent variables using multiple samples over the follow-up period. Results: Interim analysis of results identified 71 new HCC cases from 1,289 people over 15,508 person-years of follow-up. Males made up 68.8% of the population. In the final model adjusting for gender, age, cigarette smoking and alcohol consumption, serum ALT as a time-dependent variable, and HBeAg status, the risk of developing HCC was strongly associated with increasing HBV DNA level (P for trend <0.001) in a dose-dependent relationship. The HR_adj (95% confidence interval) were 6.1 (0.8—48.2), 6.2 (0.8—48.4), 7.7 (1—60.8), and 13.1 (1.7—99.6), for patients with serum HBV DNA level of 300—9,999 copies/mL, 10,000—99,999 copies/mL; 100,000—999,999 copies/mL; and ≥10^6 copies/mL respectively, compared with those who spontaneously achieved an undetectable HBV DNA level (<300 copies/mL). Other important variables included increasing serum ALT level, HBeAg-positive serostatus, age, and alcohol consumption. Conclusion: The relationship between HBV DNA level and risk of HCC remained very strong in these analyses using follow-up HBV DNA levels. The risk increased as HBV DNA level increased.

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SPECTRUM OF STEATOSIS IN CHRONIC HEPATITIS B (CHB) AND ITS RELATION TO BIOCHEMICAL, METABOLIC, VIROLOGICAL AND HISTOLOGICAL PARAMETERS
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Steatosis predicts progression of histologic injury, insulin resistance and reduced response to antiviral therapy in patients with hepatitis C. However, there is limited data in patients with chronic hepatitis B (CHB). This is relevant since response to current antiviral therapies for CHB is poor. Aim: To assess the spectrum of hepatic steatosis in CHB and to determine its relationship with biochemical, metabolic, virologic and histologic parameters. Patients and Methods: Liver biopsies of 350 patients with chronic HBV infection were blindly assessed by three experienced pathologists and categorized as: Group 1: no steatosis (≤5%) and group 2: hepatosteatosis (>5%). Demographic, biochemical, metabolic, virologic and histologic parameters were compared between two groups. HBV DNA was quantified by Hybrid capture assay and HBV genotyping was done by multiplex PCR. Results: Group 1 had 232 (66.3%) and group 2 had 118 (33.7%) patients. In group 2, 65 (55.1%) patients had mild and 53 (44.9%) had moderate to severe steatosis. More than 90% cases showed macrovesicular steatosis. Group 2 patients showed significantly higher age (35.5±10.5 yrs vs 28.1±13.9 yrs, p=0.000), male preponderance (M:F ratio-8.8:1 vs. 4.8:1, p=0.02), higher BMI (26.2±9.2 Kg/m2 vs. 20.7±3.9 Kg/m2, p=0.000), triglyceride (149.4±62.2 vs. 81.9±31.4, p=0.000) and insulin levels (13.1±9.1 vs 9.1±6.0, p=0.027). Waist circumference, blood sugar, cholesterol and leptin (2.3±1.9ng/mL vs 2.4±1.3ng/mL, p=0.95) were also higher in group 2 but not statistically significant. Mean HAI and fibrosis scores in group 2 were higher than group 1 (Group 1 vs 2, HAI, 4.5±2.9 vs 4.8±2.6, p=0.02; fibrosis 1.5±1.2 vs 1.7±1.2, p=0.17). More patients in group 1 had higher HBeAg positivity (Group 1 vs 2, 57.2% vs 47.3%, p=0.039). There was no significant difference in ALT (median, 1.14 vs 99, p=0.36) and HBV DNA levels (2.4E08±4.9E08 vs 1.9E08±4.6E08, p=0.48). In group 2, hepatic steatosis was more frequent in patients with HBV genotype D than in genotype A (58.1% vs. 42.0%, p=0.01). Conclusions: In our study steatosis was seen in one third cases with chronic HBV infection. These patients showed higher necroinflammatory activity and fibrosis. Association of steatosis with metabolic determinants and its presence in the liver suggests the role of host factors in the etiology of steatosis in chronic hepatitis B. There is no relation to viral factors like viral load and HBeAg status although there is a possibility of relation to genotype D. The efficacy of combined “antiviral and metabolic” approaches versus standard antiviral regimes in patients with steatosis and chronic hepatitis B infection needs to be evaluated.

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PREVALENCE OF FIBROSIS AND CIRRHOSIS IN CHRONIC HEPATITIS B: IMPLICATIONS FOR TREATMENT AND MANAGEMENT

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Aim: To document the prevalence and factors associated with severe fibrosis and cirrhosis in a large population of Asian chronic hepatitis B (CHB) patients. Methods: Transient elastography was performed in unselected CHB patients using Fibroscan (Echosens, France). Liver stiffness score of >8.1 kPa was used as a cut-off for the presence of severe fibrosis or liver cirrhosis. Demographics and laboratory parameters were recorded. Results: A total of 951 patients were recruited, of which 605 (64%) were males, with median age of 43 years, and positive HBeAg in 226 (24%). Overall 319 (34%) had severe fibrosis, with higher prevalence seen in males compared with females (39% vs 24% respectively, p<0.01). Severe fibrosis was seen with increasing age from 20% in patients < 25 years to 81% in those >65 years. In patients who were ≤ 45 years of age, there was no significant difference in prevalence of severe fibrosis between HBeAg-positive and HBeAg-negative patients. After the age of 45 years, higher prevalence of severe fibrosis was seen in HBeAg(+) patients compared to HBeAg(-) patients (58% vs 43% respectively, p=0.03). There was also increasing prevalence of severe fibrosis with increasing ALT levels: in patients with ALT less than 0.5 x upper limit of normal (ULN) and 0.5-1 x ULN 11% vs 30% respectively, p<0.01) and 0.5-1 x ULN and 1-2 x ULN (30% vs 48% respectively, p<0.01). There was a borderline significance in those with 1-2 x ULN and 2-5 x ULN (48% and 59% respectively, p=0.06) and no significant differences between those with ALT 2-5 x ULN and >5 x ULN. Higher prevalence of fibrosis was seen in patients with HBV DNA ≥4 log copies/ml compared to those below this level (41% vs 27% respectively, p<0.01). Using multivariate analysis by regression to determine factors associated with severe fibrosis, gender, age and ALT were significant factors (both p<0.001), whereas HBV DNA was not significant (p=0.25). In total there were 486 patients who had completely normal liver biochemistry with normal ALT levels, bilirubin ≤20 umol/L and albumin ≥40g/L. Of these, 89 (18%) had severe fibrosis. For those patients over the age of 45 years and with normal liver enzymes (n=211), 34% had underlying severe fibrosis. Conclusion: The overall prevalence of severe fibrosis in CHB patients was 34% with higher rates seen in older age groups, males, and in patients with higher ALT levels. There was a higher prevalence seen in male patients compared to female patients from age 36 to 55 years. Higher prevalence of fibrosis was also seen in HBeAg-positive patients compared to HBeAg-negative patients after the age of 45 years.

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ANALYSIS OF FULL HEPATITIS B VIRUS GENOME IN PATIENTS WITH AND WITHOUT HEPATOCELLULAR CARCINOMA

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Hepatitis B virus (HBV) genotype C and the basic core promoter (BCP) mutations were reported to be associated with the development of hepatocellular carcinoma (HCC). In this study the full sequences of HBV genomes were analyzed in order to find the other predictors of HCC development. Forty-four patients with HBsAg positive and HBV genotype C were studied. Among them, 24 cases developed HCC (HCC group) and the other 20 cases did not (non-HCC group) during the follow-up period. In the former 24 cases, the serum samples at the beginning of follow-up (HCC B) and at the detection of HCC (HCC A) were examined. In the latter 20 cases who did not develop HCC, the serum samples were obtained at the beginning of follow-up (non-HCC B) and at the end of observation period (non-HCC A). The two groups were matched in age, gender and follow-up time. All HBV sequences from serum samples were directly sequenced and compared with HBV X01587. HBV X promoter activity was examined using luciferase reporter assay. The nucleotide substitution rate in the full genome was 21.26±5.0×10⁻³/nt and 23.50±3.7×10⁻³/nt at the beginning of follow-up in the non-HCC group and HCC group, respectively (p=NS), and was 22.35±6.73×10⁻³/nt and 24.89±5.4×10⁻³/nt at the end of follow-up in the non-HCC group and HCC group, respectively (p=NS). More nucleotide and amino acid substitution rates were detected in the HCC group, compared with the non-HCC group. The substitution from G to A at nt 1317 in the X promoter was detected in 4 cases (20%) and 5 cases (25%) of 20 non-HCC B and non-HCC A cases, respectively, and in 14 cases (58.3%) and 15 cases (62.5%) of 24 HCC B and HCC A cases, respectively (non-HCC B vs HCC B, p=0.02; non-HCC A vs HCC A, p=0.02). The substitution from T to C/A/G at nt 1341 was detected in 3/2/1 cases (25%) and 3/3/1 cases (41.7%) of 24 HCC B and HCC A cases, while in none of 20 non-HCC B and non-HCC A cases (non-HCC B vs HCC B, p=0.03; non-HCC A vs HCC A, p=0.11). Pre-S2 deleted mutants were detected in B cases of 24 HCC B and 7 cases of 24 HCC A cases, respectively, while in none of the non-HCC group (HCC B vs non-HCC B, p<0.01; HCC A vs non-HCC A, p=0.01). We also examined the activity of HBV X promoter with mutations at nt 1317 and 1341 in comparison with that without mutations. The mutants at nt 1317 did not have any effect on the activity of X promoter, however, the mutants at nt 1341 from T to C/A/G resulted in 1.5, 2.3, 3.9 times increased X promoter activation, respectively. Substitutions and deletion of nucleotides of the HBV genome, especially the pre-S2 deletion and G1317A, T1341C/A/G mutations may be useful markers for predicting the development of HCC.

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HEPATITIS B VIRUS INHIBITS TLR9-MEDIATED INTERFERON ALPHA SECRETION IN PLASMACYTOID DENDRITIC CELLS

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Background and Aims: Hepatitis B virus (HBV) infection causes acute and chronic immunomodulated liver injury. Viral factors responsible for immune evasion and establishment of a persistant HBV infection remain unclear but in vivo studies highlight deficient innate responses to HBV. Plasmacytid dendritic cells (pDC) play a crucial role in innate immunity through their ability to secrete large amounts of type I interferons in response to viral pathogens in absence of active replication. Although still controversial, many studies do suggest a functional impairment of pDCs in chronic HBV infections, in both human and wood-
chucks. To investigate a direct role of HBV in this functional impairment, interactions between purified pDC and HBV particles were studied in vitro. Methods: For this purpose, HBV virions were purified using sucrose gradient from HepG2215 cells supernatants and viral load quantified by real time PCR. Interferon-alpha (IFN-α) and IL-6 secretions were determined by Elisa. Total PBMCs and purified pDCs were obtained from healthy blood donors and stimulated with oligonucleotides containing unmethylated deoxyxycytidyl-deoxyguanosine dinucleotides motifs (CpG) in the presence of HBV. Results/Discussion: Stimulation of pDC with HBV at MOI 10 to 1000 did not lead to any detectable levels of IFN-α and IL-6. When total PBMC and purified pDC were stimulated with CpG, secretion of IFN-α was reduced up to 40% by HBV. Inhibition was observed at 24 and 48h post-CpG stimulation and already detectable after 9h. Such an early inhibition suggest that mechanisms other than active viral replication are responsible for pDC dysfunction, consistent with studies suggesting an endocytosis mechanisms for HBV entry within DCs as opposed to an infection process. Contrary to the INF-α inhibition, the capacity of total PBMC to secrete Il6 was unchanged in presence of HBV. Conclusion: Overall, these findings do suggest that HBV alone is not recognized by pDC as a foreign "signal" and therefore fails to trigger this key component of innate immunity. This extent to which this pDC dysfunction plays a role in the outcome and immunopathogenesis of HBV infection warrants further studies.

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The following people have nothing to disclose: Isabelle E. Vincent, Uzma Hasan, Julie Lucifora, Christian Trepo, Isabelle Chemin

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TRACING HEPATITIS B VIRUS DNA BACK TO THE 16TH CENTURY IN A KOREAN MUMMY
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Background and Aims: It is presumed that Hepatitis B virus (HBV) has infected humans for thousands of years, but precise evidence is lacking. Korea is a country with endemic hepatitis B. A rare find of a "naturally" mummified child from the 16th century AD, in Korea, with a relatively preserved liver, enabled testing for the presence of HBV-DNA sequences. Methods: Despite the age of the mummy (verified by C14 testing of cloths from intra abdominal tissue), a parenccymatous organ was identified in the right upper abdominal quadrant by laparoscopy. Tissue samples were subjected to conventional microscopy and DNA extraction. A search for ancient HBV-DNA was conducted in three independent laboratories in Korea, UK and Israel. Pre-core, core and DNA polymerase sequences were analyzed in Jerusalem; DNA contamination was destroyed in liver tissue by laparoscopy by incubation in a fresh bleach solution. HBV DNA was extracted twice, using in-house guanidium thiocyanate or a commercial columns kit. PCR amplification and analysis of the pre C/C region was performed using a HBV Monitor kit (Roche). An HBV Genotyping kit (INNogenetics) was used for analysis of the polymerase gene Results: A 104 bp fragment from the preC/C region was amplified in Jerusalem and the genotype was determined by analysis of a 234 bp fragment from the polymerase gene. The genotype was confirmed by amplifying and sequencing a 239bp fragment from the core gene by the Korean team. A 98bp fragment from the S gene was amplified in London. These results confirm the presence HBV-DNA sequences, genotype C in material removed from the mummy. removed from the mummy. The tissue tested was also subjected to microscopic analysis. Although, as expected, no hepatic or lymphoid cells could be identified on H&E staining, the overall organization resembled the appearance of liver tissue. The architecture, as demonstrated by Masson trichrome and reticulin stains, appeared normal overall, with minimal fibrotic expansion of the portal tracts and condensation of the sinusoidal walls, as expected after the lysis of hepatocytes. Conclusion: Extraction of DNA from the a liver remnant of a 500 year old Korean mummy revealed evidence for presence of intrahepatic HBV-DNA. No statement can be made regarding the stage of HBV infection in this child presumed to be an HBsAg carrier. Other evidence obtained suggest that the child died of tuberculosis based on identification of mycobacterium tuberculosis sequences in nodules removed from intra abdominal tissue.

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The following people have nothing to disclose: Athalia Klein, Mark Spigelman, Paul Grant, Orit Pappo, Myung-Ji Kim, Dong Hoon Shin, Daniel Shouval

926
EFFECT OF ANTIVIRAL THERAPY ON THE IMMUNOHISTOCHEMICAL EXPRESSION OF BCL-XL AND BAX PROTEIN IN PATIENTS WITH HBEAG-NEGATIVE CHRONIC HEPATITIS B
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BACKGROUND: Although increasing evidence suggest that apoptosis of hepatocytes plays an important role in the initiation of liver injury/carcinogenesis, the exact role of antiviral therapy in apoptosis has never been investigated. AIMS: To evaluate the immunohistochemical expression of anti-apoptotic bcl-xl and pro-apoptotic bax proteins in hepatocytes of patients with HBeAg-negative chronic hepatitis B (CHBe-) before and after treatment. MATERIAL AND METHODS: Thirty six paired liver biopsies from CHBe- patients: between the 1st and 2nd biopsy, 29 patients received 12-month courses of antiviral therapy (interferon-alpha: 17, ade-fovir: 12) and 7 untreated patients were used as controls. In each pair of biopsies, changes in expression of apoptotic proteins (D-bcl-xL and D-bax), total apoptotic trend (D-apoptosis: difference between D-bcl-xL and D-bax), necroinflammation (D-grade) and fibrosis (D-stage) (using the Ishak score) were evaluated. RESULTS: Bax-positive compared to Bax-negative biopsies had significantly worse necroinflammation (8.2 vs 6.7, p=0.05) and fibrosis score (3.9 vs 3, p=0.036). Bcl-xL-positive compared to bcl-xl-positive biopsies had significantly lower intra lobular inflammation (1.6 vs 2.2, p=0.03). Negative, compared to stable/positive D-bax from 1st to 2nd biopsy, was associated with significantly greater improvement in necroinflammation only in treated patients (mean D-grade : 4.6 vs -1.6, p=0.06) and improvement in fibrosis in the interferon treated patients (D-stage: -0.4 vs 0.55, p=0.05). Stable/decreased compared to increased D-apoptosis was associated with significantly worsen fibrosis, particularly in patients treated with adefovir (D-stage: 2.3 vs 0, p=0.004). In the 11 patients without significant changes from 1st to 2nd biopsy (D-stage ≤1 and D-grade ≤2), positive D-apoptosis was significant more frequent in treated than untreated control
The following people have nothing to disclose: Masaya Sugiyama, Yasuhito and liver damage in vivo, which might be associated with Precore mutation of Bj could induce high replication types demonstrated in chimeric mice may influence HBV infection. Conclusion: Virological differences among HBV genotypes, while HBsAg was expressed at the highest level in HBV/Ae. The protein production of Bj_PCm transiently were expressed in a trend similar to HBV DNA levels among were expressed in a trend similar to HBV DNA levels among. HBcrAg in serum was measured by real-time detection PCR among HBV genotypes in vivo. Methods: Sera of six HBV carriers [three each for Bj_wild-type (from acute HB patients) and Bj_PCm (fulminant HB patients)] were prepared. The sera or virions of HBV recovered from culture media, that were produced by transfection with plasmids (3 strains each for Ae, Ce, Bj_wild-type and Bj_PCm) carrying 1.24-fold the HBV genome in Huh7 cells, were inoculated into uPA/SCID mice with the liver replaced by human hepatocytes (chimeric mice). HBV DNA in mice serum was measured by real-time detection PCR weekly and then histopathologic changes of liver were examined with H.E, Masson trichrome, Orcein stainings, and immunostaining using anti alpha-smooth muscle actin (alpha-SMA) antibody. Results: The replication efficiency in chimeric mice was the highest in HBV/C and Bj_PCm, followed by Ae (P<0.05). HBV DNA levels of Bj_wild-type were extremely lower than the others by 4 log levels. To exclude the possibility that immature virus particles were produced in culture media, sera of 6 HBV carriers with Bj_wild-type or Bj_PCm were inoculated with chimeric mice. The HBV DNA levels in mice serum were similar to those by injection of culture media. HBcrAg in serum were expressed in a trend similar to HBV DNA levels among HBV genotypes, while HBsAg was expressed at the highest level in HBV/Ae. The protein production of Bj_PCm transiently increased and peaked in earlier phase. Based on histopathologic examinations, chimeric mice inoculated with HBV/C or Bj_PCm for 6 months showed that human hepatocytes had somewhat ground-glass appearance and hepatic fibrosis, while chimeric mice inoculated with HBV/Ae or Bj_wild-type were not induced. Conclusion: Virological differences among HBV genotypes demonstrated in chimeric mice may influence HBV infections with distinct genotypes in clinical and epidemiological settings. Precore mutation of Bj could induce high replication and liver damage in vivo, which might be associated with development of fulminant hepatitis.

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927 DIFFERENCES OF EARLY DYNAMICS AND LIVER DAMAGE AMONG HEPATITIS B VIRUS GENOTYPES IN UPA/SCID MICE WITH HUMAN HEPATOCYTES

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Background: Hepatitis B virus (HBV) genotypes/subgenotypes indicate different clinical features, i.e. genotype C is associated with hepatocellular carcinoma and Bj with precore mutation (PCm) is associated with fulminant hepatitis. We previously showed the difference of each genotype in vitro and partially in vivo. Aim: Our purpose of this study is to investigate the differences of viral replication, protein production and liver damage among HBV genotypes in vivo. Methods: Sera of six HBV carriers (three each for Bj_wild-type [from acute HB patients] and Bj_PCm [fulminant HB patients]) were prepared. The sera or virions of HBV recovered from culture media, that were produced by transfection with plasmids (3 strains each for Ae, Ce, Bj_wild-type and Bj_PCm) carrying 1.24-fold the HBV genome in Huh7 cells, were inoculated into uPA/SCID mice with the liver replaced by human hepatocytes (chimeric mice). HBV DNA in mice serum was measured by real-time detection PCR weekly and then histopathologic changes of liver were examined with H.E, Masson trichrome, Orcein stainings, and immunostaining using anti alpha-smooth muscle actin (alpha-SMA) antibody. Results: The replication efficiency in chimeric mice was the highest in HBV/C and Bj_PCm, followed by Ae (P<0.05). HBV DNA levels of Bj_wild-type were extremely lower than the others by 4 log levels. To exclude the possibility that immature virus particles were produced in culture media, sera of 6 HBV carriers with Bj_wild-type or Bj_PCm were inoculated with chimeric mice. The HBV DNA levels in mice serum were similar to those by injection of culture media. HBcrAg in serum were expressed in a trend similar to HBV DNA levels among HBV genotypes, while HBsAg was expressed at the highest level in HBV/Ae. The protein production of Bj_PCm transiently increased and peaked in earlier phase. Based on histopathologic examinations, chimeric mice inoculated with HBV/C or Bj_PCm for 6 months showed that human hepatocytes had somewhat ground-glass appearance and hepatic fibrosis, while chimeric mice inoculated with HBV/Ae or Bj_wild-type were not induced. Conclusion: Virological differences among HBV genotypes demonstrated in chimeric mice may influence HBV infections with distinct genotypes in clinical and epidemiological settings. Precore mutation of Bj could induce high replication and liver damage in vivo, which might be associated with development of fulminant hepatitis.

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928 GENDER DIFFERENCE IN THE NATURAL COURSE OF HBEAG-POSITIVE CHRONIC HEPATITIS B

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PURPOSE: Cross-sectional age-specific seroprevalence studies from Taiwan have provided evidence to suggest that male patients have earlier spontaneous hepatitis B e antigen (HBcAg) seroconversion but, paradoxically, bear a worse prognosis in disease progression when compared to their female counterparts. On the other hand, recent studies have shown that patients infected with genotype B hepatitis B virus (HBV) have earlier HBcAg seroconversion and better prognosis than genotype C infected patients. This longitudinal study was conducted to clarify this gender-related paradox while taking account the HBV genotype factor. METHODS: HBcAg seropositive patients from 1977 to 1998 were recruited if they met the following criteria: (1) Histologic and biochemical confirmation of chronic hepatitis B; (2) Follow-up every 3-6 months and ≥ 2 years; (3) No concomitant hepatitis C or D virus infection; (4) No drug treatment during follow-up. The clinical course and biochemical/serological changes were compared between male and female patients using t test, X² and Kaplan-Meir survival analysis. RESULTS: There were 454 males and 110 females who met our entry criteria. The age, baseline serum alanine aminotransferase (ALT) level and HBV genotype distribution were comparable between male and female patients. During a mean follow-up period of 12 years, there was no significant difference in the timing and the cumulative incidence of spontaneous HBcAg seroconversion between male and female patients (97.6 vs 100%; P=0.915). To minimize the influence of other confounding factors, the 110 female patients were compared with a 1:1 group of male patients well matched for age (± 2 years), baseline ALT (± 20 U/L) and duration of follow-up (± 1 year). The HBV genotype distribution in the female and the matched male patients was comparable (P=0.864). There was no significant difference in the incidence of hepatitis flare with ALT over 5 times the upper limit of normal (66.4 vs 72.5%; P=0.326) and in the 12-year cumulative incidence of HBcAg seroconversion (85.5 vs 74.4%; P=0.129). However, 47.7% of the male patients showed persistent ALT elevation, with or without ALT flare, in contrast to 14.5% of the female patients (P<0.0001). CONCLUSION: This longitudinal study showed that there was no difference in the occurrence of hepatitis flare and spontaneous HBcAg seroconversion between males and females with active chronic hepatitis B. Male patients were found to have a higher propensity for persistent ALT elevation, which may be responsible for a more progressive course.

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The following people have nothing to disclose: Elaine Y. Lin, Yi-Cheng Chen, Shiu-Feng Huang, Chau-Ting Yeh
BOW BODY MASS INDEX (BMI) PREDICTS DISCORDANCE BETWEEN TRANSIENT ELASTOGRAPHY (TE) AND LIVER BIOPSY (LB) ASSESSMENT OF LIVER FIBROSIS

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Background and aim. In patients with chronic liver disease (CLD) liver fibrosis stage can be accurately predicted by TE. Variables associated with discordant results between TE and LB need to be defined. Methods. All consecutive patients with CLD who underwent percutaneous LB for diagnostic/therapeutic purposes were concurrently examined by TE (FibroScan®). Echo-sens, Paris, France). Grading (A) and staging (F) were assessed by METAVIR score. Steatosis was arbitrarily graded from 0 to 3 (0<5%, 1=5-24%; 2=25-50%; 3=50% of fatty hepatocytes). Only TE examinations with at least 10 validated measurements and a success rate of at least 60% were considered adequate. Statistical analysis. Determinants of TE stiffness value (log transformed) were investigated by the multiple linear regression model and variables possibly influencing discordance between TE and LB by a logistic regression model. The variables included in the models were: gender; age; body mass index (BMI, kg/m2) (<19 vs 19-25 vs 26-30 vs >30 kg/m2); etiology; AST, ALT and GGT levels (<1.5 vs 2-3 vs >3 ULN), APRI score (<0.5 vs 0.5-1 vs >1), steatosis (0-1 vs 2; 2 vs 3); A (0-1 vs 1-4) and F; LB specimen length (≤20 vs >20 mm). Discordance was the lack of correspondence between the METAVIR F and TE diagnostic cut off (7.9, 10.3 and 11.9 kPa for F=2, F=3 and F=4, respectively) identified in a previous cohort. Results: 303 males and 257 females (50±12 years) were studied. TE examination failed in 30 (5%) mainly due to high BMI. In four cases (0.7%) LB was inadequate as shorter than 15 mm or contained <11 portal spaces. By multivariate analysis, fibrosis stage (p=0.0001), BMI (p=0.0004), etiology (p=0.02), GGT (p=0.0001), APRI score (p=0.0001) and A (p=0.0001) were significantly related to TE values. TE and LB were discordant in 116 cases (22%) for the diagnosis of F=2 and in 85 (16%) for the diagnosis of F=3. The probability of discordance was positively associated with BMI (BMI<19 vs 19-25, OR=3.2, 95%CI 1.3-7.8 for F=2; OR=4.9, 95%CI 2.0-11.8 for F=3). Conclusion: In the face of many factors other than fibrosis influencing TE results, low BMI was the only predictor of discordance between TE results and LB.

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RAPID IMPROVEMENT OF INNATE IMMUNE RESPONSES AND MONOCYTE TOLL-LIKE RECEPTOR-2 (TLR2) EXPRESSION DURING LAMIVUDINE AND PEGYLATED INTERFERON THERAPY FOR CHRONIC HEPATITIS B (CHB)

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Background/Aims: Toll-like receptors (TLR’s) are critical receptors that promote innate immune responses to pathogen-associated molecular patterns. Activation of TLR’s leads to production of pro-inflammatory cytokines. We have previously demonstrated that patients with HBeAg positive CHB have suppressed TLR2 expression on peripheral monocytes and hepatocytes associated with decreased pro-inflammatory cytokine expression. Methods and Patients: The TLR2 and TLR4 expression on peripheral blood monocytes was measured from 21 patients with untreated HBeAg-positive and HBeAg-negative CHB and 10 uninfected control patients. These 21 patients were treated with lamivudine 100mg daily. Individual patient blood was stimulated with specific TLR ligands and the resultant supernatant was assayed for TNF-α and IL-6 by ELISA. In addition serum HBeAg was measured by enzyme immunoassay. Similar data was also obtained for 20 HBeAg positive patients treated with pegylated interferon Results: Expression of TLR2 on peripheral monocytes was significantly reduced in patients with HBeAg-positive CHB in comparison to HBeAg-negative CHB and to controls whilst it was significantly increased in HBeAg-negative CHB compared to controls (P=0.001). TLR2 levels increased within four weeks of effective therapy in HBeAg-positive patients (P<0.05) whilst remaining stable in HBeAg negative patients. The level of TLR4 expression did not differ significantly between the groups. The functional relevance of these findings was established by the demonstration in HBeAg positive patients of initial reduced TNF-α and IL-6 and phospho-p38 kinase production after stimulation of monocytes with specific TLR ligands. This immunosuppression improved rapidly during treatment (P<0.05). In HBeAg negative patients innate immune responses were initially increased but normalized with therapy. In the HBeAg positive patients treated with pegylated interferon TLR2 expression was further suppressed at 4 weeks (P<0.05) before improving over subsequent months. Cytokine production secondary to stimulation with Pam-3-Cys and LPS were markedly increased at week 4, dropping dramatically thereafter (P<0.05). Correlations of these results with HBV viral load and eAg levels (eAg positive patients) will also be presented. Conclusions: This is the first study to investigate how peripheral innate immune responses and TLR expression changes with different therapies. It demonstrates a potentially important interaction between HBeAg, HBV and the innate immune response and suggests that TLR2 expression and activation could represent a sensitive new biomarker in the treatment of HBeAg-positive CHB.

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931 DIFFERENTIAL EXPRESSION OF HEPATIC APURINIC/APYRIMIDINIC ENDONUCLEASE 1, A DNA REPAIR ENZYME, IN CHRONIC HEPATITIS B VIRUS AND HEPATITIS C VIRUS INFECTION

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Background: Hepatocellular carcinoma (HCC) is frequently encountered in chronic hepatitis B virus (HBV) or hepatitis C virus (HCV) infection, while HCC rarely develops in autoimmune hepatitis (AIH) and primary biliary cirrhosis (PBC). However, the mechanisms responsible for HBV- or HCV-mediated hepatocarcinogenesis have not been fully clarified, yet. Cellular DNA damage can lead to mutation induction and subsequent carcinogenesis if DNA repair processes are not completely effective. Apurinic/apyrimidinic endonuclease 1 (APE-1; also designated Ref-1) is one of the major DNA repair enzymes, and its altered expression is associated with tumorigenesis. Therefore, APE-1/Ref-1 may be involved in HBV- or HCV-mediated hepatocarcinogenesis. The aim of the present study was to examine hepatic expression of APE-1/Ref-1 in patients with chronic HBV or HCV infection, compared with those with AIH or PBC. Methods: Liver biopsies were obtained from 14 patients with chronic hepatitis B, 10 patients with chronic hepatitis C, 5 patients with AIH and 5 patients with PBC. APE-1/Ref-1 expression was assayed by Western blot or real time-PCR, and its distribution was determined by immunohistochemistry. Results: APE-1/Ref-1 protein levels were reduced by 64% in HBV-infected livers compared with AIH and PBC livers, while HCV livers had no significant difference in APE-1/Ref-1 protein levels compared with those of autoimmune chronic liver diseases. APE-1/Ref-1 mRNA levels were reduced by 58% in HBV livers compared with HCV livers. APE-1/Ref-1 had a predominant nuclear localization in the majority of hepatocytes with a panacinar distribution in all of the liver specimens. The hepatocytic nuclear APE-1/Ref-1 expression was also reduced in the HBV group. Conclusions: Patients with chronic HBV infection, not chronic HCV infection, showed reduced expression of hepatic APE-1/Ref-1 compared with those with autoimmune chronic liver diseases. Hepatic APE-1/Ref-1 expression may undergo differential regulation in chronic HBV and HCV infection. Downregulation of hepatic APE-1/Ref-1 expression may contribute to the development of HCC in chronic HBV infection.

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932 GENETIC POLYMORPHISMS AT THE APOLPOLYPEPTIDE E LOCUS AFFECT THE OUTCOME OF CHRONIC HEPATITIS B

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Introduction. Genetic variations at the apolipoprotein E (ApoE) locus are known to affect plasma lipoprotein concentrations; therefore, by modulating transport into the blood circulation and entry into the hepatocytes of hepatitis B virus (HBV), they may influence the course of HBV infection. The present study aimed to verify this hypothesis. Methods. One-hundred five HBV positive, HCV negative patients (74 males, 31 females, median age 51 years, range 22-84) were studied. Among them, a cohort of 62 patients (42 males, 20 females, median age 51 years, range 2-81) had undergone periodical clinical monitoring for a median time of approximately 20 years since first identified as HBsAg carriers. ApoE genotypes were determined by a previously described polymerase chain reaction/restriction length fragment polymorphism method. Results. Twenty-seven patients out of 105 (26%) fulfilled criteria for being inactive HBV carriers, whereas 61 had chronic hepatitis B (58%; 15 with cirrhosis) and 17 had undergone liver transplantation for hepatic failure and/or hepatocellular carcinoma (16%). The allelic frequencies of the ApoE gene were the following: ApoE2 = 15%; ApoE3 = 78%; ApoE4 = 7%. There was a strong linear trend for E3 homozygotes to be progressively overrepresented among patients with liver disease of advancing severity: E3/E3 were 13/27 (48%) among inactive HBV carriers, 36/61 (59%) among patients with chronic hepatitis B, and 16/17 (94%) among patients transplanted for hepatitis B-related sequelae (p<0.005). In the longitudinal cohort, 12/62 patients (19%) became HBsAg negative after a median follow-up time of 14.6 years (range, 1.5-32.3). Being an E3/* carrier was associated with significantly lower probability of losing HBsAg (95/56, 16% vs. 3/6, 50%, p<0.05), as well as significantly longer time to HBsAg loss (p<0.03 by Mantel-Cox test). Conclusions. The probability of undergoing disease progression is higher, and that of remaining inactive carriers or of losing HBsAg is lower among possessors of the ApoE3 allelic variant. Down-regulation and/or reduced binding involving the LDL-receptor might explain the more benign course of hepatitis B among ApoE2-E4 carriers.

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933 PREDICTIVE MODEL FOR FIBROSIS AND CIRRHOSIS IN CHRONIC HEPATITIS B USING LIVER STIFFNESS MEASUREMENT

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Aim: To correlate liver stiffness measurements with demographic and laboratory parameters, and assess the predictive value of these parameters as non-invasive markers of fibrosis and cirrhosis in chronic hepatitis B (CHB). Methods: Transient elastography was performed in 265 CHB patients using Fibroscan (Echosens, France). Liver stiffness scores of >8.1 and >10.3 kPa were used as cut-off values for significant fibrosis and cirrhosis, respectively. To derive a new index using commonly measured laboratory markers, the study sample was randomly split into a training set and a validation set. Results: Of the 265 patients, 170(64%) were male. Ninety-six patients (36%) were HBsAg-positive, and 90 patients (34%) had severe fibrosis or cirrhosis. The median liver stiffness score was 6.3 kPa (range, 3.0 to 56.3). Liver stiffness correlated positively with ALT, bilirubin and AFP, and negatively with albumin levels. Using 13 parameters (age, sex, platelet, AST, ALT, GGT, AFP, albumin, globulin, bilirubin, ALT, HBV DNA, and HBeAg), the sequence of variables in order of their associations with liver stiffness (co-effi-
HBV DNA at the end of therapy [mean ± SEM 4.31 ± 1.25 vs. 8.31 ± 0.87 log10 copies/ml respectively, p=0.04] in patients with SVR was lower when compared with those without SVR. Similarly, those with SVR had a higher, though insignificant IL-17 at the end of therapy [mean ± SEM 2.13±0.08 vs. 1.73±0.48 % respectively, p=0.47] and end of follow-up [mean ± SEM 2.57±0.28 vs. 2.35±0.74 % respectively, p=0.79] when compared with patients without SVR. Conclusion: Pegylated interferon alfa-2a induced viral load reduction results in an increase in IL-17. SVR may be associated with a higher IL-17. The role of IL-17 in CHB infection and immunopathogenesis requires further investigation.

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934 INHIBITION OF VIRAL REPLICATION WITH PEGYLATED INTERFERON ALFA-2A INCREASES IL-17 IN CHRONIC HEPATITIS B

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Background and Aim: Interleukin (IL)-17 is produced mainly by activated CD4+ T cells, currently known as Th17. Chronic hepatitis B (CHB) infection is associated with weak and narrowly focused immune response with low CD4+ and CD8+ activity. This is the first report of IL-17 in CHB infection. We assessed IL-17 expression in the CD4+ T cells (Th17) of 50 consecutive CHB patients treated with pegylated interferon alfa-2a for 48 weeks.

Methods: Peripheral blood mononuclear cells (PBMC) were stained with CD45, CD4, IL-17 and analyzed by three-color flow cytometry. PBMC of 8 hepatitis B e antigen (HBeAg) positive patients; 4 with sustained virological response (SVR) and 4 without SVR; were collected every 4 weeks during treatment until end of therapy (week 48) and every 12 weeks until the end of follow-up (week 72). Serum HBV DNA was quantified by quantitative PCR with a linear range of 100 to 109 copies/ml. SVR was defined as HBeAg seroconversion with serum HBV DNA < 104 copies/ml at the end of follow-up. HBeAg seroconversion was defined as HBeAg negativity with hepatitis B e antibody on 2 consecutive occasions 3 months apart. IL-17 was defined as CD4+IL-17+ cells/CD45+ cells.

Results: In parallel with decline in serum HBV DNA, we found an increase in IL-17 at the end of follow-up when compared with baseline [mean±standard error of mean (SEM) 2.46±0.37 vs. 1.44±0.23 % respectively, p=0.03]. There was a trend that IL-17 at the end of therapy was also higher than at baseline [mean±SEM 1.93±0.23 % respectively, p=0.05]. The serum HBV DNA at the end of therapy [mean±SEM 3.10±0.59 vs. 5.41±0.49 log10 copies/ml respectively, p=0.04] and end of follow-up [mean±SEM 4.31±1.25 vs. 8.31±0.87 log10 copies/ml respectively, p=0.04] in patients with SVR was lower when compared with those without SVR. Similarly, those with SVR had a higher, though insignificant IL-17 at the end of therapy [mean±SEM 2.13±0.08 vs. 1.73±0.48 % respectively, p=0.47] and end of follow-up [mean±SEM 2.57±0.28 vs. 2.35±0.74 % respectively, p=0.79] when compared with patients without SVR. Conclusion: Pegylated interferon alfa-2a induced viral load reduction results in an increase in IL-17. SVR may be associated with a higher IL-17. The role of IL-17 in CHB infection and immunopathogenesis requires further investigation.

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DNA in serum (P<0.005). Conclusion: (1) HBV cccDNA and pgRNA were undetectable in the serum of CHB patients; (2) HBV viral loads in PBMC were associated with the serum HBV DNA; (3) Lamivudine therapy had less effect on the HBV viral loads in PBMC compared with the serum viral loads. The inefficiency of antiviral therapy on PBMC viral loads may relate with the HBV persistence.

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936
PREDICTORS OF SIGNIFICANT HISTOLOGICAL FINDINGS IN CHRONIC HEPATITIS B PATIENTS WITH PERSISTENTLY NORMAL ALT LEVELS
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Background/aim: Current guidelines recommend antiviral therapy for chronic hepatitis B (CHB) patients with elevated ALT and high viral load. Little histological data exists for patients with persistently normal ALT levels (PNAL) and/or significant fibrosis Ishak's staged at 3 or greater) in such patients. Methods: The study of all patients with CHB who underwent percutaneous liver biopsy and who had detectable viral load (Taqman Real Time PCR, lower limit of detection is 10^3 copies/ml) was performed. 139 patients who had PNAL and/or significant fibrosis were identified. The aim of the study is to find the predictors of significant histological findings (significant necroinflammation with a score of HAI 4 or greater and/or significant fibrosis Ishak's staged at 3 or greater) in such patients. Methods: The study of all patients with CHB who underwent percutaneous liver biopsy and who had detectable viral load (Taqman Real Time PCR, lower limit of detection is 10^3 copies/ml) was performed. 139 patients who had PNAL (defined as normal ALT measured on at least 3 occasions in the intervals of more than two months over a period of 12 or more months apart prior to biopsy) were identified (group A). These patients were compared with 135 patients with abnormal ALT during the same period and who had no prior antiviral treatment. The comparisons included age, ALT, viral load, HBeAg status, and histological finding. Results: The ALT values of all 139 patients with PNAL had been normal from one year to thirty years, and the median was six years. Even though 66 (47.5%) patients with PNAL had normal liver histology, 33 (23.7%) patients were found to have significant histological findings, even 13 (9.4%) had established cirrhosis. When compared to patients within 0–0.75 × ULN ALT, patients within 0.75–1 × ULN ALT had higher rate of significant histological findings (43.5% vs. 19.8%, p=0.029). In the subgroup of patients with PNAL, significant histological findings increased sharply after the age of 40 yrs as seen in other cohorts (p=0.005). However, the subgroup of patients with PNAL and significant histological findings was not identified by viral load or HBeAg status. Conclusions: 23.7% of CHB patients with PNAL regardless of HBeAg status or viral load levels had significant histological findings including inflammation and fibrosis. Liver biopsy should be considered in CHB patients with PNAL and detectable viral load, even IFL, especially in those older than age 40 yrs and higher ALT within 0.75–1 × ULN.

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937 TREATMENT-INDUCED HBeAg SEROCONVERSION IS A POOR THERAPEUTIC ENDPOINT
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INTRODUCTION HBeAg seroconversion is considered a key therapeutic endpoint of Chronic Hepatitis B treatment, but its effectiveness as an endpoint is compromised by HBeAg negative reactivation and its durability, consequently we sought to examine these events in patients with well documented spontaneous or treatment induced HBeAg seroconversion. METHODS All patients with documented HBeAg seroconversion were followed prospectively at 3-monthly intervals. Persistent reactivation was defined as return of HBeAg with or without loss of anti-HBe, after loss of HBeAg and development of anti-HBe. The outcome events of reactivation and seroreversion were compared in spontaneous and treatment induced seroconversion using Kaplan-Meier survival analysis and Cox Regression. Data is presented as median (range). RESULTS There were 298 patients with documented seroconversion, of which 116 patients were treatment-induced and 182 were spontaneous. The patients were mainly male (71.81%), with age of 34.81 (68.7) years, with follow up duration of 39.29 (188.03) months post-seroconversion. Persistent reactivation occurred in 71 patients (Cumulative proportion=39.27%), with older age (P=0.001), male gender (P=0.002), higher ALT at seroconversion (P=0.006) being more likely to develop reactivation. No significant difference rate of reactivation was observed among different treatment groups (IFN, lamivudine & adefovir) (P=0.598). Treatment-induced seroconversion was less robust and remission of ALT was of shorter duration than spontaneous seroconverters (median 14.13 months versus 22.44 months, P=0.037). They were also more likely to develop HBeAg negative reactivation at 48 months (37.76% versus 24.96%, P=0.0482). Treatment responders are more likely to develop reactivation after adjusting for age, gender and ALT with Cox regression (Adjusted cumulative proportion: 48.5% versus 34.0% at 96 months, P=0.045). HBeAg reversion occurred in 39 patients (19.64%). Again, treatment induced seroconverters are more likely to get HBeAg reversion than spontaneous seroconverters: 24.09% versus 11.29% at 48 months (P=0.0094). After adjusting for age, gender, and ALT at seroconversion by Cox regression, the difference is consistent (P=0.010). CONCLUSION The effectiveness of treatment induced seroconversion is significantly worse than that of spontaneous seroconverters with significantly higher and faster HBeAg negative reactivation rate with less durable seroconversion over time. Treatment induced seroconversion may not be a good therapeutic endpoint in the treatment of chronic hepatitis B.

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FOUR-YEAR ENTECAVIR TREATMENT IN NUCLEOSIDE-NAIVE HBeAg(+) PATIENTS: RESULTS FROM STUDIES ETV-022 AND -901

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Background: Entecavir (ETV) 0.5 mg demonstrated superior virologic suppression compared to lamivudine (LVD) 100 mg in study ETV-022¹. One hundred and eighty-three entecavir-treated patients from ETV-022 enrolled in rlover study ETV-901. We present efficacy and safety results in a cohort of patients from ETV studies -022 and -901 who received a total of 4 years of therapy with ETV.

Methods: The nucleoside-naive HBeAg(+) 4-year cohort consists of ETV patients who completed ETV-022 and enrolled into ETV-901 with a treatment gap ≤35 days. In ETV-901, patients were treated with 1 mg of ETV. The proportions of patients with HBV DNA <300 copies/mL by PCR assay, ALT normalization, HBeAg loss and HBeAg seroconversion were evaluated among patients with available samples at week-192. Results: The nucleoside-naive HBeAg(+) 4-year ETV treatment cohort consists of 146 patients. Efficacy parameters through 4 years of ETV therapy are presented below. The safety profile of ETV was consistent with previously reported experience. Conclusions: At Week 192, 91% of patients who received ETV treatment during 4 years achieved undetectable HBV DNA and 86% had ALT normalization, with patients continuing to experience HBeAg loss and HBeAg seroconversion during Years 3 and 4. The safety profile was consistent with previously reported experience. ¹ Chang TT, et al, N Engl J Med 2006;354:1001-1010.

**Numbers/proportions represent additional patients achieving HBeAg loss or HBeAg seroconversion during treatment in ETV-901; serology tests were performed by local laboratories**

<table>
<thead>
<tr>
<th>Endpoint</th>
<th>Week 192*</th>
</tr>
</thead>
<tbody>
<tr>
<td>HBV DNA &lt;300 copies/mL, n (%)</td>
<td>98/108 (91)</td>
</tr>
<tr>
<td>ALT ≤ 1.5 x ULN, n (%)</td>
<td>96/112 (86)</td>
</tr>
<tr>
<td>HBeAg loss, n (%)</td>
<td>30/36 (81)</td>
</tr>
<tr>
<td>HBeAg seroconversion, n (%)</td>
<td>15/96 (16)</td>
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* Denominator represent patients with available samples

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THE MULTI-DRUG RESISTANT HBV RTA181T HAS A SECRETION DEFECT AND SIGNIFICANTLY REDUCES THE KINETICS OF VIRAL REBOUND

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Background/Aims: Lamivudine (LMV) [rtM204V/I] or adefovir (ADV) [rtN236T] resistance results in rebound of serum HBV DNA of at least 1.0 log10 IU/ml increase over 3-6 months (Locarnini et al. 2004. AVR:9:679). The rtA181T mutant is also associated with both LMV and ADV resistance but occurs as a mixed population with wild-type (wt) HBV. The rtA181T also encodes a stop codon in the overlapping surface gene at aa172 (sW172*) resulting in truncation and loss of the last 54 amino acids of the C-terminal hydrophobic region. The aims of this study were to examine the in vitro effects of this mutation on HBV replication, and its effect on HBV DNA levels in patients following its selection Methods: To measure HBV replication, HuH7 cells were transfected with infectious clones encoding wt HBV, rtA181T or a mixture of both. HBV replication products were detected by Western blotting (WB), Southern blotting (SB), Immunohistochemistry (IHC) and electron microscopy (EM). The clinical occurrence and accompanying viral load data from patients with rtA181T was determined using SeqHepB (Yuen et al. 2007. AVR:75:64). Results: The rtA181T change resulted in a severe secretion defect preventing egress of surface proteins and virions from the cell as demonstrated by SB, WB and IHC. WB also revealed that the surface proteins from the rtA181T transfected cells were truncated and displayed differential glycosylation and stability. When rtA181T was transfected and expressed with wt to mimic a mixed infection as found in vivo, secretion of subviral particles was restored, but virion secretion was still greatly impaired. There was also increased intracellular accumulation by IHC, although not as extensive as with rtA181T alone. EM of cell supernatant detected viral-like structures secreted from doubly-infected cells that comprised mainly of spherical particles, with few virions or filaments compared to wt. Thus, rtA181T is dependant on wt HBV for secretion but acts as a dominant negative mutant on HBV virion secretion resulting in its retention within the cell. Examination of sequential HBV DNA levels from the blood of patients where only the rtA181T change was detected by direct PCR-sequencing, revealed that viral load rebound did not occur at all or increased in incremental amounts of less than 1.0 log10 IU/ml over 12-18 months. Conclusion: The HBV rtA181T mutation causes a secretory defect and exerts a dominant negative effect on wt HBV virion secretion. The rtA181T masks viral rebound, which in the clinical situation can result in mis-diagnosis of drug resistance if only viral load is used (> 1.0 log10 IU/ml increase over 3-6 months) as criterion for breakthrough failure.

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940 CREATURE KINEASE (CK) ELEVATIONS AND MUSCLE TOXICITIES ASSOCIATED WITH CHRONIC TELBIVUDINE (LdT) USE IN PROSPECTIVE CLINICAL TRIALS

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Background: Nucleoside-associated myopathy has been well described in treatment of the Human Immunodeficiency Virus; however, CK elevations and myopathy have been a more unusual adverse event with the use of nucleosides for treatment of Hepatitis B Virus (HBV). We reviewed the LdT approval database for CK elevations and adverse event patterns consistent with muscle toxicity. Methods: Data were reviewed from trials that prospectively measured CK levels and recorded clinical adverse events, and in which LdT was used in at least one arm in New Drug Approval 22-011. We examined the time to onset and frequency of CK elevations from the pivotal Phase 3 trial (52 week data) using Kaplan-Meier plots and Fisher’s Exact Analysis (LdT vs. lamivudine (LAM)). We also reviewed detailed event narratives of clinical adverse events (AE) for CK elevations or events suggestive of myopathy, the Food and Drug Administration Adverse Event Reporting System for reports of muscle toxicity since LdT market approval (10/25/06) and preclinical evaluations for mitochondrial toxicity submitted with the Application. Results: The pivotal Phase 3 trial safety database included 680 (LdT) and 687 (LAM) subjects with HBeAg-positive and HBeAg-negative chronic HBV. More LdT subjects developed new-onset CK elevations compared to LAM subjects (Grade 1-4: 72% LdT, 42% LAM) after several months on study drug. After reviewing narratives for serious adverse events and study drug discontinuations/interruptions, there were a total of 16 LdT and 1 LAM subject with CK elevations or muscle symptoms; 6 of whom had a convincing picture of myopathy with muscle weakness, 2 of whom had biopsy confirmation, and 1 of whom was on a concomitant drug associated with myopathy. All presented after more than 300 days on study drug. History, degree and timing of CK elevations, and demographic characteristics were variable. No reports of LdT-associated CK elevations, myopathy or muscle weakness have been reported to our Adverse Event Reporting System since approval. No evidence of mitochondrial toxicity was found in review of preclinical studies. Conclusions: We found an emerging pattern of a cumulative toxicity resulting in myopathy with chronic LdT that may or may not be associated with mitochondrial toxicity. The imperfect relationship between the timing and severity of CK elevations and myopathy, coupled with the variable demography and symptom presentation in these clinical trial settings suggest that further characterization of this toxicity, its mechanism and its predisposing factors is warranted. These toxicities may occur more frequently in the general population with longer-term use of LdT.

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941 MUTATIONS IN IMMUNODOMINANT EPITOPES OF HEPATITIS B VIRUS (HBV) CORE REGION: ENHANCEMENT OF VIRAL EVOLUTION BY THE EFFECT OF INTERFERON THERAPY

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Background and aims In HBV infection, the host immune system attacks core peptides as the main target. Amino acid (aa) substitutions in immunodominant epitopes of the HbcAg sequence may allow HBV to escape immune surveillance, thereby maintaining infection. The aim of this study was to analyze aa substitution rates in HbcAg immunodominant epitopes in a group of chronic hepatitis B (CHB) patients, throughout IFNa therapy and during a period without this therapy. Patients and methods A cohort of 44 CHB patients (19 HBeAg+) (BASELINE group) was studied retrospectively, all cases with a baseline serum sample. Sixteen (1 HBeAg+) of these patients who did not respond to 24 weeks of IFNa therapy (IFN group) were longitudinally studied. Eleven of the 16 patients (1 HBeAg+) and 10 other CHB cases (5 HBeAg+) (untreated group) were longitudinally studied in the absence of antiviral treatment. In the IFN and untreated groups, serum samples were analyzed during therapy and every six months over the non-treatment period. The complete sequence of HBV core region (precore and HbcAg) was determined by direct sequencing. The viral genotyope was established by comparison of obtained sequences with consensus sequences deduced from the alignment of 185 complete sequences of the eight HBV genotypes obtained from GenBank. Yearly rates of aa changes in the immunodominant HbcAg epitopes (B cell Hbc/Hbe1-aa 74 to 84, and Th cell-aa 50 and 69) were calculated in the IFN and untreated groups. In the BASELINE group this changes were recorded in relation to the consensus sequences. Results: Rates of aa changes in the immunodominant HbcAg epitopes are shown in table. Conclusions The higher rates of aa substitutions suggest enhancement of the evolutive pressure due to IFN therapy, which may allow HBV to escape from immunosurveillance, resulting in persistent infection. This does not seem to be related to viral genotype or precore variants. In baseline samples the highest variability in Th cell epitope (aa 50-69) could be related with the absence of the immunotolerogen effect of HBeAg. This study was supported by a grant from the Spanish Ministry of Health (FISS PI061512).

<table>
<thead>
<tr>
<th>GROUP</th>
<th>Th cell epitope (aa 50-69)</th>
<th>B cell (Hbc/Hbe1) epitope (aa 74-84)</th>
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<tr>
<td>BASELINE (N=44)</td>
<td>Genotype A (N=22)</td>
<td>1.15</td>
</tr>
<tr>
<td></td>
<td>Genotype D (N=22)</td>
<td>1.36</td>
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<tr>
<td></td>
<td>Precore variant WT (N=20)</td>
<td>1.05 (e)</td>
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<td></td>
<td>Precore variant MT (N=24)</td>
<td>1.71 (f)</td>
</tr>
<tr>
<td>IFN (N=16)</td>
<td>4.5 (a)</td>
<td>3.34 (c)</td>
</tr>
<tr>
<td>Non-treated (N=21)</td>
<td>0.66 (b)</td>
<td>0.86 (d)</td>
</tr>
</tbody>
</table>

Statistical results: a vs. b P<0.001, c vs. d P<0.001, e vs. f P=0.087

Disclosures: The following people have nothing to disclose: Francisco Rodriguez-Frias, Rosendo Jardi, Maria Buti, Melanie Schaper, David Taberner, Rafael Esteban
A HIGH PREVALENCE OF SIGNIFICANT LIVER DISEASE IN ASYMPTOMATIC HEPATITIS B PATIENTS WITH HIGH VIRAL LOAD; CANDIDATES FOR ANTIVIRAL THERAPY

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Background/Aim: Current treatment guidelines recommend that antiviral therapy should be considered in chronic hepatitis B (CHB) patients with high viral load if a biopsy shows significant liver disease despite ALT levels two times or less than the upper limit of normal (ULN). We evaluated the histological findings in CHB patients with high viral load and persistently normal or slightly elevated serum ALT levels. Methods: Between January 2003 and June 2006, all consecutive treatment-naive patients with CHB who underwent ultrasonography-guided percutaneous liver biopsy, expressed HBsAg for at least 6 months, and had detectable serum HBV DNA on a direct hybridization assay, and normal or slightly elevated serum ALT levels (≤2×ULN) for 12 months were included in this study. One hundred and five patients were enrolled. Serum ALT levels were tested in at least three consecutive samples within a 12-month period, and all values were less than two times normal. Histological assessment was based on the METAVIR classification (significant histology was defined as ≥ grade A2 or ≥ stage F2). Results: All 105 patients had HBV DNA titers above 105 copies/mL. The mean (± SD) ALT level at biopsy was 42 ± 19 (range, 8-75) and the mean age was 37 ± 14 years (range, 18-68). 36 patients had persistently normal ALT levels. Significant fibrosis was observed in 63 patients (60.0%) and significant histology was found in 69 patients (65.7%). On multivariate analysis, the serum ALT levels and age at study entry were independent meaningful factors associated with significant histology. Compared to the lowest ALT levels (<0.5×ULN), 0.5-1×ULN, 1.5-2×ULN and 1.5-2×ULN had odds ratios (95% CI) for significant histology of 1.1 (0.2-5.1), 3.9 (0.7-21.6) and 8.0 (1.1-60.5), respectively. Compared to patients younger than 20 years, the odds ratios for patients in their 20s, 30s, 40s, and over 50 years old were 7.1 (1.4-36.9), 14.2 (2.1-94.2), 24.4 (4.2-141.3), and 38.8 (4.0-309.8), respectively. Conclusion: A large proportion of CHB patients with high viral load and ALT two times or less than the ULN had significant liver disease on liver biopsy. Our findings warrant a more accurate evaluation in a practical setting and, if confirmed, would have a great impact on future treatment approaches for patients awaiting potential antiviral therapy.

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ONCOLOGISTS AND HEPATITIS B: RESULTS OF A QUESTIONNAIRE SURVEY TO DETERMINE THEIR CURRENT LEVEL OF AWARENESS AND CLINICAL PRACTICE OF ANTIVIRAL PROPHYLAXIS TO PREVENT REACTIVATION

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The risk of chronic hepatitis B virus (CHBV) reactivation under the influence of chemotherapy or other immunosuppression is being increasingly recognized. However, many oncologists either have not observed this complication, and/or are not aware of current recommendations for Hep B prophylaxis.

METHODS: In an effort to determine the degree of awareness of the potential reactivation risk and to understand the current practice of screening and prevention among oncologists, we prepared a questionnaire that was administered to 131 Hematology-Oncology physicians in the Washington, DC area during March and April 2007. Questions related to their awareness of the risk of hepatitis B reactivation, screening patients for HBV, awareness of preventative treatment for HBV and how they would administer prophylaxis and follow their patients.

RESULTS: Responses to 10 questions are as follows: 1) Are you aware that reactivation of HBV can occur: Yes 78%; No 22%. 2) Are you aware that prophylactic antiviral therapy is available: Yes 56%; No 44%. 3) Have you ever seen reactivation of HBV in your practice: Yes 30%; No 70%. 4) Do you screen for HBV prior to chemotherapy: Yes 38% (86% use selective criteria vs 14% use universal screening); No 62%. 5) Which pts would you prophylax: chronic carrier 46%; active HBV 76%; resolved HBV infection 52%. 6) Which antiviral would you give: lamivudine 46%; adefovir 14%; not sure 48%. 7) How often would you monitor: q2wk 4%; q4wk 18%; q6wk 14%; q8wk 16%; q12wk 12%; not sure 36%. 8) How long would you continue prophylaxis after completing chemotherapy: 1 mo. 8%; 2 mo. 15%; 3 mo. 71%; 4 mo. 8%. 9) Would you monitor for reactivation even if you did not give prophylaxis: Yes 66%; No 6%; Not sure 28%. 10) Would you want a gastroenterologist or hepatologist to follow the pt with you: Yes 88%; No 12%.

DISCUSSION: While most oncologists are aware of the potential risk of HBV reactivation, only 30% have ever seen a reactivation and most are not screening pts for HBV prior to chemotherapy. Fewer than half of our respondents were aware that prophylactic antiviral agents are available, and even fewer were aware that prophylaxis has been shown to reduce HBV flares, and most were not sure what agent to select. A majority would, however, seek the recommendations of a gastroenterologist or hepatologist and most felt that prophylactic treatment should continue for at least 3 months after chemotherapy was completed.

CONCLUSIONS: Improving awareness of HBV reactivation and antiviral prophylaxis in the Oncology community seems warranted.

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The following people have nothing to disclose: Arash Farhadi, James H. Lewis, Omar S. Khokhar, Lisa H. McGrail

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PRE-EMPTIVE LAMIVUDINE REDUCES THE RISK OF CHEMOTHERAPY-INDUCED HBV-RELATED MORBIDITY AND MORTALITY IN HBsAg-POSITIVE CANCER PATIENTS: META-ANALYSIS

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Background: Lamivudine (LAM) has been used for preventing HBV-reactivation in hepatitis B surface antigen (HBsAg)-positive cancer patients undergoing chemotherapy but its beneficial effects have not been assessed quantitatively. Purpose: To estimate the degree of beneficial effect of pre-emptive LAM in reducing chemotherapy-induced HBV-related morbidity and mortality in HBsAg-positive cancer patients. Methods: Databases searched (until February 2007) included Medline, Ovid, Toxnet, Scopus, and Web of Science. Studies with data suitable for risk estimation of cancer chemotherapy-induced HBV reactivation in HBsAg-positive patients receiving pre-emptive LAM vs. controls that met the pre-specified criteria on outcomes and sample size. 2 investigators independently performed the literature search and data extraction, and 2 different investigators independently reviewed whether the studies met pre-specified criteria. A two-tailed p-value <0.05 was considered statistically significant. Comprehensive Meta-Analysis Software was used for the analysis. Results: Twelve studies met predefined criteria (2 RCT, 6 prospective, and 4 retrospective cohort studies). Of 12 trials in the meta-analysis, 10 were conducted in East Asia, and one each in Turkey and Israel. All studies were in English except for one in Chinese. 214 patients received pre-emptive LAM, and 399 patients served as controls receiving either no (3 studies) or deferred LAM (9 studies). The log odds ratios demonstrating risk reduction with pre-emptive LAM vs. no pre-emptive LAM in HBsAg-positive individuals undergoing cancer chemotherapy was 0.06 (95% confidence interval [CI], 0.03-0.13) for HBV reactivation, 0.07 (95% CI, 0.04-0.14) for HBV-related hepatitis, 0.20 (95% CI, 0.06-0.64) for liver failure, and 0.21 (95% CI, 0.08-0.58) for mortality. Combining results from all the studies indicated that HBV reactivation was decreased from 35.6% to 2.8% (p<0.0001) with pre-emptive LAM therapy, while HBV-related hepatitis was decreased from 32.3% to 2.8% (p<0.0001), HBV-related liver failure from 5.7% to 0.5% (p = 0.0002) and HBV-related mortality from 7.0% to 0.5% (p < 0.0001). Based upon this analysis, 1 death can be prevented by treating 15 patients with pre-emptive LAM in this clinical setting. Limitations: Small studies; only two involved a randomized-controlled design. Discussion: This meta-analysis demonstrated that pre-emptive LAM reduces the risk of HBV-reactivation, HBV-related hepatitis, HBV-related acute liver failure, and HBV-related mortality by 16, 14, 5, and 5-fold, respectively. Conclusions: HBsAg-positive patients undergoing cancer-chemotherapy warrant pre-emptive therapy with LAM.

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The following people have nothing to disclose: Rohit Loomba, Ayana Rowley, Robert Wesley, T. Jake Liang, Jay H. Hoofnagle, Frank Pucino, Gyorgy Csako
Background/Aim: Recent studies have shown that, in contrast to lamivudine, adefovir dipivoxil (ADV) therapy is associated with delayed and infrequent selection of drug resistant hepatitis B virus reverse transcriptase (RT) mutations. To date, apart from rtN236T and rtA181V ADV-resistant mutations, the impact of other mutations in the HBV RT region on the susceptibility to ADV is unknown. The aim of this study is to genotypically and phenotypically analyse emerging mutations in the HBV RT during ADV treatment. Patients/Methods: 68 patients (44±14 years) with chronic HBV (CHB)-infection treated with ADV (18±13 months) demonstrating persistent viraemia (5.5±1.0 log10 IU/ml) and 1.4±0.6 HBV IE/ml were included in this analysis. The RT-region was amplified by PCR after extraction of viral DNA from patient sera. Mutational analysis of the RT-region was done by sequencing followed by genotyping. Selected mutations were introduced into HBV replication competent constructs using site-directed mutagenesis and analyzed for sensitivity to ADV (IC50) in HepG2 cell culture experiments. Results: The ADV resistant mutations rtA181V and/or rtN236T were detected in 11 patients. Sequence exchanges at positions rtL122(F), rtN124(H), rtP130(Q), rtD131(N) in the RT-fingers domain were detected in 60% of the patients. However, these changes could be also detected in treatment naive patients and represent polymorphisms. Additionally, the exchange rtN248H in the E-domain was frequent (26/72; 36%). Statistical analysis revealed that rtN248H positively correlated with ALT (r=0.65, p=0.0001), rtP130Q (r=0.5, p=0.0001) and rtN124H (r=0.42, p=0.0001), and negatively correlated with rtL180M (r=-0.45, p=0.001). The phenotypic cell culture analysis of rtN248H showed a moderate reduction of sensitivity to ADV (IC50 <6µM ADV) compared to wild type HBV (IC50 <3µM ADV) and rtN236T (IC50 >10µM ADV). Notably, the recently published mutation rtL233V could not be detected in any of our ADV treated patients. Conclusion: Apart from the previously described rtN236T and rtA181V ADV resistant mutants, our analysis shows no evidence for further distinct ADV resistant mutations. However, antiviral treatment of CHB patients with ADV can select further exchanges in the HBV polymerase. Exchanges in the RT-fingers domain, e.g. rtL122F, rtN124H, rtP130Q, and rtD131N, may represent polymorphisms and have little or no impact on antiviral susceptibility. Phenotypic analysis of exchanges in the RT-E-domain, rtN248H, showed only a slight reduction in sensitivity to ADV. Suspected ADV resistant mutations, e.g., rtQ215S and rtL333V, were extremely rare or not detectable in our patients.

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948 ANALYSIS OF DETERMINANTS FOR SUSTAINED VIROLOGIC RESPONSE TO LAMIVUDINE MONOTHERAPY IN PATIENTS WITH HBEAG POSITIVE CHRONIC HEPATITIS B: A MULTI-CENTER TRIAL

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Backgrounds/Aims: Several reports showed inconsistent results on the durability of virologic response after successful lamivudine monotherapy. Especially, the question remains whether virologic responses can be maintained over extended follow-up period. The aim of our study was to evaluate post-treatment relapse rates for 5 years after virologic responses and to elucidate the predictive factors for sustained virologic response in patients who experienced HBeAg loss to lamivudine monotherapy. Methods: 748 patients (Male: Female = 570:178) with HBeAg positive chronic hepatitis B were treated with lamivudine from 7 medical institutions in Korea between January 1999 and August 2004. The mean duration of lamivudine monotherapy was 34 months (range, 12-88). Among 178 patients, who discontinued lamivudine monotherapy after complete response (HBeAg loss, undetectable HBV DNA and ALT normalization), 138 patients (77.5%) maintained sustained virologic response. Both host and viral factors were compared between 138 patients with sustained HBeAg response and 40 patients whose response was not sustained. Results: The cumulative relapse rates at 1, 3, and 5 years were 15.9%, 26.4%, and 30.2%, respectively. The mean time to relapse after cessation of lamivudine was 12 months (range, 2-42). Most relapses were occurred within 2 years after discontinuation of lamivudine (35/40, 87.5%). In multivariate analysis, age ≤ 40 years, additional treatment duration over 12 months after HBeAg loss were independent factors for sustained virologic response. In conclusion, only 22.5% of patients who discontinued lamivudine after HBeAg loss experienced a relapse for 5 years follow-up. Age and additional treatment are major predictive factors for sustained HBeAg loss. Key words: chronic hepatitis B, lamivudine, treatment

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949 MULTIDRUG RESISTANCE AND CROSS-RESISTANCE PATHWAYS IN HBV AS A CONSEQUENCE OF TREATMENT FAILURE

Lilly Yuen1,2, Angeline Bartholomeusz1, Anna Ayres1, Margaret Littlejohn1, Stephen Locarnini1,2; 1Victorian Infectious Diseases Reference Lab, North Melbourne, VIC, Australia; 2Evivar Medical, East Melbourne, VIC, Australia

Background/Aims: Antiviral resistance is now the single most important factor in treatment failure using nucleos(t)ide analogues (NA). Primary drug resistance mutations refer to amino acid change(s) that result in reduced susceptibility to an antiviral agent. Secondary compensatory mutations restore replication defects associated with primary drug resistance, and may be associated with low level reduced susceptibility. Several evolutionary pathways of drug resistant HBV have been observed in patients treated with NAs. It is possible that the drug resistance mutations selected with one agent may affect the efficacy of other NAs. The aim of this study was to elucidate mutation pathways associated with use of NAs and to determine the potential cross-resistance profiles selected under a particular NA. Methods: The HBV reverse transcriptase (rt) gene was amplified by PCR and sequenced from patients pre- and during-treatment. A software program (SeqHepB) was used to analyse the treatment-associated mutations (Yuen L et al. 2007. AVR;75:64). Associations between drug resistance and rt mutations found in the most recent samples from 159 patients pre- and 215 patients during failing monotherapy with either lamivudine (LMV) or adefovir (ADV) were evaluated using the Fisher’s Exact method and a pattern discovery program Magna Opus (Rule Quest Research, Australia). Results and Discussion: Therapy with LMV or Telbivudine (LdT) or entecavir (ETV) resulted in the selection of rtM204I/V. Statistical analysis identified several secondary compensatory mutations (rtL80I/V, rtV173L, rtL180M, rtT184G and rtS202I) during LMV monotherapy that can also affect response to ETV or LdT. Therapy with ADV resulted in the selection of rtN236T and/or rtA181T/V selection as well as the maintenance of rtL180M and rtM204V in a subset of patients with add-on ADV. The loss of rtL180M and/or rtM204I/V was also statistically significant among LMV resistant patients who subsequently switched to ADV monotherapy. The rtA181T/V mutation, which has been implicated with reduced sensitivity to LMV and ADV, could be either common with or separate to the “204 and 236 pathways”. Conclusion: Three major HBV evolutionary NA-resistance pathways (rtM204I/V, rtN236T and rtA181T/V) have been characterised. The rtM204V/I pathway is responsible for resistance to the L-nucleosides such as LMV, LdT and also ETV whilst the rtN236T pathway is responsible for adefovir resistance. Both are associated with clusters of secondary mutations that can affect subsequent treatment with NAs (rtT184G, rtS202I). The third pathway, rtA181T/V, is associated with resistance to LMV and ADV and is a potential multi-drug resistance pathway.

Disclosures: Lilly Yuen - Employee: Other

Stephen Locarnini - Grant/Research Support: Gilead; Consultant/Adviser: Gilead; Consultant/Adviser: Bristol-Myers Squibb; Speaker’s Bureau: Roche, Major Stockholder: Other

The following people have nothing to disclose: Angeline Bartholomeusz, Anna Ayres, Margaret Littlejohn
Different HBV variants carrying point mutations at level of the reverse transcriptase (rt) domain of the viral Pol gene had been detected in patients under treatment with nucleos(t)ide analogues. At present, few information is available about the occurrence of these drug-resistant mutants in untreated patients. Aim of this study was the genomic characterization of HBV isolates from individuals (1) naive for antiviral therapy or (2) who had developed lamivudine resistance. We analysed - by amplification, cloning and sequencing procedures - the entire rt-domain in viral isolates from 84 untreated (naive-group) and 49 lamivudine-resistant (Lam-group) consecutive HBsAg carriers with chronic liver disease. All the cases had serum HBV DNA levels > 20,000 UI/mL. Thirty-two of the 84 naive-group cases (38.1%) carried single or multiple mutations potentially predisposing to antiviral resistance. In particular, we found the following mutations: rtV173M in 2 cases (2.4%), rtA181D in 1 case (1.2%), rtV214A/E in 3 cases (3.6%), rtQ215S/P/H in 12 cases (14.3%), rtL217R in 8 cases (9.5%), rtS219S in 4 cases (4.8%), rtF221Y/L in 15 cases (17.8%), rtL233V in 4 cases (4.8%), rtP237T in 3 cases (3.6%), rtN238T/H/D in 4 cases (4.8%). In the Lam-group – besides the mutations typically inducing Lamivudine resistance – 28/49 (57.1%) carried single or multiple mutations conferring resistance to other antivirals. In particular, 4 cases (8.2%) had either the rtT184S or the rtM250V/L mutations favouring resistance to Entecavir, whereas various combined mutations predisposing to Adefovir resistance were detected in 26 cases: rtV84M in 1 case (2%), rtA181T/S in 2 cases (4.1%), rtA181T/S in 2 cases (4.1%), rtQ215S/E/P in 12 cases (24.5%), rtL217R in 3 cases (6.1%), rtS219A in 3 cases (6.1%), rtF221Y in 6 cases (12.2%), rtL233V in 1 case (2%), rtP237T/H in 2 cases (4.1%), rtN238T/H/D in 12 cases (24.5%). In conclusion, our study shows that HBV mutants predisposing to drug resistance may be present as major infecting population in untreated patients, and that variants emerging during Lamivudine treatment may also carry mutations potentially inducing resistance to either Adefovir or Entecavir. Thus, the genetic characterization of HBVs infecting patients undergoing treatment with nucleos(t)ide analogues may be a helpful tool for tailoring the most proper therapeutic strategy in each individual case.

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951 ENTECAVIR: A RESCUE THERAPY FOR CHRONIC HEPATITIS B PATIENTS WITH A LIMITED VIROLOGICAL RESPONSE TO ADEFOVIR?

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Background The frequency of detection of drug resistant HBV mutants correlates with the rapidity of initial decline of viral load. In treatment-naive patients with HBeAg-positive hepatitis B entecavir (ETV) achieves approximately 6log_{10} HBV DNA decline and PCR-negativity in 45% of the patients after 24 weeks of treatment. In lamivudine (LAM)-refractory patients who are subsequently switched to ETV, it has shown to be less effective. Here we describe 12 patients with chronic HBV infection and a persistently high viral replication after one year of treatment with ADV, who were switched to ETV as rescue therapy.

Methods Limited ADV response was defined as presence of HBV DNA levels greater than 5log_{10} copies/mL after 48 weeks of treatment. Patients were directly switched from ADV to ETV at a daily dose of 1 mg. ALT and HBV DNA levels were assessed at baseline and after 24 weeks of treatment (detection limit 400 copies/ mL). All subjects were screened for resistance-associated mutations within the HBV polymerase gene using Inno Lipa HBV DR v2 and v3 (Innogenetics).

Results The baseline characteristics of the 12 patients with limited ADV response were: median age 44 [range 23-73]; m/f: 9/3; 11 HBeAg+; 3 cirrhosis; genotype A: 3; B: 3; C:1; D: 5. Six patients had a prior history of LAM-resistance. Median period of ADV administration was 79 weeks [range 49-135]. HBV DNA levels ranged between 5.2 to 10.1 log_{10} copies/mL (median 7.7 log_{10} copies/mL). At baseline three patients had ADV-resistance substitutions [N236T ± A181V/T], and in only one patient also LAM-resistance was detected [M204I]. After 24 weeks of treatment the median decrease of HBV DNA was 3.7 log_{10} copies/mL [range 1.9-5.9]. There was no difference in decline between patients with or without a prior history of LAM-resistance (3.7 vs. 3.7). Median HBV DNA level at 24 weeks was 3.8 log_{10} copies/mL. None of the patients reached HBV DNA levels below the detection limit. The proportion of patients with ≤ 3 log_{10}, 3.1-5.5 log_{10}, > 5 log_{10} copies/mL was 33%, 42%, and 25%, respectively. HBeAg-seroconversion occurred in one patient after 12 weeks of treatment. After 24 weeks of treatment two patients showed LAM-resistance (M204I), no ETV-resistance substitutions were detected (I169, T184, S202, M250).

Conclusion Entecavir treatment leads to a suboptimal viral decline in patients with a limited response to adefovir, even in the absence of ADV or LAM resistance. Our finding that none of these patients achieved HBV DNA negativity by 24 weeks necessitates close monitoring for potential development of ETV resistance in ADV nonresponders.

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952 COMBINATION ANTIVIRAL EFFECTS OF 2', 3'-DIDEOXY-3'-FLUOROGUANOSINE WITH NUCLEOS(T)IDE ANALOGS AGAINST HEPATITIS B VIRUS IN VITRO
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Background & Aims: 2', 3'-dideoxy-3'-fluoroguanosine (FLG) is a nucleoside analogue which acts as an inhibitor of viral DNA polymerase and a chain terminator of viral reverse transcription. FLG has been shown to be active against wild-type and drug-resistant HBV in in vitro models (Jacquard et al 2006). In this study, we investigated the in vitro antiviral efficacy of combinations of FLG with lamivudine and clevudine, acyclic phosphate nucleotide analogues (PMEA, adefovir and PMPA, tenofovir) and carbocyclic analogues (entecavir). Methods: Using the HepAD38 cell line that expresses high levels of wild-type HBV as the in vitro cell model, we assayed the antiviral activities and cytotoxities of each drug alone and in combination with FLG (Tibotec Pharmaceuticals Ltd). Real time PCR was used for the measurement of HBV DNA generated by HepAD38, and the cytotoxicity was determined by 3-[4, 5-dimethylthiazol-2-yl]-2, 5-diphenyltetrazolium bromide (MTT) assay. Each combination experiment included 24 samples, 13 of which were controls (five doses of each drug alone plus three untreated wells) and 11 of which were drug combinations. Of the five concentrations tested for each drug, the middle dose was equal to the IC50; two higher doses (corresponding to three and six times the IC50) and two lower doses (responding to 0.16 and 0.33 times the IC50) were also tested. The combination data were analyzed through the Bliss independence model which is defined by the equation E_{xy}=E_x+E_y-E_{x+y}-E_xE_y, where E_x and E_y are the additive effect of drugs x and y as predicted by their individual effects E_x and E_y. The MacSynergy II program, version 1.0 (M. N. Prichard, University of Alabama) was used to evaluate antiviral data according to the Bliss independence model. MacSynergy II uses a nonparametric three-dimensional approach to quantify areas where observed effects are significantly greater (synergy) or less (antagonism) than those predicted from single-drug control data. Results: Analysis using the Bliss independence model indicated that FLG exerted significant synergistic antiviral effects when combined with lamivudine (V=41.51 µM2) or tenofovir (V=95.07 µM2) and additive effects when combined with adefovir (V=25 µM2). However, minor antagonism effects were displayed when FLG combined with entecavir (V=32.12 µM2) or clevudine (V=60.25 µM2). There was no evidence of cytotoxicity with any of the drugs when used alone or in combination at the tested doses. Conclusion: FLG exerted different kinds of combination antiviral effects when it was used with other nucleos[t]ide analogs. Future clinical study on FLG-related combination therapy is warranted.

Disclosures:
The following people have nothing to disclose: Lei Lu, Chee-Kin Hui, Hai-ying Zhang, Yui-Hung Yueng, Kwok-fan Cheung, John M. Luk, George K. Lau

953 DEMONSTRATION OF AN ASSOCIATION BETWEEN DETECTION OF IGG ANTIBODY REACTIVITY TOWARDS THE C-TERMINAL REGION OF THE PRES1 PROTEIN OF HEPATITIS B VIRUS AND THE CAPACITY TO RESPOND TO INTERFERON THERAPY IN CHRONIC HEPATITIS B
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Background: The aim of this study was to determine the predictive value of the pre-treatment presence of circulating antibodies towards a synthetic peptide mimicking the amino acids 94-117 of the preS1 protein of hepatitis B virus (HBV) and the capacity to respond to interferon–alpha (IFN-alpha) therapy. Methods: The anti-preS1 (94-117) antibodies were measured by a peptide-based enzyme-linked immunosorbent assay (ELISA) and the response to IFN-alpha therapy was judged by the effect on the viral kinetics as measured by a quantitative polymerase chain reaction (PCR) based assay during the treatment and follow-up. Results: We found a significant (p<0.001) correlation between the pre-treatment presence of anti-preS1 (94-117) antibodies and a decrease in viral levels on follow-up after the end of IFN-alpha therapy. The combined response of HBV DNA suppression (p<0.001), HBsAg loss (p<0.0001) anti-HBe seroconversion (p<0.005) and ALT normalisation (p<0.01), was also highly associated with the pre-treatment presence of anti-preS1 (94-117) antibodies. Conclusions: The positive predictive value (PPV) of anti-preS1 (94-117) in determining a virological response was 83% and the negative predictive value (NPV) was 100%, indicating that in the absence of pre-treatment anti-preS1 reactivity virtually no patient has the capacity to respond to IFN-alpha therapy. Our findings may help to improve the efficacy of IFN-alpha therapy for chronic hepatitis B (CHB) by guiding the selection of patients to treatment and optimising the clinical management of the individual patient.

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954 INCREASING ADEFOVIR AND ENTECAVIR UTILIZATION DRIVE HEPATITIS B RESISTANCE PATTERNS IN A US NATIONAL REFERENCE LABORATORY DATABASE
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INTRODUCTION An increasing number of antivirals are available for treatment of chronic hepatitis B virus (HBV) infection. Increasing use of antivirals can affect the frequency of resistance mutations in the treated population. We surveyed a large clinical database of HBV polymerase gene sequences to assess changes in the prevalence of resistance-associated mutations for lamivudine, adefovir, and entecavir over a 5-year period. METHODS HBV pol sequences for 10,800 clinical samples obtained between June 2002 and May 2007 were compiled in a database. Genotypes were determined by phylogenetic analysis of a 348-nt pol fragment. Antiviral drug prescription utilization data were obtained from NDChEalth (http://www.ndchealth.com). RESULTS The HBV sequences submitted for resistance analysis comprised genotypes C (37%), B
Background and aim: We recently described a cross-resistance pattern to adefovir (ADV) in a patient with lamivudine (3TC) resistance (EASL 2005). We aimed to further analyse the drug susceptibility of a novel mutation pattern to adefovir (ADV) in a patient with lamivudine (3TC) and entecavir-refractory patients.

Continued surveillance of emerging antiviral resistance in the clinical population is warranted.

The following people have nothing to disclose: Ersin Karatayli, Selim Karayalcin, Hayri Karaaslan, Handan Kayhan, Ahmet R. Turkylilma, Fikret Sahin, Cihan Yurdaydin, A.Mithat Bozdayi

955 DRUG SUSCEPTIBILITY TESTING OF A NOVEL MUTATION PATTERN (A181S+M204I) CONFERRING RESISTANCE TO LAMIVUDINE AND ADEFOVIR DIPIVOXIL

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Background and aim: We recently described a cross-resistance pattern to adefovir (ADV) in a patient with lamivudine (3TC) resistance (EASL 2005). We aimed to further analyse the drug susceptibility of this novel mutation pattern (A181S+M204I) against 3TC, ADV, tenofovir (PMPA), clevudine (L-FMAU), and emtricitabine (FTC). Methods: Successful suppression of HBV replication by sequential therapy of 9 MU/TIW interferon (IFN) and 3TC was followed by the genotypic resistance detected at the 19th month of treatment. ADV was added to 3TC therapy on the 44th month of antiviral treatment. However, neither ALT normalization nor a stable decrease in the HBV viral load was observed although ADV was used for more than 40 months. HBV pol region was amplified from 8 serum samples obtained before and after ADV treatment. Out of 8, complete genome was amplified in 3 serum samples and was cloned into a TA vector. DNA sequencing was performed for 8 PCR products of HBV pol gene and for 9-10 clones from 3 cloned constructs. Full genome of A181S+M204I variant was also cloned into an expression vector and its in vitro susceptibility to 3TC, ADV, PMPA, L-FMAU and FTC was determined in transiently transfected Huh7 cells. Results: Of the 29 clones sequenced, A181S+M204I pattern was detected in 14. The novel A181S mutation in the reverse transcriptase gene was associated with W172C mutation in the overlapping surface antigen gene. The results of the in vitro drug susceptibility assay revealed that this mutation pattern displays more than 1000 fold resistance for 3TC and FTC, and 28.2 fold resistance to ADV. This novel mutation pattern was relatively sensitive to the effect of PMPA and L-FMAU, displaying 5.9 and 5.6 fold resistance, respectively. Conclusion: In conclusion, A181S+M204I mutation pattern is associated with reduction in the susceptibility of all antiviral agents tested. While resistance is very high to 3TC and FTC, it is moderate to ADV and relatively mild to L-FMAU and PMPA. This study underlines the potential clinical aid of drug susceptibility assays in the area of multi-resistant viral variants.

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956 ADEFOVIR DIPIVOXIL PLUS LAMIVUDINE COMBINATION TREATMENT IS SUPERIOR TO ADEFOVIR DIPIVOXIL MONOTHERAPY IN LAMIVUDINE-RESISTANT HEPATITIS B E ANTIGEN-NEGATIVE CHRONIC HEPATITIS B PATIENTS

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The aim of this study was to evaluate the long-term efficacy of adefovir dipivoxil (ADV) in patients with hepatitis B e antigen (HBeAg)-negative chronic hepatitis B (CHB) who have developed resistance to lamivudine (LAM) treatment. In addition, we aimed to assess whether LAM should be discontinued in these patients. Sixty patients with lamivudine-resistant HBeAg-negative CHB were randomized in a 3:1 ratio to receive either ADV + LAM combination treatment (Group A; n = 45) or ADV monotherapy (Group B; n = 15). Baseline characteristics did not differ between groups. After a median follow-up time of 43 months (range, 10 to 63 months), hepatitis B virus (HBV) DNA became undetectable (< 400 copies/ml; virological response) in 37/45 patients in Group A (82.2%) and in 11/15 patients in Group B (73.3%) (p = 0.079). During follow-up, transaminases’ levels normalized in 40/44 patients in Group A (90.9%) and in 8/14 patients in Group B (57.1%) (p = 0.012). ADV-resistant mutations were identified in 2/45 patients in Group A (4.4%) and in 5/15 patients in Group B (33.3%) (p = 0.011). The 2 patients with ADV-resistance (group A) were suboptimal responders and in one of two (50%) the duplication of ADV dose to 20 mg per day resulted in disappearance of HBV DNA (< 6 IU/ml) in six months later. Virological breakthrough ( reappearance of HBV DNA after its initial disappearance) did not develop in any of the 37 Group A patients with virological response. In contrast, 3/11 Group B patients with virological response (27.3%) developed virological breakthrough (p=0.011). Treatment was well-tolerated and no adverse effects
occurred. In conclusion, ADV + LAM combination treatment is superior to ADV monotherapy in patients with lamivudine-resistant HBeAg-negative CHB, in terms of both efficacy and development of resistance to ADV. Increase of ADV dose to 20mg/day may be effective in suboptimal response patients or even ADV-resistant patients.

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Odds ratios of HBeAg loss for potential prognostic factors; multivariate analysis

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The following people have nothing to disclose: Jung Il Lee, Jin Wook Lee, Young Soo Kim, Don Haeng Lee, Seok Jeong, Hyun Joo Park, Eun Ah Choi, Yong Han Paik, Kwan Sik Lee
959

DOES THE GENOTOXIC EFFECT OF LAMIVUDINE TREATMENT IN CHRONIC HEPATITIS B TO THE HOST DNA?

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Chronic viral hepatitis B is the main cause of chronic liver disease, cirrhosis and hepatocellular carcinoma in the world. Lamivudine, a nucleoside analog, is an inhibitor of viral DNA replication. Patients are treated with lamivudine show normalization of serum alanine aminotransferase (ALT) level, and histological improvement. However, discontinuation of therapy often leads to reactivation of HBV so long-term therapy is necessary for many patients with chronic HBV infection. The aim of this study is to detect the genotoxic effects of the long-term lamivudine treatment to the host DNA. Material and Method: Fourteen chronic hepatitis B (CHB) patients (4 women, 10 men, mean age: 44 ranges: 29-64 yrs) who were treated with lamivudine were included in this study. Blood samples were collected before the treatment and 6 months and at least sixteen months (mean 27 months) of the treatment. Peripheral blood lymphocytes of these patients were cultured to make cytogenetic evaluation by observing chromosome breakage and cytologic evaluation by micronucleus (MN) test. For each individual 100 metaphase chromosome spreads were analysed. 190-1091 binucleated cells were observed and for each individual MN scores between each period of the treatment (p>0.05). Based on this data, we concluded that lamivudine treatment in patients with chronic HBV may be chromosomal instability and this instability may increase during long-term therapy especially after one year of the treatment.

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TOVFIVOR SHOWS LIMITED EFFICACY IN TREATMENT OF HBV INFECTIONS RESISTANT AGAINST ADEFOVIR

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Background: Genotypic HBV resistance against adefovir dipivoxil (ADV) develops rarely during the first two years of treatment. However with increasing treatment duration ADV resistant HBV mutants are selected in up to 28% of the patients after 5 years which is often associated with viral rebound. In vitro, these mutations cause a 3-14-fold decrease of ADV efficacy. Tenovifor (TDF), a nucleotide analogue approved for HIV therapy, is closely related to ADV, shows equipotent antiviral activity against HBV on molar basis and is cross resistant to ADV in vitro. However, due to the 25-fold higher dosage of TDF (300 mg/d) as compared to ADV (10 mg/d), TDF might still be effective in ADV resistant HBV infections. Methods: The efficacy of TDF monotherapy was retrospectively evaluated in 10 HBV monoinfected patients (m/f 9/1, mean age 47±11 [27-67] years, 6 HBeAg positive) with initial lamivudine resistance who had developed genotypic ADV resistance (rtN236T and/or rtA181T/V) after consecutive 1-45 [mean 24±9] months of ADV monotherapy. HBV DNA and ALT values were measured every 3 months (HBV Monitor, Roche, detection limit 400 copies/mL). In six patients, during TDF treatment the HBV polymerase gene was sequenced from codon r88 to r282 after PCR products were cloned in E. coli (TOPO-TA cloning system, Invitrogen; minimum of 10 clones sequenced each). Results: At the begin of TDF treatment (mean duration 16±3 [12-21] months), the mean HBV DNA was 7.5±1.3 [4.6-9.4] l copies/mL and nine patients had elevated ALT levels (mean 151±118 [34-358] U/mL). The mutations associated with ADV resistance N236T, A181T and combination of both were detected in 3, 2 and 5 patients. During TDF treatment, HBV DNA decreased by a mean of 3.6±1.2 [2-5] and 4.3±1.2 [2-6.2] log copies/mL at week 24 and week 48, respectively. HBV DNA was still detectable in 9, 8 and 6 patients while ALT levels remained elevated in 8, 8 and 6 patients at week 24, week 48 and at the end of observation. Subspecies analysis in six patients showed that ADV resistance mutations remained detectable throughout the whole observation period in increasing ratio as compared to HBV wild type. Combination therapy with TDF and lamivudine was started in two patients at month 12 and lead to undetectable HBV DNA and normal ALT levels after two months. Conclusion: Although TDF shows significant antiviral efficacy in patients with genotypic ADV resistance, HBV DNA can be completely suppressed only in a minority of the patients and the selection of ADV resistant mutations is not prevented. Therefore, patients with genotypic ADV resistance should receive combination treatment with TDF or ADV plus lamivudine or entecavir.

Disclosures: The following people have nothing to disclose: Florian van Bömmel, Jörg Trojan, Heinz-Hubert Feucht, Bernd Möller, Dietrich Hüppe, Bertram Wiedenmann, Thomas Berg

961

THE EFFECT OF LAMIVUDINE AND ADEFOVIR DIPIVOXIL ON PREVENTING HEPATOCELLULAR CARCINOMA IN HEPATITIS B VIRUS-RELATED LIVER CIRRHOSIS

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Background/Aims: We evaluated the effect of lamivudine and adefovir dipivoxil on preventing HCC in hepatitis B virus (HBV)-related liver cirrhosis. Patients and Methods: We reviewed the medical records of the patients who were diagnosed with HBV-related liver disease at Yeungnam University Hospital from Jan 1983 to Dec 2005. The number of patients treated with lamivudine over 12 months was 570 and the number of patients treated with adefovir dipivoxil after lamivudine resistance was
284 from Mar 1997 to Dec 2005. The number of patients who could not be treated with oral antiviral agent from 1983 to 1999, followed over 12 months, was 1592. In our results using Cox regression model, 4 factors were related with HCC; gender (male, OR=1.81, p<0.001), age (≥40 years, OR=4.95, p<0.001), platelet count (<150×10^3/mm^3, OR=3.48, p<0.001), and ascites (yes, OR=1.69, p=0.009). Using SPSS program, 111 patients of the oral antiviral agent-treated group and the untreated group were randomly selected, respectively, matching an age variable, a gender variable, liver cirrhosis with Child-Pugh class A and positive HBe antigen. Results: The mean follow-up period was 4.4 years in the oral antiviral agent-treated group and 5.4 years in the control group. In oral antiviral agent-treated group, HCC occurred in 5 patients (4.5%) with an annual incidence rate of 1.02% patients/year, whereas in the control group, HCC occurred in 36 patients (32.4%) with an annual incidence rate of 6.0% patients/year. The cumulative incidence of HCC in the oral antiviral agent-treated group was lower than that in the control group (Log-rank, p=0.003). Conclusion: Oral antiviral agents, such as lamivudine and adefovir dipivoxil may reduce the incidence of HCC in patients with HBV-related liver cirrhosis.

Cumulative Incidence of HCC Between the Treated Group and the Control Group in HBV-Related Child A Liver Cirrhosis with Positive HBe Antigen

![Graph showing cumulative incidence of HCC](image-url)

**Cumulative Incidence of HCC Between the Treated Group and the Control Group in HBV-Related Child A Liver Cirrhosis with Positive HBe Antigen**

Disclosures:
The following people have nothing to disclose: Jong Ryul Eun, Heon Ju Lee, See Hyung Lee, Tae Nyeun Kim, Byung Ik Jang, Jae Won Choi, Youn Sun Park, Kyung Ok Kim, Kyu Hyung Lee, Hee Jung Moon, Sang Hoon Lee

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**THE INDICATION AND LIMITATION OF INTERFERON THERAPY AS THE FIRST LINE THERAPY OF CHRONIC HEPATITIS B: FROM THE HISTOLOGICAL ANALYSIS OF 800 CHRONIC HEPATITIS B PATIENTS**

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**[AIM]** Current therapy of chronic hepatitis B does not eradicate HBV and has limited long-term efficacy. Thus, careful consideration of the patient’s age, severity of liver disease, likelihood of response, and potential adverse events is needed before treatment is initiated. The role of interferon in the first line therapy is not clearly defined. The degree of liver histology might be related to interferon’s efficacy and limitation. To prove this, we investigated the relationship between the staging score and the interferon response and durability in patients with chronic hepatitis B. [Materials and Methods] A total of 800 Japanese genotyped C treatment naive chronic hepatitis B were studied. All of them had liver biopsy before the treatment. Seven hundred patients were HBeAg positive and the remaining 100 were HBeAg negative. All patients had elevated ALT levels (>2x UNL) and increased HBVDNA (>10^6I) levels. Among these 800 patients, 269 patients with HBeAg positive chronic hepatitis B were treated with interferon alfa therapy (Sumiferon, 6 MU/d, TIW, 4 mo). The patients were stratified by age into 5 year groups beginning with 15-20 y/o until 70-75 y/o. All liver tissue specimens were obtained by needle biopsy. The grade and stage were based on international standard criteria and METAVIR. The relapse after seroconversion was defined as that HBeAg became positive again or that HBeAg remained negative but HBVDNA and ALT elevated for one year after the therapy. [RESULT] 1) The staging scores for each group were generally increased with age from 1.51 (15-20) to 2.75 (61-65), and the degree of fibrosis increased significantly especially after 30 y/o (mean, 2.2) every year at the rate of 0.04-0.06 until 45 y/o (mean, 2.54). 2) Among 269 HBeAg positive patients treated with interferon, 89 (30%) seroconverted to anti-HBe, and among them 74 (83%) had staging score either 1 or 2, and durability was 80-90%. While those with stage 3 or 4 had 50% durability. In nonresponders, the staging score was significantly higher than seroconverters (p=0.0001). [Conclusions] 1) The current study showed that progression of fibrosis appeared to increase significantly especially after 30 year old until 45 year old at the rate of 0.04 to 0.06 every year. 2) Interferon appeared to be effective in patients who had less than staging score 2 with 80-90% durability, while in patients with staging score 3 or 4, interferon did not seem to be similarly effective and the durability appeared to be poor. Thus the degree of fibrosis may be one of the important factors to indicate and limit interferon efficacy for the treatment of chronic hepatitis B.

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EMERGENCE OF HEPATITIS B VIRUS GENE MUTATION RELATED TO ENTECAVIR-RESISTANCE IN CHRONIC HEPATITIS B PATIENTS PARTICIPATED IN THE PHASE 2 CLINICAL STUDIES OF ENTECAVIR IN JAPAN

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[Background/Objective] The objective of this study was to elucidate the emergence rate of HBV DNA gene mutation related to entecavir-resistance (ETVr) in chronic hepatitis B patients participated in the phase 2 entecavir (ETV) clinical studies performed in Japan. [Method] The subjects of this study were 287 patients in total, consisted of (1) 137 nucleoside-naive patients in the ETV-047 study administered with 0.01, 0.1, 0.5 mg/day of ETV or 100mg/day of lamivudine (LVD) for 24 weeks, (2) 66 nucleoside-naive patients in the ETV-053 study administered with 0.1 or 0.5mg/day of ETV for 52 weeks, and (3) 84 LVD-refractory patients in the ETV-052 study administered with 0.5 or 1.0 mg/day of ETV for 52 weeks. After the studies, a total of 282 patients were enrolled in ETV-060 roll over study, and subsequently administered with ETV for another 2 years. Single-nucleotide-polymorphism (SNP)-PCR analysis for HBV polymerase was performed to detect the ETVr mutation for the patients who experienced viral rebound, defined as elevation in serum HBV DNA by greater than 1 log copy/mL. [Results] (Result-1: Nucleoside-naive patients). Five out of 169 nucleoside-naive patients with ETV administration (comprising 103 from ETV-047 and 66 from ETV-053), experienced viral rebound before week 148. ETV-related mutation (rtS202S/G) along with LVD-related mutation (rtL180M and rtM204V) was detected in two patients (1.2%) between week 100 and 148. The two patients who developed ETV never experienced a disappearance of serum HBV DNA. The 34 patients in ETV-047 study, who were administered with LVD for 24 weeks followed by administration with ETV 0.5mg, did not develop LVD- or ETV-related mutation throughout the study duration. [Result-2: LVD-refractory patients]. Viral rebound developed in 21 patients out of 84 in ETV-052 study, before week 148, and LVD-related mutation (rtM204I/V and/or rtL180M) along with ETV-related mutation (rtS202 and/or rtT184), was detected in 19 patients (22.6%). [Conclusions] The cumulative number of patients who developed ETVr-related mutation were 0/0/2 at years 1/2/3 in nucleoside-naive group (total n=169 at baseline), and 0/10/19 at years 1/2/3 in LVD-refractory group (total n=84 at baseline), respectively. Development of ETV-related gene mutation was rare in nucleoside-naive patients.

MUTATIONS OF THE HBV-POLYMERASE GENE ASSOCIATED WITH ADV DRUG RESISTANCE IN PATIENTS UNDERGOING A FIRST ADV THERAPY

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Background: HBV resistance mutations have been identified for all oral antiviral agents used to treat chronic hepatitis B. Whereas the rtA181V and rtN236T mutations are associated with long-term adefovir dipivoxil (ADV) treatment, the newly discovered mutation i233V is associated with primary resistance to ADV. Aim: To assess the association between existing HBV polymerase mutations and failure of antiviral therapy in patients given ADV for the first time. Patients and methods: 86 patients began ADV therapy during 2001 and 2005 for more than 12 months, of these 6 (7%) showed no significant viral suppression (less than 1 log IU/ml) and their ALT was elevated when therapy was stopped (NR group). The HBV rt amino-acid sequences from these 6 patients were compared to those of 5 patients who had virus sensitive to ADV (R group). The HBV rt gene was sequenced with in-house protocols (ABI 3100) using sera collected at baseline and at the end of treatment. Results: Phylogenetic analysis of the rt gene HBV gave genotypes A [6/11], D [4/11] and E [1/11]. The rtA181TV and rtN236T mutations were never detected at the beginning of ADV therapy. rtM204I/V and/or rtL180M were detected in 5/11 patients (45%) because most of them had been treated with Lamivudine (LVM). Two of them had lost these LVM-resistance-associated mutations at the end of ADV therapy. Only one patient in the NR group developed rtA181TV and rtN236T mutations (17%) during the ADV treatment. The rtL233V mutation, recently associated with primary resistance to ADV was not detected in any patient and did not exist in patients whose virus was resistant to ADV. But, rtL217R was detected at baseline and at the end of treatment in 3/6 of the NR patients (50%) and in 1/5 R group patients (20%). This mutation, which is more prevalent in HBV strains with genotype A, was found in 3 patients infected with genotype A. Conclusion: HBV strains that are naturally resistant to adefovir can occur, but are not frequent. The rtL233V mutation cannot explain the failure of ADV therapy in our patients, whereas rtL217R mutation seemed to be more frequent. Further studies are needed to clarify the influence of the rtL233V and the rtL217R mutations on ADV therapy.

Disclosures: The following people have nothing to disclose: Karine Sauné, Florence Abraovaneli, Guillaume Martin-Blondel, Jacques Izopet, Laurent Alric.
SUSTAINED HBEAG AND HBsAG LOSS AFTER LONG-TERM FOLLOW-UP OF HBEAG POSITIVE PATIENTS TREATED WITH PEGINTERFERON ALPHA-2B

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We evaluated the long-term sustainability of response and clinical outcome in patients treated with PEG-IFN-α 2b alone or in combination with lamivudine for 52 weeks. Methods: All 266 HBeAg positive chronic hepatitis B virus (HBV) infected patients from 41 centers enrolled in the randomized HBV99-01 study were offered participation in a long-term follow-up (LTFU) study. Initial response was defined as HBeAg negativity at the end of the initial study (26 weeks post-treatment; week 78). For the LTFU study, patients had one additional visit after the initial study (mean interval 3.0 ± 0.8 years). For analysis, retreatment was considered as non-response. Results: 172 patients (65%) from 28 centers (68%) were enrolled in the LTFU study. 91 patients (53%) received PEG-IFN-α 2b alone and 81 (47%) its combination with lamivudine. Response rates at different time points are shown in the table. At LTFU, HBeAg loss was durable in 52 of 64 initial responders (81%), of whom 67% had HBV DNA <10,000 copies/ml and 65% had normal ALT. 14 initial responders (21%) and 67 of 108 (62%) non-responders were retreated (p<0.001). HBsAg was negative in 19 initial responders (30%). Sustained HBeAg negativity was observed in 96%, 86%, 67% and 77% of initial responders with genotype A, B, C and D infection, respectively (A vs. C, p=0.04; A vs. D, p=0.07). Serum HBV DNA <10,000 copies/ml was observed in 81%, 29%, 44% and 35% of initial responders with genotype A-D, respectively (A vs. B or D, p<0.02). HBsAg negativity was observed in 58% of genotype A infected initial responders compared to 14%, 0% and 6% of those with genotype B, C or D (A vs. C or D, p<0.004). At LTFU, 3 non-responders had died and 1 developed hepatocellular carcinoma; decompensated liver disease was observed in 1 responder and none underwent liver transplantation. Conclusions: HBeAg response to PEG-IFN-α 2b ± lamivudine is durable in the majority of patients and is associated with an increase in HBeAg loss. This study further emphasizes the importance of HBV genotype in PEG-IFN therapy, with high rates of sustained virological response in genotype A infected patients. PEG-IFN should therefore particularly be considered as first line therapy in genotype A infected HBeAg positive patients.

<table>
<thead>
<tr>
<th>Week 32</th>
<th>Week 52</th>
<th>Week 78</th>
<th>LTFU</th>
</tr>
</thead>
<tbody>
<tr>
<td>PEG</td>
<td>PEG + LAM</td>
<td>PEG</td>
<td>PEG + LAM</td>
</tr>
<tr>
<td>HBeAg negative</td>
<td>21%</td>
<td>35%</td>
<td>34%</td>
</tr>
<tr>
<td>HBV DNA &lt;1,000</td>
<td>14%</td>
<td>69%</td>
<td>19%</td>
</tr>
<tr>
<td>HBV DNA &lt;400</td>
<td>9%</td>
<td>32%</td>
<td>9%</td>
</tr>
<tr>
<td>Normal ALT</td>
<td>25%</td>
<td>40%</td>
<td>36%</td>
</tr>
<tr>
<td>HBsAg negative</td>
<td>ND</td>
<td>7%</td>
<td>9%</td>
</tr>
</tbody>
</table>

*p<0.05 PEG-IFN-α 2b + lamivudine (PEG LAM) vs. PEG-IFN-α 2b (PEG); ND = not done

Disclosures:
The following people have nothing to disclose: Erik H. Buster, Hajo J. Flink, Yilmaz Cakaloglu, Christopher Simon, Jörg Trojan, Fahmi Tabak, Thomas M. So, Victor S. Feinman, Tomasz Mach, Ullus Akarca, Wanda C. Tieleman, Hanneke J. van Vuuren, Bettina E. Hansen, Harry L. Janssen.

PREDICTION OF LONG-TERM MAINTENANCE OF VIROLOGIC RESPONSE DURING LAMIVUDINE TREATMENT IN HBEAG NEGATIVE CHRONIC HEPATITIS B

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Background and Aims: It has recently been claimed that non-detectability of serum HBV-DNA by PCR after six months of treatment with telbivudine or lamivudine in chronic hepatitis B (CHB) predicts maintenance of the response after two years of treatment with a positive predictive value (PPV) for HBeAg-negative patients of > 80% The aim of the present study was to investigate in such CHB patients the relation between HBV-DNA suppression achieved during the early phases of lamivudine treatment with the long-term outcome of therapy after 4 and more years. Patients and Methods: The study included 156 patients with 56 of them (37%) in maintained virologic and biochemical responses for more than 4 years. Their virologic data was compared to those of patients who experienced a breakthrough 3 months to 5 years after starting treatment. HBV-DNA data assessed by sensitive real time PCR assays was available every 3 to 6 months and all samples negative or <1000 cp/ml were retested with the COBAS TaqMan Test (Roche Molecular Systems, CA, USA, sensitivity 50 cp/ml). Results: The positive predictive value of negative HBV-DNA after 6 months of treatment was 93% for maintenance of the response for two and 71.5% for maintenance of response for four or years more. Similar percentages were found for the results after 3 months of treatment. On the other hand, a positive result even >10,000 cp/ml at month 6 could not exclude the possibility that LAM therapy would be successful and was associated with a possibility of maintenance of response for 2 years of 36.5% and for 4 or more years of 8%, those percentages being 43.7 and 19.5% for the month three results. Overall, patients who maintained response had lower levels of HBV-DNA both at month 3 and 6 compared to patients who experienced a breakthrough. Conclusion: HBV-DNA non-detectable by sensitive PCR assays during the "early" phases of antiviral treatment of HBeAg(-) CHB are very useful in predicting maintenance of the response up to year 3 of Rx but their positive predictive value decreases to approximately 70% for response maintained longer than 4 years. Moreover their low negative predictive value cannot provide reliable information for deciding treatment discontinuation or modification.

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967 AMINO ACID VARIABILITY WITHIN HEPATITIS B SURFACE ANTIGEN AND THE OVERLAPPING REVERSE TRANSCRIPTASE REGION IN HBSAG NEGATIVE/HBCAB POSITIVE PATIENTS PRESENTING HBV REACTIVATION WHILE UNDERGOING CHEMOTHERAPY AND/OR STEM CELL TRANSPLANTATION FOR CANCER

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Background/Aim: Hepatitis B virus (HBV) reactivation has been described in HBsAg-/anti-Hbc+ patients (pts) undergoing immunosuppressive chemotherapy (CT). We previously reported HBV reactivation in 8.3% of pts (n=7) with HBV viremic profile indicating past infected who underwent CT and/or hematopoietic cell transplantation (HCT) for cancer. Baseline anti-HBs titer <100 IU/L was significantly associated with HBV reactivation in these pts. In the present study, we aimed to analyse the amino acid (aa) variability and mutations patterns within HBsAg and the overlapping HBV reverse transcriptase (rt) region of HBV strains from these pts to assess whether HBV reactivation might be associated with specific virological features. Patients/Methods: 7 male pts (mean age, 60 years), HBsAg/anti-Hbc+ (HBsAb+/−) prior to CT and/or HCT who presented HBV reactivation between 11/2003 and 12/2005. 4/7 pts received anti-C20. HBV HBsAg and rt genes were amplified/sequenced using in-house protocols. Newly-diagnosed HBsAg-positive pts (n=51) served as controls. Results: HBV genotype was D and C in 5 and 2 cases, respectively. Mean number of substitutions/100 aa was significantly higher vs controls within HBsAg major hydrophilic region (MHR) (8.8 vs 2.1; p<0.01), its “a” determinant (15.7 vs 2.5; p=0.003), and the HBsAg C-terminal region (7.5 vs 3.8; p=0.054). Substitutions D144AA/V and G145RA previously associated with altered S antigenicity were more frequent (from 2 pts each) than in controls (p=0.001 and p=0.004, respectively). Among genotype D sequences, substitutions at positions s116, s126, s134, and s144 within the MHR were significantly more frequent than in controls (p=0.05). No rt drug resistance mutation was detected at time of reactivation. However, within rt sub-domains B-CD that overlap HBsAg C-terminal region and where drug resistance mutations occur, the mean number of substitution/100 aa showed a tendency to be higher in the reactivation group (3.0 vs 1.3; p=0.081), and a significantly higher proportion of sequences harboured >1 substitutions (P=0.05). Mutations rtR/W153Q, known to restore HBV replication of lamivudine (LAM) resistant strains and corresponding to substitutions sG145R, were found in HBV from 1 pt. In 1 pt, LAM-resistance was detected 1 month after LAM introduction. Conclusions: Our data show an increased aa variability within HBsAg and HBV RT associated with HBV reactivation in AgHBs-/anti-Hbc-positive pts undergoing CT and/or HCT. Whether or not these virological patterns may play a role in the pathogenesis and frequency of HBV reactivation or might impact the response to antiviral treatments needs further investigations.

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The following people have nothing to disclose: Philippe Colson, Patrick Boretain, Diane Csoo, Aude Charbonnier, Anne-Marie Stoppa, Thérèse Auran, Erwan Bories, Anne Motte, Mireille Henry, Danielle Battia-Fridlund, Catherine Tamalet, Rene Gerolami

968 ESTIMATING THE IMPACT OF CHRONIC HEPATITIS B ON FUTURE LIVER-RELATED MORTALITY, MORTALITY AND COST IN SPAIN

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Background and aims: Chronic hepatitis B (CHB) is a common infection often producing progressive disease. Some studies suggest that HBV-related complications will increase in the future, impacting future disease costs. Our aim was to estimate future morbidity, mortality and costs associated with CHB infection in a cohort of patients in Spain. Methods: A Markov model was used to project HBV-related complications and costs over the next 20 years in a cohort of 132,028 patients representing the HBV-infected population in Spain. This cohort was stratified according to age (25-34, 35-44, 45-54, 55-64, 65-74 years), HBeAg status (13% HBeAg-positive and 87% HBeAg-negative), and the degree of liver disease. The probabilities of disease progression in the model were obtained from data published in the literature, and the cost of the disease and its complications were based on actual variable costs from the Spanish Health Care System. An annual 3.5% discount rate was applied to the costs. Results: A greater disease progression and morbidity will be observed in HBeAg-negative patients compared with HBeAg-positive patients, with a higher proportion of HBeAg-negative patients requiring liver transplant (9% vs. 5%) and a higher proportion of deaths (59% vs. 45%). The total cost of the HBV-infected cohort during the next 20 years will be US$5,007 million. The disease progression in HBeAg-negative patients requires an additional cost of 65% in relation to HBeAg-positive patients. The mean estimated cost in US$ per patient and the different stage of the disease distribution over the next 20 years are shown in the table below. Conclusions: In the future, HBV mortality, morbidity, and associated cost will increase. Treatment of the CHB-infected population potentially may control the infection, reduce progression, increase patient survival and reduce the need for liver transplantation.

<table>
<thead>
<tr>
<th>HBeAg-</th>
<th>HBeAg+</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cost per patient (%)</td>
<td>Patients (%)</td>
</tr>
<tr>
<td>CHB</td>
<td>$1,485 (5)</td>
<td>20,734 (45)</td>
</tr>
<tr>
<td>Cirrhosis</td>
<td>$636 (2)</td>
<td>1,457 (3)</td>
</tr>
<tr>
<td>Decompensated Cirrhosis</td>
<td>$341 (1)</td>
<td>323 (1)</td>
</tr>
<tr>
<td>Hepatocellular Carcinoma</td>
<td>$1,395 (5)</td>
<td>180 (1)</td>
</tr>
<tr>
<td>Liver Transplant</td>
<td>$22,430 (81)</td>
<td>2,416 (5)</td>
</tr>
<tr>
<td>Death</td>
<td>$1,236 (4)</td>
<td>20,012 (45)</td>
</tr>
<tr>
<td>Total</td>
<td>$27,523 (100)</td>
<td>44,613 (100)</td>
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</tbody>
</table>

Disclosures:
Magdalena Rueda - Employee: Gilead
The following people have nothing to disclose: Maria Buti, Max Brosa, Miguel A. Casado, Rafael Esteban
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LIVER BIOPSY: STILL ESSENTIAL IN THE MANAGEMENT OF CHRONIC HEPATITIS B

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BACKGROUND & AIMS: In the absence of an established role for non-invasive markers of fibrosis, liver biopsy remains the gold standard for the assessment and staging of disease in chronic hepatitis B virus (HBV). With growing numbers of chronic HBV patients attending hepatology clinics, the indications and timing of liver biopsy needs to be clearly defined. The aim of this study was to formally review all biopsies undertaken for a primary diagnosis of HBV in a tertiary referral centre and to assess how histology correlates with disease profile, in particular HBV DNA & ALT. Factors associated with more advanced disease or progression of disease on repeat biopsy were sought.

PATIENTS & METHODS: Between 1995 and 2007, 212 chronic HBV patients underwent liver biopsy. This comprised of 76 HBeAg+ patients (Group 1), 9 of whom underwent repeat biopsy and 136 anti-HBe+ patients (Group 2), 31 of whom underwent repeat biopsy. Two specialist liver histopathologists reviewed all biopsy material and determined Ishak fibrosis scores. A fibrosis score of 0-2 was designated mild disease, 3-4 moderate and 5-6 severe. Quantitative HBV DNA and ALT values [> or < twice upper limit of normal (2ULN)] at the time of biopsy were collected. RESULTS: Fibrosis score correlated with age at biopsy [p<0.01]. In Group 1, 43 patients (57%) [M:F 29:14] had mild disease on histology: median age at biopsy was 30 years [range 16-64]; 33 patients (43%) [M:F 27:6] had moderate/severe disease on biopsy with a median age at biopsy 37 years [range 16-73]; 4/14 (29%) patients with severe disease had HBV DNA >107 and normal ALT were, therefore, considered immuno-tolerant. In Group 2, 71 patients (52%) had mild disease on biopsy, [M:F 50:21] median age at biopsy 36 years [range 18-70]. The remaining 48% (65/136) had moderate/severe disease [M:F 50:15], median age at biopsy 47 years [range 23-73]; 27/65 (42%) with moderate/severe fibrosis had both HBV DNA>105 and ALT <2ULN. Repeat biopsy group Rate of fibrosis change in untreated patients undergoing serial biopsies was highly variable, ranging between improvements of fibrosis score of 0.44 points/year versus progression of one point per year (mean 0.22 points/year). Rate of progression did not correlate with eAg status, HBV DNA or ALT level. However, there was a trend towards faster progression in those with a BMI>25.

CONCLUSIONS: This study highlights the importance of liver biopsy in the diagnosis and management of chronic HBV. It emphasizes the inadequacy of quantitative HBV DNA and ALT values in reflecting underlying liver damage. We conclude that any decision not to offer antiviral therapy should be based on histological findings.

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NEUTRALIZING ANTIBODIES TO INTERFERON ALPHA IN CHRONIC HEPATITIS C PATIENTS NON-RESPONDING TO Pegylated INTERFERON PLUS RIBAVIRIN TREATED TO Peg-INTERFERON α-2A AND RIBAVIRIN (ANRS GAMMATI)

Philippe Halfon1, Sophie Pérusat1, Marc Bourlière4, Jean-Pierre Bronowicki5, P. Trimoulet2, Yves Benhamou6, V. Leroy7, Patrick Marcellin8, J. Foucher6, Geneviève Chène3, Patrice Couzigou2; 1Laboratoire Alphabio, Marseille, France; 2CHU Bordeaux, Bordeaux, France; 3CHU Bordeaux, Bordeaux, France; 4SISPED, Bordeaux, Bordeaux, France; 5Hôpital Saint-Jospeh, Marseille, France; 6CHU, Nancy, France; 7Hôpital Pitié-Salpêtrière, Paris, France; 8Hôpital Beaujon, Clichy, France

Background. Neutralizing antibodies to interferon (IFN)-α may explain a lack of antiviral response (Van der Eijk and al. NEJM 2006). We aimed at assessing NAb to IFN-α and IFN levels in non responders (NR) patients retreated by Peg-interferon α-2a (PEG-IFN α-2a) + ribavirin (RIBA). Methods. NR to a first-line treatment of PEG-IFN-α2a + RIBA were included to receive PEG-IFN α-2a [180µg/week] + RIBA (weight-based dosage) for 48 weeks. HCV RNA was measured at week 12 using Taq Man Roche (lower limit of detection 15 IU/ml) in a central laboratory. IFN levels and Nab to IFN-α were retrospectively measured on stored sera at baseline, weeks 4 and 12, using a quantitative sandwich ELISA for Nab to IFN-α (Bender Med-Systems Diagnostics GmbH) with a lower limit of detection of 1.38 ng/ml (similar to Van Der Eijk study). Interferon α-2a levels were measured using a quantitative sandwich ELISA for interferon α (Bender Med-Systems Diagnostics GmbH) with a lower limit of detection of 3.3 pg/ml. Results. Among 43 NR patients with baseline mean HCV RNA 6.1 log10 IU /ml (Inter-Quartile Range 4.7 to 7.4), 85% genotype 1, 53% F3-F4 Fibrosis, HCV RNA decreased greater than 2 log10 in 20 patients at week 12. Nab titers against IFN-α and IFN α titers are shown in the table. No significant association was observed between the presence nor the titers of Nab and HCV RNA levels. Median IFN levels (interquartile range) at weeks 0, 4 and 12 (pg/ml) were < 3.3 (<3.3-to-371.4); 1532 (106.8-to-5000) in the 20 responders and 84.5 (3.3-to-277.4); 1407.4 (120.2-to-2443.4); 1620.1 (120.1-to-2287.1) in the 23 non responders. Conclusion. This study, performed in non selected consecutive NR patients, do not confirm recently published case reports. Re-treatment with PEG-IFN + RIBA is able to be associated with virological response in the presence of antibody-mediated resistance to conventional IFN treatment.

<table>
<thead>
<tr>
<th>HCV RNA decrease greater than 2 Log/0</th>
<th>Undetectable HCV RNA at week 12</th>
<th>Undetectable HCV RNA at week 28</th>
</tr>
</thead>
<tbody>
<tr>
<td>yes</td>
<td>no</td>
<td>yes</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Nab to IFN-α</th>
<th>&lt;1.38</th>
</tr>
</thead>
<tbody>
<tr>
<td>(ng/ml)</td>
<td>&gt;1.38</td>
</tr>
<tr>
<td></td>
<td>11(59%)</td>
</tr>
<tr>
<td>P-value</td>
<td>0.02</td>
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</table>

Disclosures: The following people have nothing to disclose: Philippe Halfon, Sophie Pérusat, Marc Bourlière, Jean-Pierre Bronowicki, P. Trimoulet, Yves Benhamou, V. Leroy, Patrick Marcellin, J. Foucher, Geneviève Chène, Patrice Couzigou.
decompensation (ascitis, liver insufficiency, encephalopathy, gastro-intestinal bleeding) were retrospectively evaluated in a cohort of 336 HBeAg- patients referred to the Hepatology Department of Pitié Salpêtrière Hospital (Paris France) since 1980. Patients co-infected with HIV, HDV and HCV were excluded. Age, race, oral anti-HBV therapy (lamivudine, adefovir, tenofovir, entecavir), IFN based therapy, baseline ALT and serum HBV DNA were analyzed using Kaplan Meyer (KM) survival curves. A Cox regression analysis was performed to assess independent predictors of liver decompensation. Results: Main baseline characteristics of included patients are summarized in the table. Age, sex, race, oral anti HBV therapy, serum ALT, serum HBV DNA were significantly associated with liver decompensation in univariate analysis. Age (>35 years, RR 25.72 [3.08-214.80], p= 0.003) and high baseline serum HBVDNA (>6 log copies/mL, RR 11.97 [2.75-52.13], p<0.001) remained independently associated with the risk of liver decompensation in the Cox analysis (n=252). Conclusion: High HBV DNA (> 6 log copies/mL) and age older than 35 years strongly predict the risk of liver decompensation in HBeAg- patients. These patients should be actively considered for anti-HBV therapy independently of serum ALT level. This study was supported by IDENIX PHARMACEUTICAL.

### Main baseline characteristics in HBeAg- (n=336)

<table>
<thead>
<tr>
<th>Median (95 CI) follow up (months)</th>
<th>68.37 (61.6-75.25)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean (95% CI) Age (years)</td>
<td>38.12 (36.7-38.49)</td>
</tr>
<tr>
<td>Male (%)</td>
<td>237 (70.54)</td>
</tr>
<tr>
<td>Race - African n (%) - Asian n (%) - Caucasian n (%)</td>
<td>180 (53.57) / 87 (25.39) / 69 (20.54)</td>
</tr>
<tr>
<td>Alcohol consumption &gt;50 g/day n (%)</td>
<td>16 (4.88)</td>
</tr>
<tr>
<td>Median (95% CI) ALT (ULN) ALT&lt;2 x ULN²</td>
<td>34 (31-38) / 90 (26.79)</td>
</tr>
<tr>
<td>HBV DNA n copies/mL - &lt;4 &lt; 6 log copies/mL - &gt;6 log copies/mL - Undetermined</td>
<td>25 (7.41) / 99 (29.46) / 128 (38.16) / 80 (23.81)</td>
</tr>
<tr>
<td>Anti HBV therapy - IFN based n (%) - Oral n (%) - Untreated</td>
<td>20 (5.95) / 214 (63.69) / 118 (35.12)</td>
</tr>
<tr>
<td>HBeAg seroconversion n (%)</td>
<td>4 (1.83)</td>
</tr>
<tr>
<td>Liver decompensation n (%)</td>
<td>41 (12.20)</td>
</tr>
</tbody>
</table>

ULN time the upper limit of normal

Disclosures:
Yves Benhamou - Grant/Research Support: Indenix; Consultant/Adviser: Indenix; Speaker’s Bureau: Indenix
The following people have nothing to disclose: Yen Ngo, Rachel Morra, Vlad Ratziu, Vincent Thibault, Thierry Poyrand
treatment group was defined as HBe seroconversion and HBV-DNA<105 copies/ml at 24 weeks after end of treatment. Age, sex, HBe Ag, pretreatment ALT, ALT-10 and HBV-DNA levels and stage of fibrosis were examined by multivariate logistic regression analysis. Results: All patients had HBV genotype C. In LMV group, 53 patients (63.9%) [29 HBeAg +ve and 24 HBeAg –ve] achieved EVR. Pretreatment median serum IP-10 levels had no significant difference EVR between non-EVR (381 v 339 pg/ml, respectively; p=0.89). From multivariate analysis, pretreatment ALT levels (p=0.001) and negativity for HBe Ag (p=0.01) were independent predictor for EVR. Meanwhile, in sequential treatment group, 11 patients (50%) achieved SVR. Pretreatment median serum IP-10 levels had significant difference SVR between non-SVR (665 v 431 pg/ml, respectively; p=0.03). From multivariate analysis, only pretreatment ALT levels (p=0.002) was independent predictor for SVR. During sequential treatment, IP-10 levels in treated all patients showed dramatic change (median levels:pre LMV(460pg/ml), pre IFN(160pg/ml), post IFN 1week(65pg/ml), post IFN 4weeks(159pg/ml). Conclusion: Our data suggest that pretreatment serum IP-10 levels predict SVR to LMV-IFN sequential treatment in CHB. The difference of significance of pretreatment IP-10 levels predict SVR to LMV monotherapy and LMV-IFN sequential treatment may be derived from that the CHB patients with high IP-10 levels will show good IFN response which is contrary to response for IFN in CHC.

Disclosures:
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NOT HBEAG-STATUS BUT HBV GENOTYPE PREDICTS RESPONSE TO IFN IN CHRONIC HEPATITIS B
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Background and Aims: In contrast to HBeAg-positive hepatitis B, HBeAg-negative hepatitis B is considered to be associated with a poor sustained virological response towards interferon therapy. The present study investigated the influence of HBV genotype on the presumed HBeAg-dependence of IFN response. Patients and Methods: 399 patients with chronic replicative hepatitis B infected by HBV genotype A (n=141) or HBV genotype D (n=258) were treated with standard interferon-alpha or pegylated interferon-alpha for 4-12 months. HBV genotype was determined by direct sequencing of the HBV X or S gene. 44.4% of the patients had HBeAg-negative hepatitis B and 55.6% HBeAg-positive hepatitis B. Results: Sustained virologic response to standard interferon-alpha therapy was lower in HBeAg negative compared to HBeAg-positive patients (22.1% vs 35.6%; p<0.003). Prevalence of HBV genotype A was higher in HBeAg--positive hepatitis compared to HBeAg-negative hepatitis (59.3% vs. 16.2%; p<0.0001) and opposite to the prevalence of HBV Genotype D (40.7% in HBeAg-positive hepatitis vs. 83.8% in HBeAg-negative hepatitis). SVR was lower for HBV genotype D patients (19.8%) compared to HBV genotype A patients (43.3%; p=0.001). However, there was no difference in SVR between HBeAg-positive and HBeAg-negative patients of HBV genotype A (44.8% vs 38.9%, p=n.s.) neither for HBeAg-positive and HBeAg-negative patients of HBV genotype D (18.5% vs. 21.1%, p=n.s.). Multivariate logistic regression performed for a representative cohort of patients (n=208) identified HBV genotype A vs. D (p<0.002; 95%CI:1.485,43) and HBV DNA (<or ≥200pg/ml; p<0.04; 95%CI:1.01-3.82) but not pretreatment ALT levels (>2 x ULN) nor HBeAg-status as independent predictive parameters of IFN response.

Conclusions: The present data demonstrate that SVR to IFN in hepatitis B was not affected by HBeAg status but by HBV genotype. Therefore genotype dependence of IFN response and geographic variability of HBV genotype prevalence might explain the divergent SR to IFN between HBeAg-positive and HBeAg-negative patients. This should be taken into account for delineation of antiviral therapy in chronic hepatitis B.

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INTRAHEPATIC AND SERUM MARKERS OF HBV REPLICATIVE CAPACITY AND THEIR RELATIONSHIP TO SERUM HBEAG TITRES: IMPLICATIONS FOR THE USE OF QUANTITATIVE HBEAG TESTING AS A PREDICTIVE TOOL FOR TREATMENT OUTCOME
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Background: HBeAg seroconversion is one of the major therapeutic endpoints used when treating HBeAg-positive chronic hepatitis B (CH-B). Data from clinical trials suggests a potential role for quantitative HBeAg titres in guiding therapy. However, a commercial assay is not available and HBeAg titres have not been well-characterized in vivo. The relationship of HBeAg titre to viral load (VL) and cccDNA in vivo remains unresolved; interpretation may be confounded by the presence of the basal core promoter (BCP) or precore (PC) variants in the quasispecies pool. Although shown to reduce/abolish HBeAg production in vitro, their impact on HBeAg titre in vivo has not been defined. Aims: 1) To define the normal range of HBeAg titres seen in patients with HBeAg-positive CH-B in the immunoequilibration phase of disease. 2) To correlate HBeAg titres with VL, liver cccDNA, viral genotype and the presence of PC/BCP mutations. Methods: A high throughput commercial HBeAg ELISA kit (Architect, Abbott Laboratories, Ill) was optimised for use as a quantitative assay using the Paul-Ehrlich (PE) reference standard. HBeAg titre was correlated with VL in all patients. Detailed virological characterization was performed in a subset; this included genotype, BCP/PC sequence, and intrahepatic cccDNA (Werle-Lapostolle, Gastro, 2004). Results: The assay was validated using a cohort of 85 untreated HBeAg-positive patients in the immunoequilibration phase of disease. Sequencing was performed in 30 patients. Intrahepatic cccDNA was measured in 17 patients. The linear range of the assay was 0.5 – 90 PE IU/ml. The observed HBeAg titres followed a log-normal distribution (median 1-14236 PE IU/ml). Median VL was 3.43x108 IU/ml. Median cccDNA was 3.65 copies/Geq. VL correlated strongly with...
cddNA (r = .81, p < .05). HBcAg titre correlated modestly with VL (r = .50, p-value < .001) and cddDNA (r = .48, p-value = .05). The lack of a stronger correlation was attributed to the presence of BCP and PC variants in the quasispecies pool (dominant virus WT/WT in 17/30, BCP/WT in 9/30, and WT/PC in 4/30; median HBcAg titres 3095, 209 and 149 respectively, p < .05 vs WT). The effect of the BCP/PC variants was independent of VL HBcAg titre did not differ between genotypes A-D. Conclusion: We have optimised a commercial HBcAg assay for use as a sensitive and accurate quantitative tool. HBcAg titres were log-normal distributed and modestly correlated with VL and cddDNA, which was due to the presence of BCP/PC variants reducing HBcAg titre independent of VL. BCP/PC sequencing should be incorporated into research protocols evaluating the utility of HBcAg titres in predicting treatment outcome.

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976 DRUG RESISTANCE MUTATION ANALYSIS BY DIFFERENT METHODS IN A HIGHLY TREATMENT-EXPERIENCED CHRONIC HEPATITIS B PATIENT POPULATION

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Background: Antiviral drug resistance in patients with chronic hepatitis B has become a major drawback, ultimately leading to antiviral treatment failure, subsequent biochemical breakthrough, and hepatic decompensation. To optimally monitor treatment of HBV patients, a highly sensitive assay is required that can detect resistance mutations early. Patients and Methods: 133 adult HBV patients attending the liver clinics of University Health Network (Toronto, Canada) were prospectively monitored for antiviral resistance mutations using INNO-LiPA HBV DR assays (Innogenetics, Belgium) and sequencing. The majority was Asian (78%), HBcAg positive (57%), and had mean HBV DNA upon resistance testing 5.4±2.1 log10 IU/μl. Ninety percent had received lamivudine therapy, 40% adefovir, and a few tenofovir or entecavir. INNO-LiPA HBV DR v2* probes cover mutations at codons 80, 173, 180, 181, 204 and 236; a prototype INNO-LiPA HBV DR v3 strip covers codons 184, 194, 202, 233 and 250. Sequencing and LiPA were performed on the same amplicon. Concordance between the LiPA and sequencing was calculated. Results: Viral loads ranged from 1.29-7.7 log10 IU/μl. All genotypes except F were found, with the majority being genotype C (51.1%). Interpretable LiPA patterns were obtained for 133/133 samples (100%); sequence information for 131/133 samples (98.5%). Thirty-four of the 131 samples (26%) showed no mutations at any one of the 11 codon sites with LiPA or sequencing, whereas 97 samples (74%) displayed at least one mutation with one or both methods. Of resistance mutations detected by sequencing, 70% were known mutation at codon 204 after LiPA analysis. With sequencing, 45 patients (86%) and 70 patients (92%) showed these respective mutations. Conclusions: Both LiPA and sequencing are useful and accurate methods for drug resistance mutation detection. However, LiPA enables detection of minor mutant populations as mixtures earlier than sequencing. Earlier detection of resistance mutations using LiPA followed by prompt salvage therapy could help optimize the management of patients with antiviral-resistant HBV.

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977 RESTORATION OF HBV-SPECIFIC T CELL RESPONSES IN LAMIVUDINE LONG TERM RESPONDER

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Background: Hepatitis B virus (HBV)-specific T cell responses appear to play a pivotal role in infection control and disease outcome. The benefit of Lamivudine (LAM), a nucleoside analogue used for HBV treatment, is compromised by progressively increasing emergence of drug-resistant strains (up to 70% after 5 years). However, 20-30% of patients do not develop resistance despite long-term therapy. While LAM has been shown to induce early but only transient restoration of CD4 and CD8-specific T cell responses, the immunological profile of LAM long-term responders (LAM-LTR) has not yet been analyzed. Aim: To assess the HBV-specific T cell responses in LAM-LTR compared to LAM resistant (LAM-R) and to evaluate if successful long-term LAM therapy restores HBV-specific immunity. Methods: Seven LAM-LTR (male/female: 5/2; median age: 60; range: 42-67), defined by sustained HBV suppression during prolonged LAM administration (median years of treatment: 9; range: 7-11) and 13 LAM-R patients (male/female: 12/1; median age: 52; range: 30-60), defined by occurrence of viral breakthrough, were tested with a panel of overlapping peptides (18-20 mers) spanning the entire HBV sequence. The frequency and magnitude of HBV-specific T cell responses was assessed by an IFN-γ ELISPOT assay after in vitro stimulation with the synthetic HBV peptides. Results: HBV-specific immune responses were detected in all LAM-LTR (100%) and in eight of the 13 subjects with LAM-R (61%). The frequency of HBV-specific T cell responses were significantly higher in LAM-LTR compared to LAM-R (median frequency: 5 vs 1, respectively, p=0.001). Moreover, LAM-LTR presented significantly stronger IFN-γ responses (median: 2793 vs 1740 SFC/1,000,000 PBMC, p=0.03). Polymerase-specific immune responses dominated the HBV-specific T cell repertoire in both groups of patients, although LAM-LTR presented multiple responses to all four HBV proteins. Interestingly, two regions, in the core protein and in polymerase, were targeted by 3 LAM-LTR (42%), suggesting two new regions of immunogenicity. Conclusions: LAM-LTR are characterized by a multispecific pattern of HBV specific T cell responses. Moreover, the immune repertoire of responses is stronger in terms of both frequency and magnitude in long term responders than resistant ones. These results can contribute to...
better characterize HBV patients with LTR to antiviral therapy and to help for their clinical management.

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TWENTY-FOUR WEEKS THERAPY WITH PEGINTERFERON ALFA-2A IS SIMILAR TO 48 WEEKS THERAPY IN PATIENTS WITH HBEAG POSITIVE CHRONIC HEPATITIS B AND GOOD PREDICTORS OF RESPONSE

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Background: 48 week therapy with Peginterferon alfa-2a has demonstrated to be effective in about one third of patients with HBeAg-positive chronic hepatitis B. Although the recommended treatment duration for these patients is 48 weeks, there is no enough data supporting 48 weeks of therapy over 24 weeks of therapy. Treatment might be shortened particularly in patients with good predictors of response. Aim: To compare the efficacy of 48 weeks vs 24 weeks of therapy with Peginterferon alfa-2a, in patients with chronic hepatitis B who had good predictors of response. Patients and Methods: 19 patients with high baseline ALT levels (> 3 ULN) and low viral load (HBV DNA < 109 cp/ml) were treated with Peginterferon alfa-2a 180 mcg/week, during 48 weeks. Virological, biochemical and serological responses were compared with those obtained in 16 patients with similar baseline characteristics treated with Peginterferon alfa-2a for 24 weeks. All patients had a follow-up period of 24 weeks after end of therapy. Results: At end of follow-up, HBeAg seroconversion was observed in 7/19 (36.8 %) of patients treated for 48 weeks and in 6/16 (37.5 %) of patients treated for 24 weeks (p=ns). Patients treated for 48 weeks evidenced a significantly higher decrease in HBV DNA at the end of therapy than patients treated for 24 weeks (-4.8 logs vs -3.6 logs respectively, p<0.05). However, the % of patients with HBV DNA < 100.000 cp/ml was similar in both groups at the end of follow up (42.1 % vs 43.7 %, ns). No significant differences between both groups were observed regarding ALT normalization, HBSAg loss or seroconversion. Serious adverse events occurred in 1 patient from each group. Conclusion: The results from this study indicate that 24 weeks of therapy with Peginterferon alfa-2a is similar to 48 weeks therapy in patients with HBeAg positive chronic hepatitis B who have good predictors of response.

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LONG-TERM FOLLOW-UP OF HBSAG CLEARANCE IN PATIENTS WITH HBEAG-NEGATIVE CHB TREATED WITH PEGINTERFERON ALFA-2A: INCREASE IN HBSAG CLEARANCE RATE FROM 3% 6 MONTHS POST-TREATMENT TO 8% AFTER 3 YEARS

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Clearance of HBsAg in patients with CHB is associated with long-term clinical outcome and improved survival. It can be induced by treatment with interferon-based therapy but is extremely rare following treatment with direct antivirals. The HBsAg clearance rates 24 weeks post-treatment in patients with HBeAg-negative CHB who were treated for 48 weeks treatment with peginterferon alfa-2a, peginterferon alfa-2a plus lamivudine or lamivudine alone in a multinational randomized trial were 3%, 4% and 0%, respectively. All patients included in this trial were offered entry into an observational study of long-term response 3 years post-treatment. The proportion of peginterferon alfa-2a-treated patients losing HBsAg increased from 3% 24 weeks post-treatment to 8% at 3 years; 9 patients treated with peginterferon alfa-2a monotherapy cleared HBsAg and 9 in the combination therapy group. Loss of HBsAg was observed across all the major HBV genotypes. Eight of the 18 patients developed anti-HBs antibodies. We analyzed the quantitative on-treatment HBsAg levels using the Architect assay (Abbott Diagnostics) for 198 patients treated with peginterferon alfa-2a +/- lamivudine who were included in the long-term follow-up study for whom 3-year post-treatment follow-up data were available. An HBsAg level <10 IU at week 48 was significantly associated with HBsAg loss 3 years post-treatment (relative risk [RR] 22.8; 95% CI 8–64.9; p<0.0001). Of the 23 patients with HBsAg <10 IU/mL at week 48, 12 (52%) had cleared HBsAg by year 3 vs 4/171 patients (2.3%) with HBsAg >10 IU/mL at the end of treatment. An on-treatment reduction of >2 log IU/mL from pre-treatment level to week 48 was significantly associated with HBsAg clearance at year 3 (RR 14.6; 95% CI 5.5–38.5; p<0.0001). Of the 26 patients with an on-treatment HBsAg decline of >2 log IU/mL, 11 (42.3%) had HBsAg at year 3 compared with only 5 of 172 patients (2.9%) with an on-treatment HBsAg decline <2 log IU/mL. Despite the fact that at the end of the 48-week treatment period patients who cleared HBsAg had HBV DNA suppressed to levels <400 cp/mL, an HBV DNA level <400 cp/mL was not predictive of HBsAg clearance 3 years post-treatment. In conclusion, the ability to induce HBsAg clearance, an outcome that is associated with long-term improvement in survival, supports the use of peginterferon alfa-2a as a first-line treatment for patients with HBeAg-negative CHB. Potent HBV DNA suppression appears to be required, but is not sufficient, for subsequent clearance of HBsAg. The ability of interferon-based therapy to induce HBsAg clearance may be associated with its dual immunomodulatory and antiviral mode of action.

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Maurizia Brunetto - Grant/Research Support: Roche
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VERY HIGH PREVALENCE OF PRECORE (PREC) AND/OR BASAL CORE PROMOTER (BCP) MUTATIONS (MUT) IN HBEAg-POSITIVE (EAG+) AND NEGATIVE (EAG-) CHRONIC HEPATITIS B (CHB) ESPECIALLY AMONG OLDER PATIENTS

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PURPOSE: Relapse is common following anti-HBe seroconversion and discontinuation of therapy for eAg+ CHB. Presence of an eAg+ HBV with preC/BCP Mut among patients who “appear” to have eAg+ CHB may be responsible for such treatment failure. Our goal is to examine the prevalence of preC and BCP Mut in eAg+ CHB patients.

METHODS: We performed a cross-sectional study of 330 CHB patients with genotypic Mut analysis done between 11/05-5/07 at a U.S. clinic. Mut analysis was performed by Quest Diagnostics, San Juan, PR. The following people have nothing to disclose: Mindie H. Nguyen, Huy N. Trinh, Ruel T. Garcia, Jeanine Phan, Gloria H. Nguyen, Khanh Nguyen, Huy Nguyen, Emmet B. Keeffe.

RESULTS: A total of 330 cases were identified and included. All but 4 were Asians. Mean age=46±14, 61% male, median ALT=35 (range:7-1070) U/L. Treatment history was as follows: naive=242 (73.3%), adefovir only=27 (8.2%), lamivudine=adefovir=57 (17.3%), and entecavir=4 (1.2%). Mean HBV DNA was lower in patients with preC/BCP Mut (3.6±107±2.7±108 vs. 1.9±108±4.2±108 IU/L, p=0.0002). Distribution of HBV genotypes was as follows: A=4%, B=67%, and C=28%. eAg+ was seen in 38% and eAg- in 62%. Overall prevalence of preC/BCP Mut was 77%, 95% in eAg- and 49% in eAg+. Mut prevalences increase with age (Figure). The majority of preC Mut detected were G1698A (87%) with G1896A/G, G1896G/A, 1839, 1841, 1845, 1874, or mixed in the remaining. The majority of preC Mut detected were A1762T(A)+G1764A(G,A/G)(90%) with the remaining having single Mut. There were no significant differences PreC/BCP Mut prevalence by gender or treatment groups.

CONCLUSIONS: The prevalence of PreC/BCP Mut is very high among CHB patients, especially among older patients. PreC/BCP Mut prevalence among eAg+ patients is 49% which also increases with age. Patients with mixed wild-type and preC-BCP Mut may be at higher risk for relapse after anti-HBe seroconversion. Baseline Mut analysis may help identify pts who would benefit from long-term therapy despite anti-HBe seroconversion.

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clinical isolates from 2 patients of group A undergoing long term LAM treatment. In the other 5 patients with detectable HBVDNA no ADV major resistant mutations were disclosed

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FACTORS AFFECTING RESPONSE TO ADEFOVIR TREATMENT IN PATIENTS WITH CHRONIC HEPATITIS B AND LAMIVUDINE RESISTANCE
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Background & Aims: Adefovir (ADV) may not always gain control of lamivudine resistant (LAM-R) chronic hepatitis B (CHB). To identify factors that predict adequate ie. undetectable HBV DNA (<60 IU/mL) response to ADV in the treatment of CHB. To identify factors that predict adequate ie. undetectable control of lamivudine resistant (LAM-R) chronic hepatitis B and ADV resistance in 1 yr while on ADV.

Methods: A retrospective study of CHB with genotypic or phenotypic LAM-R treated with ADV 10mg daily in an Add To or Switch To/Add Back Lam treatment strategy. Adequate response to ADV was defined as undetectable HBV DNA (<200copies/ml or <60IU/ml) at 1 yr. Factors evaluated included eAg status, treatment strategy, pre-treatment (just prior to starting ADV) HBV DNA level (PreTxDNA), whether ADV was added at phenotypic or genotypic resistance, and rapid HBV DNA decline at 6 months, defined as >50% logarithmic decline from PreTxDNA. A multivariate analysis was performed both for all patients and for the subset with cirrhosis. Results: Only 45 of 95 (47%) patients achieved undetectable HBV DNA at 1 yr. In multivariate analysis, only PreTxDNA level and >50% decline in HBV DNA at 6 months predicted achieving undetectable HBV DNA at 1 yr. Though patients with a PreTxDNA >7 log IU/ml were only 12% (3% to 43%) as likely to achieve an undetectable HBV DNA at 1 yr compared to patients with HBV DNA <7 log IU/ml, this effect continued below this cutoff. For each 1 log IU/ml increase in PreTxDNA a patient was only 40% (25% to 64%) as likely to attain an undetectable HBV DNA at 1 yr. Patients who at 6 months had >50% logarithmic decline in HBV DNA compared to pre-treatment were 25.4 (3.1 to 206.1) times more likely to reach undetectability at 1 yr. These same factors significantly predicted response at 1 yr when only patients with cirrhosis were evaluated. Three of 21 patients with a <50% decline in HBV DNA at 6 months developed ADV resistance in 1 yr while on ADV. Conclusions: ADV should be started at the first emergence of LAM-R when HBV DNA is lowest to maximize the probability of achieving an adequate response. Assessing the 6 month rate of decline in HBV DNA may identify those who should have anti-viral therapy changed earlier.

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PREDICTORS OF VIROLOGIC RESPONSE AND RESISTANCE TO ADEFOVIR IN PATIENTS WITH LAMIVUDINE-RESISTANT CHRONIC HEPATITIS B VIRUS INFECTION
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Background: Adefovir (ADV) can be used as initial therapy in patients with HBeAg positive or HBeAg-negative chronic hepatitis B or as additional therapy in those with lamivudine (LAM)-resistant HBV. ADV resistance was common in LAM-resistant patients than in treatment-naive patients. We evaluated the virologic response to ADV in patients with LAM-resistant chronic hepatitis B virus infection, and the potential role of primary non-response in prediction of virologic response and resistance.

Methods: Liver panel, HBeAg/anti-HBe, HBV DNA PCR (COBAS Amplicor HBV Monitor assay) were measured every 2 or 3 months during ADV treatment. Genotypic mutation was detected using mass spectrometry-based genotyping assay (RFMP). Primary non-response was defined as a decrease in serum HBV DNA by < 2 log10 copies/ml at 24 weeks of therapy. Virologic breakthrough (VBT) was defined as a >1 log10 copies/ml increase in serum HBV DNA from nadir after achieving virologic response. Virologic response was defined as a decrease in serum HBV DNA to undetectable levels by PCR, and loss of HBeAg. Results: 146 HBeAg positive CHB patients with LAM resistance were received ADV for at least 12 months. Mean treatment duration was 25 months (range 12-42). Mean pretreatment HBV DNA and ALT levels were 7.7 log10 copies/ml and 357 IU/L, respectively. 115 patients were chronic hepatitis, 31 were liver cirrhosis. Serum HBV DNA became undetectable and ALT normalized in 59 (44%) and 121 (82.9%), respectively. Thirty-four (23.3%) patients represented primary non-response. Virologic response was archived in 34 (23.3%) patients. The cumulative rates of virologic response were 9, 22, and 40% at 12, 24, and 36 months, respectively. In multivariate analysis, the absence of primary non-response (OR, 3.34; 95% CI, 1.017-10.965; P<0.001), higher pretreatment ALT (OR, 100; 95% CI, 1.000-1.002; P<0.05), and lower pretreatment HBV DNA (OR, 1000; 95% CI, 1.000-1.000; P<0.05) were independently associated with virologic response. VBT and genotypic ADV resistance were observed in 24 (16.9%) and 18 (12.3%), respectively. The rtA181T, rtN236T, and rtA181V and rtN236T were detected.

Predictive Factors For Effective ADV Therapy In LAM-R CHB (Multivariate Analysis)

<table>
<thead>
<tr>
<th>Predictive Factor</th>
<th>1 yr All Patients OR (95% CI)</th>
<th>1 yr Cirrhotos OR (95% CI)</th>
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<tr>
<td>eAg Status</td>
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<td>ns</td>
</tr>
<tr>
<td>Treatment strategy</td>
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<tr>
<td>Phenogenotypic Resistance</td>
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<td>ns</td>
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<tr>
<td>PreTxDNA (&gt;7 vs &lt;7 log IU/mL)</td>
<td>0.13 (0.05-0.43)(^a)</td>
<td>0.23 (0.05-1.00)(^a)</td>
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<tr>
<td>PreTxDNA (per 1 log IU/ml)</td>
<td>0.40 (0.25-0.64)(^b)</td>
<td>0.45 (0.26-0.76)(^b)</td>
</tr>
</tbody>
</table>

>50% decline at 6 months
25.1 (3.1-206.1)\(^c\) | 24.6 (2.4-290.3)\(^c\) |
The cumulative rates of genotypic ADV resistance at 12, 24, and 36 months were 3, 13, and 26%, respectively. In multivariate analysis, the predictive factor associated with VBT was only the presence of primary non-response (OR, 2.46; 95% CI, 1.089-5.547; P<0.05). Conclusions: Primary non-responder to ADV for LAM rescue therapy showed not only lower rate of virologic response, but also higher rate of virologic breakthrough than those of primary responder.

Disclosures: The following people have nothing to disclose: Neung Hwa Park, Jung Woo Shin, Jin Woo Park, Seok Won Jung, In Du Jeong, Sung Jo Bang, Da Ha Kim

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MANAGEMENT OF CHRONIC HEPATITIS B VIRUS (HBV) INFECTION BY PRIMARY CARE PHYSICIANS IN URBAN HOSPITALS AND CLINICS IN NEW YORK CITY

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Current primary care physician practices with respect to the management of persons with chronic HBV infection are largely unknown and comprehensive recommendations directed to primary care physicians are lacking. In this pilot study we examined chronic hepatitis B disease management practices in primary care settings in New York City HHC South Manhattan Network Hospitals and clinics. Analysis was conducted, retrospectively, through electronic medical record review. We present preliminary results for patients 19-49 years of age who first visited primary care clinics in affiliated facilities during January 2003-December 2006. For the purposes of this analysis, chronic HBV infection was defined as a positive test for hepatitis B surface antigen (HBsAg). The minimum adequate evaluation for chronic HBV infection was defined as testing for HBsAg, VL and ALT. Among the 18,457 patients, 42.4% (7,826) were screened for HBsAg. 893 were HBsAg+. The mean age of HBV-infected patients was 31.8 ± 7.4 years. 57.8% were men. The HBV-infected patients were overwhelmingly APIs (83.9%), followed by Blacks (7.8%), Hispanics (3.6%), and Whites (0.9%). 97.9% of HBV-infected persons were tested for ALT, 71.0% for HBeAg, 68.2% for viral load, and 63.6% for AFP. The rates of testing for HCVAb, HAVAb, HDVAb and HIV were 52.0%, 47.3%, 3.8% and 22.8%, respectively. Approximately one third of the patients (37.8%) received an abdominal ultrasound exam, 9.9% had a CT scan and 0.6% had a MRI. 564 patients (63.2%) had HBeAg, VL and ALT measurements and 306 patients (34.3%) had all three measurements and an ultrasound exam. 83.1% of those with abnormal ALTs had HBeAg and VL tests, higher than 58.5% among those with normal ALTs, p<0.01. Management practices did not differ by patient sex (p=0.51), race/ethnicity (p=0.07), age (p=0.18) or country of birth (p=0.82). Patients at hospital facilities were more likely to receive the minimum adequate evaluation (68.6%, and 64.9%, respectively), compared to patients at community clinics (45.5%, and 29.3%, respectively), p<0.01. In conclusion, the management of patients with chronic HBV infection by primary care physicians in this study was suboptimal according to practice guidelines established by the AASLD. Additional education and training of primary care physicians in the proper management of these patients needs to be implemented and evaluated in order to decrease the long-term morbidity and mortality of persons chronically infected with HBV.

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HEPATITIS B SURFACE ANTIGEN TITER WAS DECREASED DURING CLEVUDINE THERAPY

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Background: Clevudine therapy in chronic HBV patients resulted in a significant reduction of serum HBV DNA level. The purpose of this abstract is to determine if reduction of HBV DNA with clevudine therapy may lead to decrease of serum hepatitis B surface antigen (HBsAg). Methods: A total of 50 naive patients with chronic hepatitis B received clevudine 30 mg daily for 24 weeks followed by clevudine 10 mg daily for another 24 weeks. Serum HBV DNA was determined by Digene hybrid capture II assay [limit of detection of 4700 copies/ml] and Amplicor PCR assay [limit of detection of 300 copies/ml]. Serum HBsAg was quantified by the ARCHITECT HBsAg assay. Results: Based on the level of HBsAg titer at baseline, patients were stratified into four groups: group I (n = 18), < 10,000 IU/ml; group II (n = 15), 10,000 – 20,000; group III (n = 9), 20,000 – 40,000; group IV (n = 8), > 40,000. Median baseline HBV DNA were 6.1, 7.1, 8.1 and 9.0 log 10 copies/ml and baseline HBsAg levels were 2922, 14577, 25200 and 102605 IU/ml in the group I, II, III and IV, respectively. During first 24 weeks of clevudine treatment, HBsAg titers of group II, III and IV except group I showed a rapid decrease which showed the similar pattern of HBV DNA suppression. At the end of 1-year treatment, median HBV DNA reduction from baseline was 3.6, 4.6, 5.6 and 4.9 log 10 copies/ml and the change of HBsAg level from baseline of 1061, -2887, -5573, and -83909 IU/ml in the group I, II, III, and IV, respectively. Particularly, median serum HBsAg level in group IV at week 48 dropped to 18.2% of pretreatment level. Combined data of HBsAg reduction from group II, group III and group IV at week 48 showed statistically significant decrease (P = 0.002). Con-
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A HEPATITIS B VIRUS MUTANT IMPLICATED IN NON-RESPONSE TO SEQUENTIAL TREATMENT WITH ADEFOVIR, LAMIVUDINE AND ENTECAVIR SHOWS CROSS-RESISTANCE TO MULTIPLE ANTIVIRAL AGENTS

Tim Shaw1, Tina Sozzi1, Fee Wong1, Stephen Locarnini1,2; 1Victorian Infectious Diseases Reference Lab, North Melbourne, VIC, Australia; 2Evisar Medical, East Melbourne, VIC, Australia

Background: Development of safe and effective nucleos(t)ide analogue reverse transcriptase inhibitors (NRTI) has revolutionised chemotherapy for chronic hepatitis B (CHB), but prolonged treatment engenders viral resistance and sequential treatment with different NRTI can promote the emergence of multi-drug resistant mutants, which are refractory to continuing therapy and may be cross-resistant to new drugs. Consequently, control of multi-drug resistant HBV is becoming a major challenge. We describe the phenotype of an HBV mutant isolated from a patient with CHB who responded poorly to sequential treatments with adefovir, lamivudine, and entecavir. Isolates from this patient were genotype D and HBeAg negative due to the presence of the precore [pc] G1896A mutation and encoded multiple substitutions in the polymerase region including rtA181T, rtI233V, rtM250L and rtM250L. Cloning established that these were encoded on a single genome. Aims: To determine antiviral drug resistance phenotype of the putative multi-drug resistant rtA181T/rtI233V/rtM250L mutant, and determine how rtI233V and rtM250L influence phenotype. Methods: Mutant HBV clones encoding rtI233V, rtM250L, rtA181T/rtI233V/rtM250L and rtA181T/rtI233V/rtN236T/rtM250L were generated by site-directed mutagenesis from a wild type (WT) genotype D HBV isolate. WT and derived clones harboured the [pc] G1896A mutation, which may increase replication efficiency but does not significantly affect drug resistance. The phenotypes were determined by comparing viral replication in transfected HuH-7 cells. Virus replication and its inhibition were monitored by measuring changes in the amounts of intracellular core-associated viral DNA in cell lysates using a commercial assay kit (Bayer Versant HBV 3.0). Results: The rtA181T/rtI233V/rtN236T/rtM250L substitutions in combination imparted a severe replication defect but conferred high level resistance to all L-nucleosides (lamivudine, telbivudine, clevudine and emtricitabine) as well as high level resistance to the deoxyguanosine analogues diaminopurine, 2',3'-dideoxy-3'-fluoroguanosine and abacavir, and conferred lower level, but still significant, resistance to adefovir and tenofovir. The rtI233V and rtM250L substitutions in isolation did not confer significant drug resistance and significantly reduced replication capacity in the absence of selection pressure, but presumably act to compensate for the replication defects associated with acquisition of drug resistance. Conclusions: Sequential treatment with NRTI promotes multi-drug resistance. It is likely that development of multi-drug resistance could be reduced by combination therapy optimised to individual viral phenotypes.

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COST-EFFECTIVENESS OF NEW TREATMENT PARADIGMS FOR E-AG NEGATIVE CHRONIC HEPATITIS B

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BACKGROUND: A number of new drugs for chronic hepatitis B have become available in recent years, and several studies show that treatment with either interferon or the oral nucleos(t)ide analogs is cost-effective compared with no treatment. However, the cost-effectiveness of recent treatment algorithms has not been evaluated. METHODS: We considered the base case of a forty year old patient with newly-diagnosed, treatment-naive, e-Ag negative chronic hepatitis B. Using a decision analysis model, we examined seven treatment strategies: (1) no treatment (natural history arm), (2) Adefovir (ADV) with switch to entecavir (ETV) upon emergence of resistance (3) ETV with switch to ADV upon emergence of resistance, (4) ADV with addition of lamivudine (LAM) upon emergence of resistance, (5) ETV with addition of ADV upon emergence of resistance, (6) ADV with addition of ETV upon emergence of resistance, and (7) Peginterferon alfa-2a for one year. We examined the cost-effectiveness of each option from a third-party payer perspective, using a sixty year time horizon and an annual discount rate of 3%. We then separately considered the case of a similar patient with pre-existing LAM resistance. RESULTS: All treatment options yielded more quality-adjusted life years (QALY’s) than the “no treatment” arm. The oral agents demonstrated extended dominance over pegylated interferon. For treatment-naive patients, the most cost-effective option was initial ADV with switch to ETV upon emergence of resistance, yielding an incremental cost effectiveness ratio (ICER) of $6,697 over the next most cost-effective strategy. Among the “add-on” regimens, initial ETV with addition of ADV was a dominant strategy, giving the highest number of QALY’s at the lowest cost. For patients with pre-existing LAM resistance, adding ADV was preferred over switching to ADV or ETV, with an ICER of $37,118 per QALY gained. CONCLUSION: New treatment recommendations for the treatment of e-Ag negative chronic hepatitis B are cost-effective. Initial ADV with switch to ETV upon emergence of ADV resistance is the most cost-effective treatment strategy. Initial ETV with ADV add-on is superior to other “add-on” strategies due to a relatively longer allowable period of monotherapy. In patients who have already developed LAM resistance, adding ADV is cost-effective compared with switching to ADV or ETV, due to a lower rate of subsequent resistance.

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The following people have nothing to disclose: Tim Shaw, Tina Sozzi, Fee Wong
HBV reactivation is needed for clinicians treating patients with chronic HBV as pre-emptive therapy against reactivation. Current practice among oncologists is unknown in regard to screening and prophylaxis strategies for HBV. Aims: To assess the practice of oncologists in screening and management of HBV prior to initiating chemotherapy. Methods: A survey was sent to AMA registered oncologists in the United States assessing practice demographics and HBV screening practices. Fischer’s exact test was used for analysis. Results: Responses were obtained from 265 individuals: 38% (n=101) from an office-based practice, 55% (n=145) from a university/academic/research institution, and 6% (n=17) from other practice setting. Office-based physicians were less likely to screen for HBV prior to chemotherapy (p<0.001). Years in practice varied: 51% with <5 years (n=136), 29% with 5-15 years (n=77), and 18% with >15 years (n=49). No difference was found in screening practices based on years of experience (p=NS). Surveyors screen for HBV as follows: never-20% (n=54), abnormal liver tests-30% (n=80), risk factors or history of hepatitis-38% (n=101), randomly-11% (n=28), always-13% (n=35). Most oncologists used HB surface antigen as a screening test (76%), in conjunction with surface antibody (64%), and core antibody (46%). In pts with HBV, 75% (n=201) of oncologists refer to specialists and 7% (n=18) initiate therapy themselves, while 15% (n=39) do not refer or initiate therapy, most of whom are in an office setting (p=0.02). Conclusions: 20% of oncologists never screen for HBV prior to initiating chemotherapy, and fewer than 40% screen even if patients have risk factors or a history of hepatitis. Screening practices were not influenced by years in practice, however office-based physicians were less likely to screen, treat, or refer to a specialist prior to chemotherapy. Greater education and awareness regarding risk of HBV reactivation is needed for clinicians treating patients with immunosuppressive therapies.

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The following people have nothing to disclose: Tram Tran, Mina Oh, Fred Poorداد, Paul Martin

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PROFILES OF HBV DNA, ALT AND HBEAG STATUS AFTER STOPPING LAMIVUDINE IN PATIENTS WITH HBEAG SEROCONVERSION

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Aim: To determine the virological and biochemical relapse rates in Chinese CHB patients with lamivudine-induced HBeAg seroconversion after stopping lamivudine therapy, and to identify significant viral or biochemical factors predictive of subsequent relapse. Methods: All patients from all Hepatology Clinic with lamivudine-induced HBeAg seroconversion with subsequent cessation of lamivudine therapy were recruited. Liver biochemistry, HBeAg, anti-HBe, and serum HBV DNA by PCR (COBAS Taqman) was determined at the time of stopping lamivudine, at 3 months, 6 months, 1 year, and yearly thereafter. Results: Twenty-two patients were included, of which 16(73%) were male with a median age of 32 years (range, 21-55). The median duration of follow-up was 104 months (range, 31-150). Six (27%) patients had undetectable HBV DNA by PCR at the time of stopping lamivudine treatment. In total, 7(32%) patients had subsequent flare (as defined by ALT >2x upper limit of normal), with associated virological rebound of HBV DNA (as defined by 1 log increase from time of stopping therapy). The cumulative incidence of flare at 5 years was 44%, all occurring before 25 months. There was no significant difference in the cumulative incidence of flares between those patients that continued lamivudine for 6-12 months, 1-2 years and over 2 years after HBeAg seroconversion (p=0.58). In patients who had normal ALT and undetectable HBV DNA, their cumulative incidence of flare was not significantly different to those that had detectable HBV DNA or abnormal ALT at time of stopping lamivudine (p=0.73). Two patients underwent HBeAg seroconversion at 5 and 10 months, with resulting flares. The cumulative incidence of HBeAg seroconversion after stopping lamivudine was 9% at 5 years. Fourteen (64%) patients underwent virological rebound, with a cumulative incidence of 82% at 4 years. There was no significant difference in the cumulative incidence of virological rebound in patients with detectable HBV DNA at the time of stopping lamivudine therapy compared to those with detectable HBV DNA. Of those with virological rebound, 8(57%) had HBV DNA >10^5 copies/ml. Neither patient age or gender, genotype, presence of precore or core promoter mutations were significant factors in subsequent flare or virological rebound after stopping lamivudine therapy. Conclusion: Despite the high sustained HBeAg seroconversion (81%) after stopping lamivudine, HBV DNA rebound occurred in 64%, of which 57% had HBV DNA >10^5 copies/ml and 32% had ALT flares. Patients who had both normal ALT and undetectable HBV DNA by PCR at the time of stopping lamivudine therapy did not have a lower cumulative incidence of flares.

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REDUCTION IN SERUM HBsAg LEVEL IN PATIENTS WITH CHRONIC HEPATITIS B INFECTED WITH GENOTYPE D INDUCED BY (PEGYLATED)INTERFERON ALFA-2A ALONE OR IN COMBINATION WITH NUCLEOS(T)IDE ANALOGS: A LONG-TERM SINGLE CENTRE COHORT STUDY

Maurizio Brunetto1, F. Moriconi1, D. Cavallone1, F. Oliveri1, A. M. Maina1, P. Ciccossi1, P. Columbatta1, B. Coco1, G. Moscato2, Ferruccio Bonino2, 1UO Gastroenterologia ed Epatologia, Pisa, Italy; 2Laboratorio Centrale, Azienda Ospedaliera Universitaria Pisana, Pisa, Italy; 3Foundation IRCCS Policlinico of Milan and University of Pisa, Pisa, Italy

In patients with CHB undergoing antiviral therapy, clearance of serum HBsAg appears to be associated with better long-term clinical outcome. In clinical practice, quantification of serum HBsAg might provide a valuable tool to identify the best therapeutic strategy for individual patients. We quantified serum HBsAg (Architect, Abbott Diagnostics) in a total of 80 consecutive HBV genotype D patients treated at our chronic viral hepatitis reference center. The patients received interferon (IFN) alfa-2a [recombinant or pegylated] monotherapy (11 patients), PEG-IFNα-2a + lamivudine [LMV] (24 patients), LMV or adefovir [ADV] monotherapy (6 patients) or LMV + ADV (39 patients). Of the 80 patients, 45 were resistant to LMV; we treated 39 of these with LMV + ADV and 6 with LMV + PEG-IFNα-2a). Baseline characteristics such as gender, HBeAg, viral load and ALT level were not significantly different between the 2 groups of patients receiving (PEG)-IFN +/- nucleos(t)ide analogs (NA) or NAs alone. Patients treated with NAs had lower HBsAg baseline levels (mean 3.39 vs 3.77 log IU/mL, p=0.025) and were older (mean 57 vs 45 years, p<0.001). Patients were followed up for a mean duration of 40 months (24–84 months). The overall serum HBsAg delta variations (log decline from baseline to last observation) according to treatment group are reported in the Table. Almost all patients treated with NAs alone had no or minimal reduction in HBsAg level and none of these cleared HBsAg during follow-up. In contrast, the majority of patients who received interferon-based therapy showed a reduction in HBsAg level of at least 0.5 log and 4 patients achieved HBsAg clearance. In conclusion, IFNα-2a (conventional or pegylated) appears to be more effective in suppressing serum HBsAg independently of baseline host and virologic features, also in patients who are resistant to LMV therapy. Quantification of serum HBsAg could provide a useful tool to predict the long-term outcome of interferon-based therapy and its ability to induce HBsAg clearance in CHB patients infected with genotype D.

<table>
<thead>
<tr>
<th>Reduction in HBsAg level (log)</th>
<th>&lt;0.5 n (%)</th>
<th>≥0.5 &lt;1 n (%)</th>
<th>≥1 n (%)</th>
<th>≥2 n (%)</th>
<th>≥3 n (%)</th>
<th>HBsAg loss n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(PEG)-IFN +/− NA n=35</td>
<td>17 (46.6%)</td>
<td>5 (54.6%)</td>
<td>1 (22.2%)</td>
<td>5 (11.1%)</td>
<td>4* (11.1%)</td>
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</tr>
<tr>
<td>NA alone n=45</td>
<td>33 (73.3%)</td>
<td>12 (26.7%)</td>
<td>2 (4.4%)</td>
<td>1 (2.2%)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total n=80</td>
<td>50 30 12 0 5 4</td>
<td></td>
<td></td>
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* 1 patient cleared HBsAg during PEG-IFNα-2a rescue therapy for LMV resistance.

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HIGH RATES OF HBsAg SEROCONVERSION IN CHRONIC HEPATITIS B PATIENTS RESPONDING TO INTERFERON THERAPY: A LONG TERM FOLLOW-UP STUDY

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Background and Aim Loss of HBsAg with seroconversion to anti-HBs is the most desired treatment endpoint in chronic hepatitis B (CHB), and can be considered a complete response indicating resolution of hepatitis B. The difficulty is that HBsAg loss is rare, occurring spontaneously in less than 1% per year in inactive carriers and in patients receiving a one-year course of nucleos(t)ide analogue therapy. HBsAg loss is more frequent with interferon based therapy, occurring in up to 8% of patients three years post-treatment. The aim of this study was to assess the long term HBsAg response in a cohort of HBsAg-positive CHB patients treated with interferon. Patients and Methods All consecutive patients with HBeAg-positive CHB treated with conventional interferon between 1987 and 2000 were retrospectively evaluated. There were 97 patients. Sustained virological response (SVR) was defined as persistent HBsAg seroconversion and undetectable HBV DNA in serum 24 months after treatment discontinuation and during the follow-up period. Cumulative incidence of HBsAg seroconversion was assessed during the follow-up period and compared between patients with and without SVR. Results The baseline characteristics of the study population were: male gender (88%), mean age (39±13 years), mean ALT level 4.5±3.5 ULN, mean serum HBV DNA level 7.3±0.9 log10 copies/ml. Liver histology showed moderate-severe necroinflammation (META VIR A2-A3) in 42%, and severe fibrosis (META VIR F3-F4) in 44% of patients. SVR was achieved in 25 patients (26%). At multivariate analysis, SVR was independently associated with age > 45 years (p=0.008, OR=4.6) and serum HBV DNA level < 6 log10 copies/ml (p=0.002, OR=9.4). During a median follow-up period of 11 years (4.20), HBsAg seroconversion developed in 28 patients (29%), and was significantly more frequent in patients who achieved SVR than in those who failed interferon treatment (64% vs. 17%, p<0.0001). The cumulative incidence of HBsAg seroconversion was significantly higher in patients who developed SVR compared with those who failed interferon treatment during the whole follow-up period (40% vs. 8% at year 5, and 60% vs. 18% at year 10 and 80% vs. 30% at year 15). Five patients developed hepatocellular carcinoma (HCC) after a median period of 7 years (5-12) post interferon treatment. None of these patients had developed HBsAg seroconversion (p=0.02). Conclusion Patients with HBsAg-positive CHB achieved a 26% SVR rate following treatment with conventional interferon. Long-term follow-up showed that SVR was associated with a very high rate of HBsAg seroconversion (60% at 10 years) and a significant decrease in the incidence of HCC.

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The following people have nothing to disclose: Anneke Korevaar, Rami Moucari, Tarik Asselah, Olivier Lada, Nathalie Boyer, Michèle Martinot-Peignoux, Pierre Bedossa, Patrick Marcellin.
HBSAG SEROCONVERSION: DIFFERENT KINETICS OF SERUM HBSAG LEVEL DECREASE IN INACTIVE CARRIERS AND CHRONIC HEPATITIS B PATIENTS TREATED WITH INTERFERON

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Background and Aim HBsAg is used typically as a qualitative serological marker for diagnosing an ongoing HBV infection. HBsAg clearance is the most desired treatment endpoint in hepatitis B and considered as resolution of disease. Recently, a quantitative, fully automated chemiluminescent microparticle immunoassay (Abbott Architect HBsAg QT) has become available for the quantitation of HBsAg at a wide range of concentrations. The aim of this study was to assess the kinetics of HBsAg level decrease in serum of patients who have cleared HBsAg spontaneously or following interferon therapy. Patients and Methods 22 HBV-infected patients who have cleared HBsAg have been retrospectively studied. Five patients were inactive carriers while 17 have been successfully treated with interferon. HBsAg clearance occurred after a median period of 6 years (2-14) after treatment discontinuation. Serum samples collected at yearly interval up to 4 years prior to HBsAg clearance and adequately kept frozen were retrospectively analysed. Samples were diluted 1:20 and 1:500 with the Architect HBsAg diluent in order to expand the upper limit of quantitation in serum at wide range of concentrations. These data also suggest that interferon treatment accelerates HBsAg clearance. Further studies are ongoing to explore the clinical relevance of HBsAg quantitation for the monitoring of patients treated either with interferon or analogues.

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HIGH PREVALENCE OF SIGNIFICANT HISTOLOGIC DISEASE IN PATIENTS WITH CHRONIC HEPATITIS B (CHB) AND NORMAL ALT

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PURPOSE: Current guidelines recommend therapy for CHB with elevated ALT. However, many patients presenting with “normal” ALT may not have persistently normal levels on f/u. Our goal was to define the spectrum of histologic findings in such patients. METHODS: We performed a retrospective cohort study of all patients with active CHB (HBV DNA≥10000 copies/ml) & ALT<UNL during liver biopsy at a GI clinic. Significant histology was stage II or grade 2+ stage I or higher. RESULTS: Of the 136 potential cases, we excluded 4 with +anti-HCV and 3 with significant EtOH use and included 129 cases. Mean age=45±11, mean weight =62±14 kg, 65%=male, 28%=smokers. 6% had DM, 40% had hyperlipidemia, 18% had FH of liver CA/liver-related death. All were Asian: 58% were HBeAg+. At biopsy, mean HBV DNA=6.2±1.9 log10 copies/ml and mean ALT=45±18 U/L. Average number of ALT levels per patient =4.1±2.5 over a median f/u of 17 months (range:1-113), when 38% had at least one ALT>UNL. Mild steatosis was seen in 28% and moderate in 11% but this finding was not a significant predictor. The overall prevalence of significant histology was 43.4% & increased with age (Figure): 31% in those with persistently normal ALT and 63% in those without. On univariate analysis, predictors of significant histology were age, weight, HBeAg negativity, & abnormal ALT on f/u. On multivariate analysis, predictors were elevated ALT (OR=5.1, p=0.0001) and age (OR=5.2, p=0.037 for age 35-49; OR=11.2, p=0.005 for age 50-64; & OR=28.0, p=0.007 for age≥65). CONCLUSION: Only 62% of patients with normal ALT at evaluation have persistently normal levels on follow-up, a third of whom can still have significant histology. Older age starting at 35 is the strongest independent predictors of significant histology. Asian patients with normal ALT should be followed closely and histologic evaluation should be considered if patients are ≥35 or if ALT becomes elevated.

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BASELINE PARAMETERS PREDICT BOTH EARLY Virologic RESPONSE AND LONGER TERM OUTCOMES FOR TELBIVUDINE-TREATED PATIENTS WITH CHRONIC Hepatitis B (THE GLOBE STUDY)

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Background: Viral load at 24 weeks of telbivudine treatment has been linked to efficacy outcomes after 1 and 2 years. Studies have shown that pretreatment, elevated ALT and low HBV DNA levels predict higher responses to interferon, however few corresponding data are available for nucleos(t)ide therapies for hepatitis B. Here we report that baseline disease variables predict virologic responses to telbivudine at 24 weeks, which in turn predict efficacy outcomes at 2 years. Methods: The GLOBE trial enrolled 1,367 HBeAg+ and HBeAg- hepatitis B patients with HBV DNA >6 log10 copies/mL, ALT 1.3-10 xULN, and compensated liver disease, randomized to receive telbivudine or lamivudine for 2 years. The influence of baseline parameters and early HBV DNA suppression on subsequent efficacy outcomes and resistance were evaluated by univariate and multivariate regression analyses. Results: Pretreatment serum HBV DNA and ALT levels were significant predictors of virologic response at Week 24 and of efficacy outcomes and viral breakthrough at Week 104. Among HBeAg+ telbivudine recipients with ALT ≥2 xULN and HBV DNA <9 log10 copies/mL, 47% and 14% experienced HBeAg seroconversion and viral breakthrough at Week 104, respectively, vs. 30% and 29% among all HBeAg+ telbivudine recipients. 71% of patients in this subset were PCR-negative after 24 weeks of telbivudine, vs. 44% of HBeAg+ telbivudine recipients overall. HBV genotype had minor, generally nonsignificant effects on outcomes. Similar relationships were observed in HBeAg- patients with elevated ALT and serum HBV DNA <7 log10 copies/mL at baseline, however due to the higher overall rate of viral clearance in this group, baseline parameters had a less pronounced effect on virologic responses at Weeks 24 and 104, compared with HBeAg+ patients. After initiation of treatment, multivariate analyses showed a diminished impact of baseline parameters on subsequent outcomes, and confirmed viral load at Week 24 as the most significant predictor of outcomes at Week 104. Among patients with HBV DNA <9 log10 copies/mL and elevated ALT at baseline, PCR negativity at Week 24 resulted in 52% HBeAg seroconversion and 3.6% viral breakthrough at 2 years. Conclusions: High rates of efficacy responses after 2 years of telbivudine, and low resistance, occurred in patients with elevated ALT and HBV DNA <9 log10 (HBeAg+ patients) or <7 log10 (HBeAg patients) copies/mL pretreatment, coupled with optimal viral load reduction at Week 24. These results define patient characteristics associated with optimal responses to telbivudine and may contribute to on-treatment patient management strategies.

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ON-TREATMENT Virologic SUPPRESSION AT WEEK 24 DECREASES THE RISK OF HISTOLOGIC PROGRESSION AT 1 YEAR; DATA FROM THE GLOBE TRIAL

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Background: Serum HBV DNA and ALT level have been correlated with active liver disease in patients with chronic hepatitis B (CHB). However, on-treatment predictors of liver disease progression have been inadequately studied. Objective: To identify on-treatment predictors of worsening of HBV-related liver disease. Methods: The GLOBE trial enrolled 1367 compensated CHB patients with baseline HBV DNA >6 log10 copies/mL, ALT 1.3-10 xULN. Patients were randomized 1:1:1 to treatment [telbivudine (600 mg/d PO) or lamivudine (100 mg/d PO)] and stratified by HBeAg status and baseline ALT level. Predictors of histologic progression at Week 52 (WS52) were evaluated using baseline (BL) and 24-week (W24) data from the GLOBE trial. Progressors (P) exhibited [1-unit increase in Ishak Fibrosis Score (IFS)] OR [2-unit increase in Knodell Necroinflammatory Score (KNS)] with no decrease in IFS at WS52. All other patients were deemed non-progressors (NP). Stepwise logistic regression analysis of data from 349 telbivudine patients and 535 lamivudine patients compared P (n=142) to NP (n=942) after 52 weeks of treatment; p-values and odds ratios of being P at W52 were calculated. Factors were entered into the model using α=0.25, and retained in the model using α=0.1. BL factors included IFS, KNS, viral load, e-antigen status, genotype, ALT, BMI, age, gender, and treatment assignment. On-treatment factors were viral load reduction, ALT decline, and PCR negativity (all at week 24). Results: BL IFS (OR=5.0) and KNS (OR=2.7) were the only significant factors predicting P (p<0.0001 for both), with lower scores being more predictive of P than higher scores, possibly due to higher IFS and KNS responding better to antiviral treatment. The only significant on-treatment predictor of P (p≤0.05) was failing to achieve a decline in HBV DNA of ≥5 log10 copies/mL by W24 (p=0.0058), which increased the likelihood of P by 57%. Genotype, baseline BMI and treatment arm were retained in the model but were not significant at p<0.05. However, significantly more telbivudine-treated patients achieved ≥5 log10 decline in HBV DNA compared with lamivudine (69% vs. 55%, p<0.05). Conclusion: Although baseline histology status was the most important predictor of disease progression at 1 year, the only significant on-treatment factor found to reduce the likelihood of progression was attaining HBV DNA decline ≥5 log by W24. These data suggest that using a more potent antiviral agent can result in histologic improvements over periods as short as one year. Longer term studies are needed to evaluate the effects over time. Acknowledgment: D Walczak, Cayuga Consulting & V Mats, Quartesian LLC

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IMPACT OF ADEFOVIR DIPIVOXIL ON LIVER FIBROSIS AND ACTIVITY ASSESSED WITH FIBROTEST-ACTITEST IN PATIENTS INFECTED BY HEPATITIS B VIRUS
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Background: The aim was to assess the usefulness FibroTest-ActiTest (FT-AT) as surrogate markers of histological features in patients with chronic hepatitis B based on a correlation analysis with biopsy data in pivotal studies with adefovir dipivoxil.

Methods: Patients with chronic hepatitis B randomized in two pivotal trials of adefovir versus placebo, with available paired liver biopsy and FT-AT at baseline and after 48 weeks of treatment were included. Fibrosis and Activity were assessed blindly according to ISHAK scoring system. Diagnostic value of FT-AT was assessed using the area under the receiver operating characteristic curves (AUROCs) for the diagnosis of bridging fibrosis, cirrhosis, and moderate-severe necro-inflammatory activity; sensitivity analyses take into account race, length of biopsy, HBsAg status and sample date. The impact of treatment versus placebo was assessed on liver injury according to baseline stage, and virological response at 48 weeks. For baseline discordant cases, 48 weeks repeated estimates in adefovir virological responders and placebo non-responders permitted to estimate the ratio due to FT-AT or biopsy failures. Findings: 462 patients were included, 304 treated with adefovir and 158 received placebo with paired FT-AT and biopsies. The analysis of 924 estimates showed for the diagnosis of bridging fibrosis, cirrhosis, and moderate-severe necro-inflammatory activity very significant FT-AT AUROCs: 0.76±0.02, 0.81±0.02 and 0.80±0.01 respectively. Similar impacts on liver fibrosis and activity were observed both with biopsy and FT-AT, with greater efficacy in patients with baseline advanced fibrosis, treated with adefovir and virological responders. The discordances analyses suggested that 66% of discordances were attributable to biopsy failure and 34% to FT-AT failure. Conclusion: In patients with chronic hepatitis B, FibroTest-ActiTest is a simple and non-invasive quantitative estimate of liver fibrosis and necroinflammatory activity, which could be used as a surrogate marker to reduce the need for liver biopsy.

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LONG-TERM FOLLOW-UP OF ENTECAVIR TREATED PROTOCOL-DEFINED NON-RESPONDERS IN ROLLOVER STUDY ETV-901
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Background: Entecavir’s (ETV) efficacy in nucleoside naïve HBeAg(+) [ETV-022] and HBeAg(-) [ETV-027] patients with chronic hepatitis B (CHB) has been demonstrated.¹,² Protocol-defined patient management criteria dictated that ‘Non-Responders’ in ETV-022 and ETV-027 discontinued study therapy. However, ‘Non-Responders’ who enrolled in rollover study ETV-901 continued to be monitored and are reported here.

Methods: 679 nucleoside-naïve HBeAg(+) and HBeAg(-) patients were enrolled and treated with ETV in studies ETV-022 and ETV-027. Patients who failed to achieve HBV DNA <0.7 MEq/mL by bDNA at week 48 or during year-2 were classified as ‘Non-Responders’, discontinued study and were offered continued entecavir (1 mg) treatment in rollover study ETV-901 or at the discretion of the investigator, alternative off-study anti-HBV therapy. The proportions of patients with HBV DNA <300 copies/mL by PCR assay, ALT normalization and HBeAg seroconversion were assessed during long-term follow-up in ETV-901. Results: At 48 weeks, 19 HBeAg(+) and 3 HBeAg(-) ETV-treated patients were classified as ‘Non-Responders’ (3%). Among patients treated during year-2, 8 additional HBeAg(+) patients became ‘Non-Responders’ (8/269 [3%]). Twenty-one HBeAg(+) patients from ETV-022 enrolled in ETV-901. Median HBV DNA and ALT at entry into ETV-901 were 3.3 log₁₀ copies/mL and 37 IU/L respectively. The table below shows proportions of patients who achieved HBV DNA <300 copies/mL by PCR, ALT normalization and HBeAg seroconversion as well as the proportions of patients who maintained these endpoints at their last observation. Four patients experienced a virologic breakthrough. However, genotypic and phenotypic analysis failed to show any evidence of resistance. No patients discontinued due to adverse events. Conclusions: Fewer than 5% (30/679) of nucleoside-naïve HBeAg(+) and HBeAg(-) patients treated in ETV-022 and ETV-027 met protocol-defined criteria of ‘Non-Response’. Substantial proportions of ‘Non-Responders’ subsequently achieved undetectable HBV DNA and ALT normalization in ETV-901. Approximately one third of patients also experienced HBeAg seroconversion. References 1. Chang TT, et al. NEJM 2006;354:1001-1010. 2. Lai CI, et al. NEJM 2006; 354:1011-1020

<table>
<thead>
<tr>
<th>Endpoint</th>
<th>Achieved endpoint in ETV-901</th>
<th>Maintained endpoint at last observation</th>
</tr>
</thead>
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<tr>
<td>HBV DNA &lt;300 copies/mL, n (%)</td>
<td>15/21 (71%)</td>
<td>12/21 (57%)</td>
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<tr>
<td>ALT normalization, n (%)</td>
<td>20/21 (95%)</td>
<td>13/21 (62%)</td>
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<tr>
<td>HBeAg seroconversion, n (%)</td>
<td>7/21 (33%)</td>
<td>7/21 (33%)</td>
</tr>
</tbody>
</table>

* serology tests were performed by local laboratories

Disclosures:
PROGNOSTIC FACTORS THAT ASSOCIATED WITH HEPATITIS B VIRUS DNA BREAKTHROUGH IN CHRONIC HEPATITIS B PATIENTS WITH LAMIVUDINE TREATMENT
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Objective: To early determine the prognostic factors associated with hepatitis B virus DNA breakthrough in chronic hepatitis B patients with lamivudine treatment. Methods: Clinical data, YMDD genotyping, HBV DNA loads, ALT level, HBeAg titer and lamivudine-resistant HBV mutant ratio, before and during therapy collected from 64 patients throughout lamivudine treatment 48 weeks were retrospectively analyzed by using non-parametric test and logistic regression model test. HBV DNA viral loads were determined by real-time PCR, ALT level were determined by automatic biochemistry analysis system, HBeAg titer were measured by microparticle enzyme immunoassay, and the lamivudine-resistant HBV ratio were determined by Pyrosequencing. Results: the ratio of YMDD and rtL180M mutants were significantly higher in patients who developed HBV DNA breakthrough after lamivudine therapy than before, although they were not significantly associated with HBV DNA breakthrough, for 24 weeks data, multivariate logistic regression analysis revealed that HBeAg titer (24 weeks) was the independent factor for HBV DNA breakthrough (p=0.004, 48w), and was correlated with HBV DNA loads(r=0.493). Conclusions: Pre-existing lamivudine-resistant HBV ratio was not the prognostic factors associated with hepatitis B virus DNA breakthrough, while HBeAg titer after 24 weeks of lamivudine treatment was the independent factor that influenced HBV DNA breakthrough after 48 weeks of lamivudine treatment. Dislosures: The following people have nothing to disclose: Zheng Zeng, Guobao Tian, Di Tian, Jianjun Cui, Haiying Lu

1000 ADEFOVIR DEPIVOXIL FOR DECOMPENSATED LIVER CIRRHOSIS PATIENTS WITH LAMIVUDINE-RESISTANT HEPATITIS B: MULTI-CENTER LONG-TERM RESULTS
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Background/Aim: The effect and safety of adefovir dipivoxil for patients with decompensated cirrhosis were not well known. Long-term efficacy and safety of adefovir dipivoxil in decompensated cirrhosis patients with lamivudine-resistant hepatitis B (HBV) were studied. METHODS: One hundred nineteen cirrhotic patients who developed hepatic decompensation (any cirrhosis complication or Child-Pugh (CP) score ≥ 7) after lamivudine treatment were enrolled from eleven university hospitals and treated with 10 mg of adefovir dipivoxil for a median 33 months between January 2003 and October 2006. Lamivudine was switched to adefovir dipivoxil with or without overlap period (median 2 months). We evaluated the overall survival...
HBV reactivation usually occurs on patients with HBsAg positive, with HBsAg positive developed abnormal liver function easily and especially those with higher viral load. HBV reactivation delayed on the NHL patients with HBsAg negative and anti HBc-IgG positive or even with anti HBsAb positive. Our results suggest that the importance of HBV viral load and liver function surveillance routinely during chemotherapy and necessity of prophylactic antiviral therapy to patients with hepatitis B carriers at the onset of chemotherapy.

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1001 CLINICAL FEATURES OF LYMPHOMA CHEMOTHERAPY ASSOCIATED HEPATITIS VIRUS B REACTIVATION
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Objective: Investigate the incidence of Hepatitis Virus B reactivation and its related severity of hepatitis after the Non-Hodgkin’s Lymphoma (NHL) patients had been administered with chemotherapy, recognize the significance of detecting HBV DNA and employing prophylactic antiviral therapy. Methods: Retrospective study was conducted on 252 NHL patients who had received chemotherapeutics, among which 166 cases with HBV serologic test negative, 86 cases with HBV positive (60 with HBsAg positive, 26 with HBsAg negative and anti HBc-IgG positive). Results: After these 252 NHL patients received chemotherapy, 56 cases occurred hepatitis biochemical abnormality, among which 11 with HBV negative (6.6%), 45 with HBV positive (52.3%), there was a statistically significant difference between these 2 groups (P<0.01). In addition, within the HBV positive group, 39 cases with HBsAg positive (65.0%) and 6 cases with HBsAg negative and anti HBc-IgG positive (23.1%). There was also a statistical difference between these 2 groups (P<0.05). The prevalence of HBV reactivation in patients with HBsAg positive was much higher when compared with those with HBsAg negative and anti HBc-IgG positive (53.3% vs. 7.7%, P<0.01). 32 among 39 patients with HBsAg positive occurred liver function abnormal within three cycles of chemotherapy, nevertheless 4 among 6 patients with HBsAg negative and anti HBc-IgG positive occurred liver function abnormal following five cycles of chemotherapy. Conclusions: NHL patients with HBV positive have a higher incidence of hepatic biochemical abnormality than those with HBV negative. Patients with HBsAg positive developed abnormal liver function easily than those with HBsAg negative and anti HBc-IgG positive. HBV reactivation usually occurs on patients with HBsAg positive and especially those with higher viral load. HBV reactivation delayed on the NHL patients with HBsAg negative and anti HBc-IgG positive or even with anti HBsAb positive. Our results suggest that the importance of HBV viral load and liver function surveillance routinely during chemotherapy and necessity of prophylactic antiviral therapy to patients with hepatitis B carriers at the onset of chemotherapy.

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1002 PREDICTING SUSTAINED HBEAG LOSS AFTER TREATMENT WITH PEGINTERFERON ALPHA-2B: DEVELOPMENT AND VALIDATION OF A PRACTICAL MODEL
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We developed and validated a model based on readily available factors for the prediction of response to peginterferon in individual patients. Methods: Patients randomized to PEG-IFN α-2b 100µg alone (n=136) or in combination with lamivudine 100mg (n=130) for 52 weeks were analyzed. Univariate logistic regression analysis, followed by backward stepwise selection was used to identify predictors of HBeAg loss at 26 weeks post-treatment among the variables age, sex, HBV genotype (A-D), serum HBV DNA, ALT, GGT, treatment allocation and previous antiviral therapy. Interactions between variables and nonlinear relationships were investigated. Discrimination was quantified by the area under the receiver-operating characteristic curve (AUC). Bootstrap sampling was used for internal validation to reduce overfit bias. Results: 23 patients were excluded because of missing values or infection with HBV genotype other than A-D, leaving 233 patients for analysis. HBV genotype, serum HBV DNA, GGT and previous IFN therapy were found to be independent predictors of HBeAg loss (p<0.05). Since the influence of ALT was dependent on HBV genotype, an interaction between these variables was included (p=0.03). A multivariable model based on the above mentioned variables had adequate discriminative ability (AUC 0.73). After bootstrapping, the discriminative ability of the model was found to be somewhat lower (AUC 0.69). A nomogram was generated from the logistic regression formula (figure). The probability of HBeAg loss is calculated by drawing a vertical line from each of the 4 variable axis to the top Points axis. The sum of these 4 points is put on the Total score axis, from which a vertical line is drawn to the Chance of HBeAg loss axis. Conclusion: A multivariable model based on HBV genotype, serum HBV DNA, ALT, GGT and previous IFN therapy provides an adequate prediction of PEG-IFN induced HBeAg loss and will be a useful tool to guide treatment choice of PEG-IFN vs. nucleos(t)ide analogues in individual patients.
1003
THREE-YEAR ASSESSMENT OF ENTECAVIR RESISTANCE IN GENOTYPE C CHRONIC HEPATITIS B PATIENTS IN JAPAN REVEALS DIFFERENT CLINICAL OUTCOMES REGARDING BREAKTHROUGH HEPATITIS PREDICTED BY THE RESISTANCE SUBSTITUTIONS USING RECENTLY DEVELOPED INNO-LIPA HBV ASSAY

Motakazu Mukaida, Yasuhiro Tanaka, Etsuro Orito, Fuat Kurbanoğlu, Kenichi Fukai, Osamu Yokosuka, Michio Sata, Tatsuya Ide, Karino Yoshiyasu, Kohsaku Sakaguchi, Masashi Mizokami.

Background/Aim: Currently, entecavir (ETV) is being considered as a first- or second-choice treatment for nucleoside-naive or lamivudine (LVD)-refractory chronic hepatitis B patients. To assess frequency of ETV-resistance (ETVr) and the specific substitutions associated with breakthrough hepatitis (BTH) during 3-years ETV therapy, recently developed INNO-LIPA HBV DR v2 and ETV TDF assay was applied. Methods: Forty-five patients including 25 chronic LVD-refractory genotype C patients (n=22/23, mean age: 41.6 yrs, ALT: 129.1 HBeAg: 36 and HBV-DNA (TMA): 6.98) and 20 naïve patients were enrolled. Drug resistance was assessed by detection L80I, V173L, M204I and L259F for LVD and T184A/C/G, A194T, S202G/C for ETV. Results: In 25 LVD-refractory patients, the ETVr substitutions (SCGA184, ILMF184, G202, C202, VL250) were present in 3/25 (12.5%) at baseline and emerged in 5/25 (20.8%), and 7/25 (29.4%) and 5/17 (29.2%) at weeks 48, 96, and 144, respectively, versus 8%, 8%, 0%, and 13% in naïve patients, respectively. Five of the 25 LVD-refractory (20%) patients treated with 1.0 mg ETV had evidence of BTH after 72 to 136 weeks of the therapy. Of the all BTH patients, LVDr substitutions (M180 and V204) were detected at baselines, and an additional G202 ETVr substitution occurred (0-96 weeks), resulting in BTH 40-72 weeks later. Other substitutions emerged during ETV therapy, but none of them seemed to affect susceptibility to ETV or associated to BTH. Conclusions: INNO-LIPA is useful in early ETVr detection. In LVD-refractory, genotype C, Japanese patients, LVD (M180 and V204) plus G202 ETVr substitutions resulted in BTH. High and long-term efficacy of ETV was demonstrated in naïve patients, whereas adefovir add-on LVD should be considered as a first-, or second-line treatment option for LVD-refractory chronic hepatitis B patients.

Table 1. ETVr associated substitutions emerged in LODr cohort during follow up

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<th>ETVr-refractory patients</th>
<th>weeks</th>
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<th>ILMF184</th>
<th>G202</th>
<th>C202</th>
<th>VL250</th>
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<tr>
<td>0</td>
<td>25</td>
<td>1(4%)</td>
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<td>1(4%)</td>
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<td>1(4%)</td>
</tr>
<tr>
<td>48</td>
<td>25</td>
<td>1(4%)</td>
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<tr>
<td>96</td>
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<td>6(24%)</td>
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<tr>
<td>144</td>
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<td>2(11.8%)</td>
<td>1(5.9%)</td>
<td>3(17.6%)</td>
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Table 1004
THE EFFECTS OF α-GALACTOSYLCERAMIDE ON CHRONIC HEPATITIS B INFECTION IN A RANDOMIZED PLACEBO CONTROLLED PHASE I/II TRIAL


Background: The glycosphingolipid alpha-galactosylceramide (α-GalCer) has been shown to have an immunomodulatory effect mediated via the activation of natural killer T (NKT) cells and is able to inhibit hepatitis B virus replication in HBV transgenic mice. Aim: The aim of this dose-escalating randomized placebo-controlled phase I/II trial was to investigate the antiviral activity and safety of α-GalCer as a novel class of treatment for chronic hepatitis B patients. Methods: Twenty-seven patients were allocated to a α-GalCer dose of 0.1 μg/kg (n=8), 1 μg/kg (n=9), 10 μg/kg (n=6) or to placebo (n=10). All patients had an HBV DNA level above 10^5 copies/ml at screening and ALT values >1.2 times the upper limit of normal. The treatment period consisted of intravenous injections with α-GalCer activated NKT cells are able to inhibit hepatitis B virus replication in HBV transgenic mice. Aim: The use of this dose-escalating randomized placebo-controlled phase I/II trial was to investigate the antiviral activity and safety of α-GalCer as a novel class of treatment for chronic hepatitis B patients. Methods: Twenty-seven patients were allocated to α-GalCer dose of 0.1 μg/kg (n=8), 1 μg/kg (n=9), 10 μg/kg (n=6) or to placebo (n=10). All patients had an HBV DNA level above 10^5 copies/ml at screening and ALT values >1.2 times the upper limit of normal. The treatment period consisted of intravenous injections with α-GalCer at 0, 4 and 8 weeks. Thereafter, patients were monitored for an additional 16 weeks. Results: Although some patients showed an HBV-DNA decrease after administration, no clinically relevant suppression of viral replication was observed. Four patients exhibited a more than 0.5 log_{10} copies/ml decrease in HBV DNA fol-
following the first administration (1 µg/kg n=2, 10 µg/kg n=2) with a median decline in the first week of 1.1 log_{10} copies/mL (range 0.5 – 3.0) from baseline, but this decline was not sustained and also not observed after the second and third administration of α-GalCer. There were no clear changes in ALT levels. In three patients of the highest dose level, a rise in IL-6 production in serum was observed four hours after drug administration. In four patients (1 µg/kg n=1, 10 µg/kg n=3) α-GalCer was discontinued prematurely because of an episode of fever and severe rigors shortly after drug administration. Otherwise there were no significant side effects. Conclusion: α-GalCer used as monotherapy for chronic hepatitis B infection at the doses (0.1-10 µg/kg) used in this trial had no clear effect on HBV DNA and ALT levels, was poorly tolerated, and is unlikely to provide an alternative as monotherapy for the current treatment of peg-IFN and nucleos(t)ide analogs.

Disclosures:
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1005 CROSS-RESISTANCE CHARACTERIZATION OF THE MAIN HBV DRUG RESISTANT MUTANTS
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Introduction - Currently, four nucleoside analogues are approved for the treatment of chronic hepatitis B: lamivudine, adefovir, entecavir and telbivudine. They suppress viral replication through inhibition of HBV DNA polymerase and chain termination. However, prolonged treatments with these analogues result in the emergence of resistant virus harbouring mutations within the HBV polymerase gene which are associated with resistance to treatment. Objectives - We isolated and cloned HBV DNA genomes from patients who developed viral resistance following various therapies. We determined in vitro the antiviral susceptibility of the main resistance mutants to nucleoside analogues approved for chronic HBV treatment, and tested new nucleoside analogues on these mutants. Results - A bank of mutants, constructed by site directed mutagenesis or from patients serum samples were intensively analyzed. We could observed that all the mutants harbouring the rtM204V substitution, except the rtM204V mutation alone, showed a >1000 fold decrease in susceptibility to lamivudine as compared to wild-type (wt) HBV. We also characterized different mutants harbouring the rtA181V mutation and all these mutants were >2.7 fold less sensitive to adefovir than wt HBV. This in vitro decrease in susceptibility to adefovir seems to be sufficient to induce in vivo viral breakthrough. Concerning entecavir, all the mutants harbouring the rtS202G substitution, but only in addition to rtL180M+M204V main lamivudine resistance mutations showed a >200 fold resistance to this drug in comparison with wt HBV. Tenfovir seems to be one of the most efficient drugs on these mutants, since only two of them (rtN236Ts and rtL180M+M204V+N236T) showed a decrease in susceptibility to tenofovir as compared to wt HBV. We also tested new nucleoside analogues for their capacity to inhibit replication of drug resistant mutants and demonstrated that PMEO, a novel pyrimidine analogue, showed a better activity than adefovir on all the tested mutants except the rtN236T mutant. Phenotypic characteristics of additional complex mutants were also determined. Conclusion - These data on antiviral susceptibility of the main HBV polymerase mutants observed in patients provide useful information to guide anti-HBV therapy.

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The following people have nothing to disclose: Stephanie Villet, Fabien Zoulim

1006 DETECTION OF HEPATITIS B VIRUS YMDD MUTANTS BY MULTIPLEX-PCR USING DUAL PRIMING OLIGONUCLEOTIDE (DPO)
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Background: The mutants of hepatitis B virus (HBV) emerge selectively during long-term lamivudine therapy. Since lamivudine resistance has cross-resistance with other antiviral agents, initial choice of antiviral agents for prevention of multi-drugs resistance is very important. According to recent studies, early detection of HBV mutants at 3 months is useful to predict the long-term outcomes of lamivudine therapy. In addition, predominance of mutants is significantly associated with viral DNA breakthrough. Therefore, early detection and periodical testing of HBV mutants is needed. However current methods of detecting mutants are time-consuming and labor intensive which require multiple steps and expensive instruments. Aims: To determine the effect of recently developed YMDD genotype method using Multiplex-PCR technology, we compared this method with restriction fragment mass polymorphism (RFMP) and direct sequencing method. Patients and Methods: Total eighty chronic hepatitis B patients treated with lamivudine more than 6 months participated in this study. YMDD mutants were genotyped by RFMP using MALDI-TOF mass spectrometry, sequencing and Multiplex-PCR using dual priming oligonucleotide (DPO). Results: The Multiplex-PCR method with DPO was available for sensitive, specific and simultaneous detection of 4 types of HBV mutants (rtM204V/I/S, rtL180M) by only one time PCR. Additionally, this assay enabled to estimate relative abundance of mutants in variant/variant mixed or variants/wild type mixed serum by agarase gel electrophoresis. The Multiplex-PCR assay detected as few as 1000 copies/mL of HBV genome plasmids, also detected as little as 2% mutant plasmids. The total protocol was carried out easily within 4 hours. The patients with breakthrough (virologic or biochemical), early viral response to lamivudine therapy, HbeAg positivity, HBV-DNA >100,000 copies/ml and elevated ALT (>2 times upper limit of normal) had higher mutation rate. For these patients, Multiplex-PCR with DPO was the most sensitive method for detection of the mutants (especially mixed variants). Interestingly, 18 of 80 patients (22.5%) had single or mixed variants, these mutants only detected by the Multiplex-PCR, but not by RFMP or sequencing. These results indicate that Multiplex-PCR with DPO is more sensitive than RFMP and sequencing to detect variants. Conclusions: Multiplex-PCR with DPO is rapid, sensitive and cost-effective method for detection of the YMMD mutants and relative quantitation as well. Therefore, this method can be useful diagnostic tool for early detection of HBV mutants and estimate their predominance, thereby preventing drug resistance and early rescue therapy.

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Background/Aims: The growing number of HBV nucleos(t)ide inhibitors, the widening of anti-HBV sequential and/or combination therapies, and the viro-clinical impact of drug resistance mutations warrant surveys of HBV genotypic drug resistance. Therefore, we performed a 5-year retrospective survey during the 2002-04/2007 period aiming to analyse the prevalence and dynamic of HBV reverse transcriptase (rt) drug resistance mutations patterns in patients (pts) chronically-infected with HBV followed-up in Marseilles public hospitals. Patients/Methods: 381 HBV rt sequences from 312 pts (70% male; mean age, 47) were analysed. 39 pts have been tested twice, and 12 pts >2 times. For each year from 2002 to 2007, only one sequence per >18 year-old pt was selected. Serum HBV DNA direct sequencing was performed using in house protocols. 25 amino acid (aa) positions within domains A-E of HBV rt and previously associated with drug resistance were analysed, which included all positions at which association of aa substitution with drug resistance is clearly established. Results: A nearly 1.7-time increase of the number of HBV sequences was observed in 2006 (n=96) as compared to 2002-2003 (55 and 57, respectively). The mean number of aa substitutions per sequence ranged between 2.1 in 2003 and 1.7 in 2007, without significant change. The proportion of non-A HBV genotypes increased from 53.54% in 2002-2003 to >60% in 2006-04/2007 (p=0.24). The proportion of sequences harbouring >3 aa substitutions was significantly higher for genotypes A vs genotypes D or non-A/non-D (23% vs 11 and 9%, respectively; p<0.05). In contrast, the proportion of aa substitutions was significantly higher at positions 80, 181, 215, and 237 for genotypes D vs genotypes A (p<0.02). The most variable aa positions were rt204 and rt180. Substitution rtM204I/V was found in 71 sequences (19%), associated with L180M in all but one case for M204V and with A181V/T in two cases. Substitution rtA181V/T was found in 4.7% of sequences (n=17) and 6.4% in 2007. The proportion of mutated sequences significantly increased between 2002 and 2007 at positions 236, 237, and 238 (p<0.05). Substitution rtN236T was observed in 6 sequences (1.8%), always associated with rt204 wild-type aa, and was significantly associated with rtA181V and rt169L (in 2 sequences each; p<10-3). Conclusions: Our data underline the changing pattern of HBV drug resistance mutations and the association or exclusion of rt aa mutations in the setting of emerging nucleos(t)ide inhibitors and combination and/or sequential anti-HBV therapies. They also suggest that genotypic drug resistance patterns might differ according to the HBV genotype.

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The following people have nothing to disclose: Philippe Colson, Rene Gerolami, Christian Tourres, Anne Motte, Isabelle Ravaux, Isabelle Poizot-Martin, Jacques Moreau, Patrick Borentain, Mireille Henry, Catherine Tamalet
ECTOPIC EXPRESSION OF NEURAL AUTOANTIGEN IN LIVER SUPPRESSES AUTOIMMUNE NEUROINFLAMMATION BY INDUCING ANTIGEN-SPECIFIC CD4+ CD25+ FOXP3+ REGULATORY T CELLS

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CD4+ CD25+ Foxp3+ regulatory T cells (Treg) are important mediators of immune tolerance to self-antigens, and their inactivation may cause autoimmune disease. Therefore, immunotherapy for autoimmune diseases may aim at restoring or expanding autoantigen-specific Treg activity. Here we report that Treg-mediated suppression of autoimmune disease could be achieved in vivo by taking advantage of the liver’s ability to promote immune tolerance. Ectopic expression of the neural autoantigen myelin basic protein (MBP) in the liver, which was accomplished constitutively in liver-specific MBP-transgenic mice, or transiently after hydrodynamic gene transfer to liver cells in vivo, induced protection from experimental autoimmune encephalomyelitis (EAE), a mouse model for human multiple sclerosis. A role of central tolerance in the observed resistance to EAE was excluded by neonatal thymectomy. Protection from EAE was mediated by MBP-specific CD4+CD25+Foxp3+ Treg cells, as demonstrated by adoptive transfer to non-transgenic mice and by their ability to suppress conventional CD4+CD25- T cells after antigen-specific stimulation with MBP in vitro. Our findings indicate that autoantigen expression in the liver may generate autoantigen-specific Treg cells. Thus, targeting of autoantigens to hepatocytes may be a novel therapeutic approach to prevention or treatment of autoimmune diseases.

A) Transient hepatic MBP expression facilitated by hydrodynamics-based gene transfer to hepatocytes with MBP-encoding pcDNA3.1 expression vector (black circles, unbroken line), but not by empty control vector (white circles, broken line) suppresses EAE.

B) Protection of wild-type mice from autoimmune encephalomyelitis by adoptive transfer of 10^5 splenic CD25+ CD4+ T cells from MBP-transgenic mice (black circles, unbroken line), but not by transfer of 10^5 splenic CD25- CD4+ T cells from MBP-transgenic mice (white squares, broken line).

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CRITICAL ROLE FOR CD1D-RESTRICTED Vα14 INKT CELLS IN STIMULATING INTRAHEPATIC CD8 T CELL RESPONSES TO LIVER-EXPRESSED ANTIGEN

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Background and aims. Activation of CD1d-restricted Vα14 invariant (i) NKT cells rapidly induces their secretion of effector cytokines. As Vα14 iNKT cells are localized in peripheral tissues such as the liver rather than lymphoid tissues, their role in modulating the stimulation of conventional, MHC-restricted T cell responses has remained ambiguous. We here describe a role for Vα14 iNKT cells in modulating conventional T cell responses to antigen expressed in liver, using transferrin-mOVA (Tf-mOVA) mice. Methods. Naïve ovalumin-specific Class I MHC-restricted T cells (OTI) were adoptively transferred into Tf-mOVA mice in the presence or absence of CD1d-ligand α-galactosylceramide, after which their priming, antigen-specific cytokine production and liver damage were analyzed. Results. Transfer of OTI cells resulted in robust intrahepatic, antigen-specific proliferation of T cells. OTI T cells were activated in liver, and IFNα production by OTI T cells was stimulated by co-activation of Vα14 iNKT cells using αα-galactosylceramide. This stimulation was absent in CD1d-/- Tf-mOVA mice, which lack Vα14 iNKT cells. OTI T cells induced hepatitis and liver damage was corroborated by α-galactosylceramide injection. Conclusion. CD1d-restricted Vα14 iNKT cells stimulate intrahepatic antigen-specific effector CD8 T cell responses. Our findings elucidate a previously unknown intervention point for targeted immunotherapy to autoimmune and possibly infectious liver diseases.

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The following people have nothing to disclose: Dave Sprengers, Fenna C. Sillé, Gurdyal S. Besra, Harry L. Janssen, Eckart Schott, Marianne Boes

CTS-1027, A POTENT MMP INHIBITOR, PROTECTS AGAINST TNFα- AND α-FAS-INDUCED LIVER INJURY

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Background. CTS-1027 is an orally bioavailable small molecule that is a potent inhibitor of MMP-2, 3, 8, 9, 12, 13 and 14, but not MMP-1. In the liver, in response to a variety of insults, excessive MMP activity may play a role in destruction of the extracellular matrix, and recruitment of inflammatory cells. CTS-1027 was previously evaluated in an extensive phase 2 clinical trial in which a well tolerated chronically administered dose was identified. Thus, it was of interest to evaluate a MMP inhibitor whose safety profile was known in models of acute liver injury in mice. Methods. CTS-1027 (0.001-30 mg/kg) was administered PO to male mice (C57BL/6), 30 minutes prior to treatment with TNFα/D-Galactosamine (D-Gln) or the Fas activating antibody (α-Fas). Six hours later, animals were anesthetized with Nembutal and blood taken by cardiac puncture. Plasma ALT activity was determined using a commercially available kit. For survival studies, mice were observed up to 24 hours post-injection. Pharmacokinetic analysis of CTS-1027 was
conducted in treatment-naive mice to determine plasma and liver levels. **Results:** CTS-1027 dose-dependently decreased plasma ALT activity in the TNFαr model. The average ED50 from 4 studies was 0.26 ± 0.08 mg/kg. Twenty-hour survival was also increased by CTS-1027 (10 mg/kg). The average 24 hour survival from 3 studies was 27 ± 7.3% and 55 ± 7.6% (p = 0.03) in the TNFαr/D-Gln control mice and CTS-1027-treated mice, respectively. CTS-1027 (10 mg/kg; PO) was also protective against α-Fas, significantly (p<0.05) reducing the elevation in plasma ALT activity in 2 independent studies by an average of 49%. In a separate group of mice, PK analysis of CTS-1027 in plasma vs. liver indicated that CTS-1027 was rapidly absorbed with Tmax of 0.25-0.5 hour in liver and plasma. AUCs increased in a dose-dependent manner and were 1.7-fold greater in liver than plasma. The AUC in plasma at the αED50 dose in the TNFαr/D-Gln model (~0.3 mg/kg) was 13-fold lower than the AUC at a dose previously determined to be well tolerated in man. **Conclusions:** CTS-1027 potently antagonized the liver toxicity induced by treatment with TNFαr/D-Gln or α-Fas as indicated by reductions in plasma ALT and/or lethality/morbidity at doses lower than the well tolerated dose in man. CTS-1027 is an attractive candidate for further development and is expected to enter clinical trials in patients with liver disease by the end of 2007.

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Patricia C. Contreras - Employee: Other
Karen Valentino - Employee: Other

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**1012 INVESTIGATING THE ROLE OF AUTOACTIVE CD8+ AND CD4+ T CELLS IN THE PATHOGENESIS OF AUTOIMMUNE HEPATITIS IN A TRANSGENIC MOUSE MODEL**

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Autoimmune hepatitis (AIH) is a chronic active liver disease of unknown cause. Autoreactive lymphocytes are thought to mediate the liver injury but the pathogenesis of AIH remains poorly understood. To investigate liver-specific CD4+ and CD8+ T cell responses and the role of autoreactive T lymphocytes in the pathogenesis of AIH, we have generated transgenic mice that express the influenza virus hemagglutinin (HA) neoantigen in the liver under the control of albumin regulatory elements (Alb-HA mice). Adoptive transfer of HA-specific naive CD8+ T cells into Alb-HA mice leads to an activation of autoreactive CD8+ T cells in the liver or in liver-draining lymph nodes and elicits a transient hepatitis characterized by portal and lobular inflammation and elevated serum level of alanine aminotransferase (ALT). Adoptive transfer of HA-specific naive CD4+ T cells into Alb-HA mice leads to an activation of autoreactive CD4+ T cells in liver-draining lymph nodes or in the spleen and causes mild transient necroinflammatory lesions of the liver. These data suggest that both, CD8+ and CD4+ T cells play an active role in the pathogenesis of AIH and that tolerance mechanisms keep autoreactive T lymphocytes specific for a liver antigen normally in check.

**Disclosures:**
The following people have nothing to disclose: M. Zierden, E. Kühnen, M. Odenenthal, H. P. Dienes
1014 DELETION OF CD39 INHIBITS DEVELOPMENT OF METASTATIC LIVER CANCER
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New vessel formation and tumor infiltrating lymphocytes (TIL) influence host responses to malignant tissues. Extracellular adenosine-mediated pathways promote both vascular endothelial cell proliferation and inhibit cytotoxic T cells, thereby potentiating cancer growth. CD39 is the dominant ectonucleotidase of vascular and T regulatory cells and has the potential to generate high levels of adenosine locally. We have previously shown that deletion of Cd39 results in angiogenic failure and T regulatory cell dysfunction with loss of immune suppressive functions. Aim: Investigate impact of CD39 upon development of hepatic metastases. Methods and Results: We studied the development of metastatic liver deposits following portal vein infusion of 1.5x105 melanoma B16/F10 cells, with luciferase expression, in wild type and Cd39-null C57BL/6 mice (n=24). Tumor formation in liver was directly examined and animals imaged at days 7-17 after tumor cell implantation. As predicted, the formation of hepatic malignant foci was markedly suppressed in Cd39-null mice, at all time points examined. To test whether the major impact of Cd39-deletion was upon neo-vasculature formation or immune responsiveness, adoptive transfer experiments were conducted. Bone marrow transplants (BMT) from Cd39-null or wild type BL/6 mice were placed in lethally irradiated control and/or null mice, in a crossover manner (total n=24 for each group, respectively). Eight weeks post-adoptive transfer, melanoma cells were infused via portal vein as before and tumor growth studied. The Cd39-null mice that received wild type BMT mirrored the wild type phenotype with progressive tumor growth observed (n=8 per time point; p=0.015). In contrast, metastases were significantly inhibited in both number and size and ultimately became necrotic in the wild type mice that had received Cd39-null BMT. Conclusions: Bone marrow derived cells mediate the major inhibitory effects of Cd39 deletion on tumor growth. Pharmacological inhibition of CD39 may find utility as an adjunct therapy in the management of hepatic malignancy.

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1015 EVIDENCE FOR ALTERNATIVE- AND CLASSICAL-PATHWAY INDEPENDENT ACTION OF COMPLEMENT FACTOR C3 DURING LIVER REGENERATION
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The liver regenerates itself following injury. Recently, the complement pathway has been implicated as important for the regenerative response. For example, both complement factor C3 and C5-null mice show impaired hepatic regeneration that can be rescued by exogenous supplementation of the respective factors. However, the specific mechanism by which complement pathway signaling is activated during liver regeneration (classical, alternative, or lectin-dependent) has not been defined. The classical and lectin dependent pathways of complement activation are absolutely dependent on complement factor C4, while the alternative pathway of activation requires factor B. In the studies reported here we investigated whether either C4 or factor B is required for normal liver regeneration using the mouse partial hepatectomy model. Methods: 2-3 month old wildtype (WT) C57Bl/6 and C3-, Factor B-, and C4-null mice on C57Bl/6 backgrounds were subjected to partial hepatectomy (PH) or sham surgery, allowed to recover, and then sacrificed for plasma and tissue harvest. 3-6 animals were examined per time point and genotype. Liver tissue was formalin-fixed, paraffin-embedded, and stained with H&E or for bromo-deoxyuridine (BrdU). Cyclin D1 protein expression was assessed by protein immunoblot. Results: Compared to WT mice, C3-null mice had reduced hepatocellular proliferation and delayed peak proliferation, as well as suppressed induction of hepatic cyclin D1 protein expression. At 48h after PH, hepatocellular mitotic body frequency was significantly reduced in C3-null mice, compared to WT mice. In contrast, both C4-null and Factor B-null mice showed normal hepatocellular BrdU incorporation, cyclin D1 expression, and mitotic body frequency. Neither C3-, C4- nor Factor B-null mice had increased mortality or increased liver tissue injury. Conclusion: Our results suggest that neither the C4-dependent classical or the lectin-dependent pathways nor the factor B-dependent alternative pathway of complement cascade activation is absolutely required for normal liver regeneration. These data raise the possibility that other mechanisms exist by which C3 and C5 can be activated during the regenerative response.

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1016 HEPATOCYTE SURVIVAL IN ACUTE HEPATITIS IS DUE TO C-JUN/AP-1-DEPENDENT EXPRESSION OF INDUCIBLE NITRIC OXIDE SYNTHASE
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Analysis of the molecular factors determining hepatocyte survival or death in response to inflammatory stimuli is essential for understanding the pathogenesis of inflammatory liver disease and for identifying novel therapeutic approaches. Jun N-terminal kinase (JNK) is a major mediator of cytokine-induced cell death during hepatitis, but the signaling pathways downstream of JNK remain less well defined. Here we show that the transcription factor c-Jun/AP-1, a prototypic target of JNK, is strongly expressed in the liver of patients with acute liver injury of viral or toxic etiology. The contribution of c-Jun to cell death was further analyzed in mice using the Concanaavalin A (ConA) model of TNFα-dependent hepatitis. Mice lacking c-Jun in hepatocytes display increased liver cell death and mortality upon ConA injection. This phenotype is due to impaired expression of inducible nitric oxide synthase (nos2, also known as iNOS), which we identified as direct transcriptional target of c-Jun during hepatitis, and reduced production of hepatoprotective nitric oxide (NO). The increased hepatotoxicity and hepatocyte necrosis in mutant mice are likely caused by hypoxia and oxidative stress and can be rescued pharmacologically by liver-specific delivery of NO or by the antioxidant N-Acetylcysteine. These findings demonstrate that c-Jun/AP-1 mediates hepatocyte survival during acute hepatitis by regulating nos2 expression and thus functionally antagonizes the cell death-promoting functions of JNK. Moreover, our data provide a strong rationale for further exploring NO-based therapies for the treatment of severe liver injury.
EFFECTS OF COPY NUMBER VARIANTS (CNVS) OF THE CHEMOKINE GENE CCL3L1 ON Susceptibility to HCV INFECTION

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Recently a novel class of genomic diversity, copy number variants (CNVs), of the CCL3L1 gene have been associated with susceptibility to HIV infection (Gonzales et al. Science 2005;307:1434-40). The chemokine CCL3L1 is a potent ligand for CCR5, which plays a central role in anti-viral lymphocyte activity. Of note, CCR5 deficiency has been associated with differences in susceptibility to hepatitis C virus (HCV) infection and the hepatic fibro-inflammatory reaction. Hence, variation in the gene dose of CCL3L1 due to CNVs may also contribute to differences in the overall susceptibility to HCV infection and/or the inflammatory response. Thus, our aims now were to compare the CCL3L1 CNV distributions between patients with chronic HCV infection, HCV/HIV co-infection and non-infected controls, and to test for association with histological grades of inflammation and stages of fibrosis in patients with chronic HCV infection.

Patients and methods: Patients with chronic HCV infection (n=254), HCV/HIV co-infection (n=144) and non-infected controls (n=210) were recruited. CNVs of the CCL3L1 gene were determined using a real-time fluorescent-dye labelled PCR adapted from Gonzales et al. (Science 2005;307:1434-40), and CNVs were compared between groups using tests and non-parametric Mann-Whitney-U tests. Results: Copy numbers of the CCL3L1 gene are in line with previously published data for non-Africans, ranging from 0 to 12 copies (mean 2.5 ± 1.4 copies). Consistent with previous studies, CCL3L1 copy numbers of HCV/HIV co-infected patients are significantly lower compared to HCV patients (2.2 vs. 2.6; P < 0.01) and controls (2.2 vs. 2.9; P < 0.001). Of note, CCL3L1 copy numbers of HCV mono-infected patients are also significantly lower compared to controls (2.6 vs. 2.9; P = 0.011). Accordingly, CNV distributions are shifted to lower copy numbers in HCV mono-infected patients compared to controls (P = 0.036) and in HCV/HIV co-infected patients compared to HCV mono-infected patients or controls (both P < 0.01). Patients with two or less copies of the CCL3L1 gene are over-represented in the HCV cohort compared to controls (OR = 1.54; 95% CI 1.07 - 2.55; P = 0.02). HCV/HIV patients show a similar over-representation of less than two or two CCL3L1 copies (OR = 3.42; 95% CI 2.18 - 5.38; P < 0.001). However, no association is found between CCL3L1 CNVs and grades of inflammation or stages of fibrosis in HCV mono-infected patients. Conclusions: HCV patients carry lower CCL3L1 gene copy numbers than non-infected controls. Our findings indicate for the first time that susceptibility to HCV infection might be influenced by copy number variation within the host’s genome.

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INHIBITION OF NKT CELL ACTIVATION AND THE DEVELOPMENT OF HEPATITIS BY A GLYCOSAMINOGLYCAN-BINDING DEFICIENT RANTES MUTANT

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Background: Hepatitis is associated clinically with an increase in hepatic chemokine expression, including RANTES/CCL5, as well as the activation of NKT cells within, and recruitment of lymphocytes to, the liver. Recently, a glycosaminoglycan-binding deficient mutated version of CCL5 has been developed (GAG-CCL5) and demonstrates anti-inflammatory properties, via a mechanism which remains unclear. Methods/Results: Concanavalin A-induced hepatitis is a well characterized murine model of T cell mediated hepatitis which is critically dependent upon hepatic NKT cell activation. Using this model we have documented a significant inhibition of hepatitis in mice treated with GAG-CCL5 prior to concanavalin A (con A) administration, as reflected by serum ALT levels and histology [serum ALT 8 hrs post-con A: veh 8186 ± 489[SEM] U/L vs GAG-CCL5 1514 ± 230; p<0.01;n=5/group]. This inhibition of hepatitis was associated with a significant suppression of hepatic NKT cell activation as reflected by a > 50% reduction in the number of hepatic NKT cells producing either IL-4 or IFNγ in GAG-CCL5 vs vehicle treated mice receiving Con A (p<0.05 vs respective controls for both; NKT cell cytokine production determined by flow cytometry). We then used the specific in vivo NKT cell activator α-galactosylceramide (given as an intraperitoneal injection) to confirm that GAG-CCL5 was directly inhibiting the activation of hepatic NKT cells (as reflected by a > 65% reduction in hepatic NKT cell IFNγ production in GAG-CCL5 treated vs vehicle treated mice, as determined by flow cytometry; p<0.05). We next examined the effects of GAG-CCL5 in acetaminophen (APAP)-induced hepatitis which is known to be dependent upon both hepatic NKT cell activation and NK cell recruitment into the liver. In this model, GAG-CCL5 again significantly suppressed hepatitis [serum ALT 24 hrs post-APAP: veh 2378±275 vs GAG-CCL5 1094±145; p<0.05;n=4/group] and inhibited hepatic NKT cell activation (reflected by a >50% reduction in the numbers of IFNγ-producing hepatic NKT cells in GAG-CCL5 vs vehicle treated mice which received APAP; p<0.05) and also significantly inhibited the recruitment of NK cells, and IFNγ-producing NK cells, into the liver (>50% reduction in number of NK cells/liver in GAG-CCL5 treated mice vs vehicle treated mice receiving APAP; p<0.05). Conclusion: In summary, GAG-CCL5 inhibits the development of experimental hepatitis by preventing hepatic NKT cell activation, indicating that GAG-CCL5 may represent a novel therapeutic approach for the treatment of patients with hepatitis by targeting the innate immune system within the liver.

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The following people have nothing to disclose: Maureen N. Ajuebor, Amanda Proudfoot, Cory Hogaboam, Tai Le, Mitch Kronenberg, Mark Swain
1019 CONTRIBUTION OF TRIF TO SECRETION OF MATURE IL-1β AND IL-18 VIA THE TLR-MEDIATED CASPASE-1 ACTIVATION DURING EXTRACELLULAR BACTERIAL INFECTION IN MICE

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Innate immune cells (such as macrophages) participate in early host defense against pathogens by production of proinflammatory cytokines. We previously reported that Propionibacterium acnes-primed mice are highly susceptible to LPS and develop endotoxin shock or liver failure by their hyper-production of and hyper-responsiveness to TNF, IL-1β and IL-18. Both IL-1β and IL-18 are synthesized as precursor form and become active after being cleaved with caspase-1. Caspase-1 is also produced as precursor form and become enzymatically active when innate cells are stimulated with LPS or other appropriate stimuli. Two distinct pathways are activated for caspase-1-dependent release of IL-1β- and IL-1β- by stimulation with LPS. LPS alone induces caspase-1-dependent release of IL-1β- and IL-18 via activating extracellular sensor, TLR4 but independently of MyDD8. On the other hand, LPS plus ATP can induce those responses through activating intracellular sensor NOD-like receptors (NLRs), but not TLR4, which is due to intracellular influx of LPS by ATP. Since TRIF and TRAM are responsible for transducing MyDD8-independent signaling, we investigated whether these common signal adaptors are involved in LPS-induced caspase-1 activation. LPS stimulation of wild type Kupffer cells accelerates caspase-1 activation, while TRIF or TRAM deficient Kupffer cells fail to do it, indicating that both adaptors are essentially required. However, LPS plus ATP could induce activation of caspase-1 in TRIF-deficient Kupffer cells, indicating the dispensability of TRIF. After infection with Escherichia coli, extracellular bacterium, wild-type mice, but not tlr4-/-, trif-/- or caspase 1-/- mice, showed elevated serum levels of IL-18, indicating the importance of TLR/TRIF-mediated caspase-1 activation in vivo. These results demonstrate that mammalian host equips two caspase-1 activation machineries, i.e. TLR-mediated TRIF-dependent and NLR-mediated TRIF-independent pathways, and that TRIF links extracellular bacterial pathogen with caspase-1 activation would provide new regimen for the treatment of inflammatory liver diseases associated with aberrant activation of caspase 1.

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1020 ALCOHOL CONSUMPTION INDUCES EXPRESSION OF SOCS3 AND SOCS1 AND INHIBITS SIGNALING VIA STAT3 AND STAT1 PATHWAYS IN HUMAN MONOCYTES

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Introduction: Acute alcohol consumption is associated with downregulation of pro-inflammatory responses to various pathogens, induction of immunoinhibitory cytokines, and poor responses to IFNα treatment in HCV infection. We previously reported that alcohol activates JAK1-STAT3 signaling leading to IL-10 induction. The JAK-STAT pathway also activates its own negative regulator, suppressors of cytokine signaling, SOCS1 and SOCS3. SOCS proteins are inducible inhibitors that negatively regulate STAT3/STAT1 signaling pathways induced by cytokine, IL6 or IFNα. Aim: To explore the effect of alcohol on induction of SOCS1/SOCS3 and regulation of STAT3/STAT1 pathways induced by IL6 or IFNα in human monocytes in vitro and in vivo. Methods: Monocytes were isolated from blood samples of treatment-naive patients with chronic hepatitis C infection. Blood samples from normal volunteers were collected before and 24 hours after consumption of 2 ml vodka/kg body weight. In vitro experiments human monocytes were treated with 25-50mM of alcohol with or without cytokines for 45 min-5 h. Whole cell lysates were analyzed by western blot, nuclear proteins by EMSA, mRNA by real time RTPCR. Results: Alcoholic treatment of monocytes for 48h downregulated SOCS3 mRNA expression in monocytes. Alcohol treatment resulted in increased phosphorylation of STAT3 at tyr705 and ser727 residues and increased STAT3 DNA binding. Activation of STAT3 has been shown to induce expression of SOCS1 and SOCS3. We hypothesized that induction of SOCS proteins by alcohol may also lead to modulation of cytokine signaling through STAT1 and STAT3. Indeed, we observed significant downregulation of both IL6- and IFNα-induced STAT3 and STAT1 DNA binding when alcohol was added to monocytes 3 hours prior to the cytokine stimulation. To address whether inhibition of IL6-induced STAT3 DNA binding had downstream effects, we measured mRNA levels of IL6-dependent genes, MCP-1 and ICAM-1. We detected significant inhibition of IL6-induced expression of MCP-1 and ICAM mRNA in monocytes after acute alcohol exposure. Finally, we found increased baseline as well as inducible STAT1 and STAT3 DNA binding in monocytes of patients with chronic HCV infection compared to normal controls. Importantly, acute alcohol inhibited both IL6- and IFNα-induced STAT1/3 activation in monocytes of HCV patients. Conclusion: Alcohol activates STAT3 signaling pathway and induces SOCS3 and SOCS1 levels in monocytes, thus leading to downregulation of IL6- and IFNα-induced signaling via STAT1/STAT3 pathways. These mechanisms may be involved in attenuated responses to IFNα therapy after alcohol use in HCV infection.

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1021 ACTIVATION OF CYCLIC-AMP RESPONSE ELEMENT BINDING PROTEIN CONTRIBUTES TO ADIPONECTIN-STIMULATED INTERLEUKIN-10 EXPRESSION IN RAW 264.7 MACROPHAGES

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Adiponectin, an adipokine predominantly secreted from adipose tissue, has potent insulin-sensitizing effects in liver and muscle, as well as potent anti-inflammatory properties in macrophages. Adiponectin concentrations in the circulation are decreased in response to chronic ethanol feeding; treatment with adiponectin during chronic ethanol exposure protects mice from the development of ethanol-induced liver injury (Xu, et al., 2003). The mechanisms for the protective effects of adiponectin are likely complex. However, recent data suggests that adiponectin's anti-inflammatory properties play a critical role in its protective effects against ethanol-induced liver injury, since adiponectin treatment normalizes LPS-stimulated TNFα produc-
tion by isolated Kupffer cells after chronic ethanol exposure. We have recently reported that increased production of interleukin-10 (IL-10), a potent immune-modulatory cytokine, is involved in the anti-inflammatory actions of adiponectin in macrophages (Park, et al., 2007). Here we have further investigated the molecular mechanisms by which adiponectin increases IL-10 production in macrophages. Globular adiponectin (gAcrp) increased IL-10 promoter activity and IL-10 mRNA accumulation in RAW 264.7 macrophages. Deletion of the sequences from –416 and –369 in the IL-10 promoter, containing a cyclic AMP-response element binding protein (CREB) binding site, prevented gAcrp-induced IL-10 promoter activation. Treatment of RAW 264.7 macrophages with gAcrp increased the phosphorylation of CREB at Ser133, as well as enhanced the DNA binding activity of CREB. Further, overexpression of a dominant negative form of CREB prevented gAcrp-stimulated transcriptional activation of IL-10. gAcrp-stimulated CREB phosphorylation was mediated by the activation of both ERK1/2- and PKA-dependent pathways. Inhibition of either ERK1/2 or PKA activity prevented gAcrp-stimulated CREB phosphorylation, as well as gAcrp-stimulated IL10 promoter activation. Taken together, these data identify gAcrp-stimulated CREB as a key transcription factor responsible for gAcrp-induced IL-10 promoter activation. Supported by NIH AA11975 and AA013868.

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1022 TRIMMING OF BRANCHED OLIGOSACCHARIDE STRUCTURES ON HBV INHIBITS DC-SIGN BINDING
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Background: Currently, a high affinity receptor for Hepatitis B virus (HBV) is still unknown. For HCV, both DC-SIGN, a mannose recognizing lectin expressed on dendritic cells, and L-SIGN, a liver expressed homologue, are known to bind glycoprotein E2. The oligosaccharide structures on HBV surface antigen (HBsAg) may also allow DC-SIGN/L-SIGN interaction. Therefore, we aimed to investigate the role of both DC-SIGN and L-SIGN in binding of HBV. Methods: Interaction between HBV or HBsAg and DC-SIGN/L-SIGN was studied by ELISA, using recombinant DC-SIGN-Fc and lysates of DC-SIGN/L-SIGN transfected Raji cells. Specificity was determined by blocking with mannan (100 µg/ml) or EGTA (10 mM). As a positive control, HCV virus like particles (VLP) were used. Highly mannosylated HBV was generated by treating HBV producing HepG2.215 cells with the mannosidase inhibitor kifunensin (5 µg/ml). Upstream of HBsAg by DC-SIGN/L-SIGN transfected Raji cells was studied by flow cytometry and compared to lectin mediated uptake of dextran-FITC. Results: DC-SIGN-Fc specifically interacted with HCV VLP, but no interaction with either HBsAg or HepG2.215 derived HBV was detected. HBV binding to DC-SIGN was observed after kifunensin treatment, indicating that HBV oligosaccharide trimming inhibits interaction with DC-SIGN. In theory, the recombinant DC-SIGN-Fc protein might be misfolded, therefore binding of HBsAg to native DC-SIGN/L-SIGN was also studied with lysates of transfected Raji cells. No interaction was observed between HBsAg and either DC-SIGN or L-SIGN. Also, cultured transfected Raji cells did not bind HBsAg. In contrast, both Raji DC-SIGN/L-SIGN showed mannan sensitive binding of dextran-FITC. Conclusions: Although both DC-SIGN and L-SIGN are known receptors for HCV, they do not seem to play a role in recognition of HBV. Inhibition of mannos trimming during HBV replication renders the virus susceptible to recognition by DC-SIGN. Whether HBV might use mannos trimming as a way to escape recognition by DC-SIGN and L-SIGN remains to be elucidated.

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1023 CONCANAVILIN A-INDUCED HEPATITIS IS DEPENDENT ON TOLL-LIKE RECEPTOR 4 (TLR4) SIGNALING PATHWAYS
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Background: Concanavalin A (Con A) administration in mice leads to T cell mediated hepatitis, which involves cytokines interferon (IFN)-γ and tumor necrosis factor (TNF)-α. Lipopolysaccharide (LPS)/TLR4 signaling pathway has been shown to be involved in underlying mechanism in a number of liver pathologies ([L/R]-induced injury, alcohol-induced hepatitis). Involvement of TLR4 in T-cell mediated hepatitis has not been elucidated. In the current study the impact of TLR4 gene deletion (TLR4-/- mice) and a defective TLR4 (C3H/HeJ mice) was investigated. Methods: Mice with normal TLR4 (C3H/HeN and C57BL/6), C3H/HeJ and TLR4-/- were injected with Con A (20 mg/kg). Plasma ALT was measured to assess liver injury. Plasma TNF-α and IFN-γ were measured. Results: Following Con A administration, C3H/HeN and C57BL/6 mice developed a severe liver injury indicated by increased increased plasma ALT (Fig). However, no ALT increase was seen in C3H/HeJ or TLR4-/- mice. Histological examination confirmed signs of necrosis following Con A in the wildfire animals that was absent in the C3H/HeJ and TLR4-/- mice. TNF-α was significantly elevated at 2 hours post-Con A in C3H/HeN (1478±160 pg/ml) and C57BL/6 mice (7302±3500 pg/ml) but not in C3H/HeJ mice (347±199 pg/ml). IFN-γ levels were significantly higher in C3H/HeN (770±160 pg/ml) and C57BL/6 mice (113±15 pg/ml) compared to C3H/HeJ mice (40±30 pg/ml). Conclusions: Our results show that Con A causes liver injury and proinflammatory cytokine production in mice with functional TLR4. In the absence of TLR4 signaling pathway, cytokine production is attenuated and injury is prevented (C3H/HeJ) and TLR4-/-.
were revealed to be responsible for the suppression. These mechanisms may contribute to the clinical persistency of HCV infection and might constitute a novel antiviral therapeutic target.

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1025 EXPRESSION OF HEPATITIS C VIRUS PROTEINS IS SUFFICIENT TO INDUCE RECOGNITION BY RESIDENT HEPATIC AND INVARIANT CD1D-RESTRICTED T CELLS

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BACKGROUND: The liver is selectively enriched for innate immune cells, including natural killer T cells (‘NKT’), defined by co-expression of NK markers and T cell antigen receptor. The corresponding presenting molecule for a diverse subset of NKT, CD1d, is expressed at very low levels in healthy liver, but is specifically up-regulated in certain inflammatory conditions, including chronic HCV infection. The CD1d:NKT system is highly conserved across mammalian species. CD1d-restricted NKT are a major rodent hepatic subset and we identified analogous but distinct pro-inflammatory Th1 human populations. NKT cells positively or negatively regulate immune responses, partly through cytokine production. Their activation can be protective against certain viral and other infections, but excessive stimulation causes liver damage. METHODS: Human intrahepatic lymphocytes (IHL) and hepatocytes were isolated from subjects with HCV or other conditions along with hepatocytes from HCV transgenic mice. Blood-derived ‘invariant’ NKT served as control effectors and hepatocytes and other CD1d+ cells were used as targets. Responses of hepatic NKT were measured ex vivo and in vitro by cytokine production. An HCV-expressing Huh-7 hepatocyte line was also used as stimulator. CD1d expression was monitored on potential target cells. RESULTS: IHL CD1d-reactive NKT could be readily detected ex vivo, particularly in chronic hepatitis C, confirming their presence as a major population. Hepatocyte surface CD1d was specifically up-regulated in chronic HCV infection. Expression of HCV proteins in a hepatocyte cell line was sufficient for CD1d-dependent recognition by human IHL as well as blood invariant NKT. Furthermore, human and murine peripheral as well hepatic NKT responded efficiently to HCV transgene-expressing murine hepatocytes, suggesting a direct role for HCV protein expression in activating these cells in disease. CONCLUSIONS: HCV protein expression by hepatocytes can induce CD1d+ for surveillance by resident Th1 hepatic CD1d-restricted NKT. While potentially of benefit in acute hepatotropic infections, HCV appears to have evolved to exploit this system, thereby facilitating chronic inflammation.

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1026 PROSPECTIVE ANALYSIS OF HEPATIC CD1D-RESTRICTED T CELLS IN ADVANCED HCV LIVER DISEASE

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BACKGROUND: The liver is selectively enriched for innate immune cells, including natural killer T cells (NKT), defined by co-expression of NK markers and T cell antigen receptor. The corresponding presenting molecule for a diverse subset of NKT, CD1d, is expressed at low levels in healthy liver, but is specifically up-regulated in certain inflammatory conditions including chronic hepatitis C. The CD1d:NKT system is highly conserved across mammalian species. CD1d-restricted NKT are a major rodent hepatic subset with analogous but distinct pro-inflammatory Th1 human populations. NKT cells can control immune responses, partly through cytokine production, and their excessive stimulation causes liver damage. To test for a role in HCV-mediated liver disease progression, we performed prospective analysis of hepatic CD1d-restricted NKT in advanced HCV-mediated liver disease from samples obtained within the HALT-C trial. METHODS: Human intrahepatic lymphocytes (IHL) were expanded from up to 3 biopsies over 48 months from HALT-C subjects and tested for CD1d responses. Blood-derived ‘invariant’ NKT served as control effectors. Responses of hepatic NKT were measured by cytokine production. RESULTS: There was an increase in hepatic NKT cell Th2 pro-fibrotic IL-4 production (5±2.5 vs 6; 6±6 at tested again at Month 48) but not IFNγ from hepatic CD1d-restricted NKT. Cross-sectional analysis of samples from a total of 19 subjects to date with 1 or more time point analyzed for IL-4 also support an increase in this prototypical Th2 cytokine over time. Notably, in the non-seral samples also, there was at least as much IFNγ at Month 48. Whether treatment (in this by definition treatment refractory cohort) selectively impacts hepatic CD1d-restricted NKT IFNγ responses is under study. However, unlike the steady (likely treatment refractory) IL-4 rise for 5/6 subjects, IFNγ dropped at Month 24 (4/4 informative subjects; most proximal point post-therapy) in those who actually received ribavirin / IFNa and then rebounded to baseline biopsy level at the Month 48 biopsy. Other pro- and anti-fibrotic cytokines are being analyzed. CONCLUSIONS: HCV infection of treatment refractory patients was associated with a switch from Th1 hepatic CD1d-restricted NKT toward mixed Th1 and Th2 cytokine production. While potentially of benefit in control of HCV infection, NKT cells may facilitate HCV-mediated liver disease progression.

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1027 BLyS/BAFF RECEPTOR-LIGAND SYSTEM IN HCV INDUCED B-CELL CLONAL DISORDERS

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Background & Aims: Chronic hepatitis C virus (HCV) infection induces B lymphocytes clonal disorders, including mixed cryoglobulinemia (MC)-vasculitis and B-cell non-Hodgkin’s lymphoma (B-NHL). The aim of this study was to examine the activity of the B-Lymphocytes Stimulator (BLyS) receptor-ligand system in HCV-induced B-cell disorders. Methods: A group of 94 patients with chronic HCV infection (28 HCV+ without MC, 23 HCV+ with asymptomatic MC, 35 HCV+ MC-vasculitis and 9 HCV+ B-NHL) and 15 healthy volunteers participated in this study. BLyS studies included serum BLyS measurement, membrane bound BLyS (mBLyS) staining on PBMC, BLyS binding capacity on CD19+ cells, BLyS receptors staining on CD19+ cells, CD3+ cells and clonal Vh1-69+ population and BLyS anti-apoptotic and proliferative culture studies. Finally, we evaluated BLyS measurement following treatment with interferon-alpha and Ribavirin (64 patients) or Rituximab (14 patients). Results: Serum BLyS concentration was elevated in HCV induced MC-vasculitis and B-NHL compared with HCV+ patients without vasculitis or healthy controls (p<0.05). Healthy controls and HCV patients without MC-vasculitis had similar levels of mBLyS expression with a trend towards lower expression in HCV-induced MC vasculitis. Compared with healthy controls BLyS binding to CD19+ cells was decreased in HCV patients without vasculitis, with a further decrease in patients with HCV-induced MC vasculitis (MFI, 41±6.7, 20±8±1.9, and 7.9±4.3, respectively, p<0.01 and p<0.05, respectively). BR3 staining showed a step wise decrease with highest values in healthy controls and HCV+ without MC, and lowest in HCV-induced B-NHL (p<0.0001). BR3 staining levels were further decreased in Vh1-69+ clonal B-cells. BLyS anti-apoptotic effects were maintained in culture despite this decrease in BR3 staining. Complete clinical remission in HCV-induxed MC-vasculitis or B-NHL was associated with a decrease in serum BLyS concentration, as well as with a marked increase in BR3 staining on CD19+ cells. Rituximab treatment in HCV induced MC-vasculitis or B-NHL was associated with a remarkable five-fold increase in serum BLyS concentration (p<0.001). This increase mirrored the depletion of CD19+ cells. BR3 staining in repopulating B-cell sub-populations was significantly decreased from 19.8±2.5 to 6.9±2.5 (p<0.005). Conclusion: The results of this study suggest that BLyS is significantly involved in HCV-induced B-cell clonal disorders.

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1028 HCV-SPECIFIC CELLULAR RESPONSES IN ANTIBODY NEGATIVE, HCV RNA NEGATIVE INJECTION DRUG USERS

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Background & Aims: Hepatitis C virus (HCV) infection resolves spontaneously in 15-50% of adult patients, while the remainder becomes persistently viremic. However, growing evidence suggests that some people exposed to HCV may develop adaptive cellular immune responses without becoming viremic or developing antibodies. The aim of this study was to examine the prevalence of HCV-specific interferon (IFN)-γ cellular responses in high-risk individuals that are both HCV RNA and HCV antibody negative. Methods: We evaluated HCV-specific T-cell responses in 13 HCV RNA and HCV antibody negative young injection drug users (IDUs, aged 18-35 years) by IFN-γ enzyme-linked immunospot (ELISPOT) assay using 429 overlapping HCV peptides that cover the entire HCV genome. Peptides were pooled in 21 mixes before incubation with patients’ peripheral blood mononuclear cells. Nine IDUs who were HCV antibody
positive and HCV RNA negative (who had presumably spontaneously resolved HCV infection) were used as positive controls. Fourteen people at low-risk for HCV acquisition were used as negative controls. Responses >2 standard deviation above the mean of the 14 controls for each respective mix were considered positive. Results: HCV-specific cellular responses in IDUs negative for both HCV RNA and HCV antibodies were similar to those measured in spontaneous resolvers. While we detected very strong responses to nearly all peptide mixes in 3/13 (23%) patients, 4/13 (31%) additional patients had moderately strong responses to at least 7 antigenic combinations. Conclusions: Intermediate to strong IFN-γ responses to multiple HCV epitopes were detected in approximately half of analyzed patients, suggesting their prior exposure to HCV despite the lack of detectable HCV antibodies. The high frequency of cellular responses against HCV in seronegative IDUs supports the hypothesis that multiple low-level exposures to HCV may promote the development of cellular immunity without the development of viremia as has been shown previously in older IDUs, and suggests that this immunity may develop after a shorter duration of exposure. The potential protective role of these immune responses remains to be determined. *Drs. Edlin and Talal have contributed equally to the project.

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1029 DETECTION OF CD4+ T CELL RESPONSES IN PATIENTS WITH ACUTE HCV INFECTION IRRESPECTIVE OF CLINICAL OUTCOME

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Background and aims: HCV specific proliferative CD4+ T-cell responses have been demonstrated to be associated with the outcome of acute HCV infection. In contrast, little is known about the frequency and phenotypic properties of the HCV-specific CD4+ T cell response. Direct characterization of circulating CD4+ T cells has represented a significant challenge in human immunology, despite the recent development of MHC Class II tetramers. The purpose of this study was to longitudinally study CD4+ responses directly ex vivo by class II tetramer staining and by expanded CD4+ T cell lines. Methods: Multicolor ex vivo class II tetramer analysis was performed in a large cohort of 39 subjects with acute HCV infection. In a subset we were able to track the HCV specific CD4+ responses longitudinally. Additionally, we assessed the breadth of the response using expanded HCV specific short term lines of these subjects. Results: Using novel class II tetramers we could analyze HCV-specific CD4+ T cells directly ex vivo. We were able to track up to 3 different specificities by class II tetramers in a single patient. The frequency of tetramer positive cells reached up to 1/250 of all CD4+ cells during acute infection and declined significantly after resolution of HCV. Surprisingly, we found specific CD4+ cells by ex vivo Tetramer stainings or after short-term stimulation with IL2 also in most subjects with chronically evolving HCV, despite the absence of a proliferative response. These responses disappeared over time, but were occasionally preserved in patients in whom treatment with Interferon-alpha was started early. Preliminary phenotypic studies also did not reveal a T-cell marker clearly associated with the outcome of infection. Discussion: HCV specific CD4+ T cells are detectable at early timepoints of HCV infection in the vast majority of subjects, irrespective of clinical outcome and independent of the results in standard proliferation assays. Direct ex vivo analysis of those cells using class II tetramers will be a powerful tool to understand the mechanisms by which CD4+ T cells fail to control HCV infection and to elucidate the fate of the T cell response when HCV persists.

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the ability to develop an (intermediate) effector memory phenotype. While, in a subgroup of patients, we were able to demonstrate antigen specific FoXP3+ regulatory T-cells, which were associated with the down regulation of HCV-specific CD4+ T-cell responses, their occurrence was short lived and not related to the evolution of chronic hepatitis C.

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1031
HIGHER PERCENTAGES OF INTRA-HEPATIC REGULATORY T CELLS ARE PRESENT IN CHRONIC HEPATITIS B PATIENTS WITH A HIGH VIRAL LOAD

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CD4+CD25+FoxP3+ regulatory T cells (Treg) play a key role in the impaired immune response observed in patients with a chronic hepatitis B virus (HBV) infection. Most studies concerning Treg and HBV are performed with cells isolated from peripheral blood, while the liver is the main site of infection. To gain more insight in the role of Treg in chronic HBV we have monitored intra-hepatic Treg. Peripheral blood and liver biopsy samples were obtained from 40 patients with a chronic HBV infection. The liver tissue was collected in culture medium and was digested with collagenase for 15 minutes to create a single cell suspension. The percentage of Treg was determined by flowcytometry using antibodies to CD4, CD25 and FoxP3. The results show that patients with a high viral load of >1 x 10^5 geq/ml have a significantly higher percentage of intra-hepatic Treg as compared to patients with low viral load <1 x 10^5 geq/ml (8.20% ± 0.50% vs. 6.18% ± 0.54% respectively; p< 0.05), while the percentage of peripheral blood Treg was similar in both groups (9.38% ± 0.39% vs. 8.94% ± 0.43% respectively). When patients were divided into two groups based on liver inflammation, indicated by their serum ALT levels (ALT < 2 x ULN and ALT > 2 x ULN) no differences in percentages were observed between the two patient groups for intra-hepatic Treg (7.61% ± 0.56% vs. 7.03% ± 0.52% respectively) as well as for peripheral blood Treg (9.34% ± 0.31% vs. 8.89 ± 0.60% respectively). There was also no relation observed between the proportion of Treg and the Metavir Fibrosis score. Next to CD4+CD25+FoxP3+ Treg the liver also contained a large population of CD4+CD25-FoxP3+ cells (2.40% ± 0.21% (intra-hepatic) vs. 0.89% ± 0.06% (peripheral blood); p < 0.05). Additionally, approximately 40% of liver Treg expressed the immunoregulatory marker programmed death-1 (PD-1) while peripheral blood Treg hardly express PD-1. PD-1 expression does not seem to be specific for Treg, since the liver contains more PD-1+CD4+ T cells compared to peripheral blood. In conclusion: Liver CD4+ T cells have a higher expression of the immunoregulatory receptor PD-1. There was no relation between previous liver damage or liver inflammation and the presence of Treg. On the other hand, an increased proportion of intra-hepatic Treg was observed in patients with a high viral load, suggesting the importance of Treg in HBV tolerance and persistence of viral replication.

Disclosures:
The following people have nothing to disclose: Jeroen N. Stoop, Andrea M. Waltman, Rekha S. Binda, Johannes G. Kusters, Pieter E. Zondervan, Ernst J. Kuipers, Harry L. Janssen, Renate G. van der Molen

1032
ANTIGEN-PULSED DENDRITIC CELLS ARE CAPABLE OF INDUCING BOTH INNATE AND ADAPTIVE IMMUNITY: A LESSON LEARNT FROM CROSS TALK BETWEEN NATURAL KILLER CELLS AND DENDRITIC CELLS AND THEIR APPLICATION FOR IMMUNE THERAPY OF CHRONIC HEPATITIS B VIRUS INFECTION

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Purpose: The magnitudes of both innate and adaptive immunities are decreased in chronic infections and cancers. Accordingly, immune therapy against these diseases have been formulated to activate innate immunity targeting natural killer (NK) cells and adaptive immunity targeting dendritic cells (DC). This study was conducted to develop insights about crosstalk between NK cells and DCs and to assess if antigen-pulsed DCs can activate both innate and adaptive immunity. Methods: Both normal C57BL/6 mice and hepatitis B virus transgenic mice (that express hepatitis B surface antigen (HBsAg), HBV DNA, and hepatitis B e antigen) were used. NK cells were depleted by administrating anti-asialo GM1 antibody, 100 microliter/mouse, intraperitoneal, twice at an interval of 48 hours. Normal C57BL/6 mice and NK-depleted mice were immunized with hepatitis B vaccine containing HBsAg, intraperitoneally and the levels of antibody to HBsAg (anti-HBs) and HBsAg-specific T cells were estimated 28 days after immunization. Spleen DCs from normal C57BL/6 mice and hepatitis B virus transgenic mice were cultured with HBsAg to prepare HBsAg-pulsed DCs and administered to NK-depleted mice, twice, at an interval of 2 weeks. We assessed if HBsAg-pulsed DCs activated innate immunity in NK-depleted mice by inducing proinflammatory cytokines (interleukin-12p70, interferon-gamma, tumor necrosis factor-alpha, and interleukin-6), 3 days after administration of HBsAg-pulsed DCs. Also, we evaluated if HBsAg-pulsed DCs induced anti-HBs and HBsAg-specific lymphocytes in NK-depleted mice. Results: Flow cytometric analyses revealed that injection with anti-asialo GM1 in mice resulted in depletion of >90% NK cells from liver, spleen and bone marrow. Compared to normal C57BL/6 mice, DCs from NK-depleted normal C57BL/6 mice produced significantly lower levels of all proinflammatory cytokines (p<005) and could not activate HBsAg-specific lymphocytes in vitro indicating that NK cells are essential for proper functioning of DCs. High levels of anti-HBs were detected in the sera of all normal mice (10 of 10, 100%), but very low levels of anti-HBs were found in only 2 of 10 NK-depleted mice due to immunization with HBsAg (p<0.05). Also, the levels of HBsAg-specific T cells were significantly lower in NK-depleted mice compared to those in normal C57BL/6 mice (p<0.001). Administration of HBsAg-pulsed DCs induced higher levels of all proinflammatory cytokines, anti-HBs and HBsAg-specific lymphocytes in NK-depleted mice. Conclusions: This study provides the rationale for using antigen-pulsed DCs for treatment of chronic infections when both innate and adaptive immunities are impaired.

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1033 HLA CLASS II AND RACE-DEPENDENT DIFFERENCES IN IMMUNE RESPONSE TO HCV, SUSCEPTIBILITY TO CHRONIC INFECTION, AND RESPONSE TO THERAPY
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A hallmark of HCV infection is its tendency to develop into chronic disease. The response to therapy is more effective for Caucasian (CAU) than African American (AA) patients. The goals of the present study within the University of Tennessee Cooperative Hepatitis C Research Center have been to identify HLA associated genotypic differences among chronically infected AA and CAU patients that are 1) correlated with susceptibility to chronic infection, 2) correlated with response to combination therapy, and 3) determine class II-dependent immune responses by patient PBMC. HLA genotypes have been accumulated for 57 CAU and 85 AA patients. All patients had genotype 1a or 1b and received pegylated interferon-alpha2b and weight-based ribavirin. The difference in inheritance of HLA-DRB1*11 among African Americans (AA) chronically infected with HCV and the inheritance of DRB1*11 among AA in the Southeast is highly significant (Binomial Probability p = 0.000097). There is no DRB1*11-dependent significant difference in response to therapy among AA enrolled in our study. Among chronically infected Caucasians (CAU), we have observed a highly significant difference in the frequency of inheritance of HLA-DRB1*03 (Binomial Probability p = 0.00022). Both associations have been reported. What has not been reported is the difference in response to therapy among CAU with DRB1*03. Among CAU patients, 34% initially responded to therapy with elimination of circulating virus to below detectable levels for at least 24 consecutive weeks only to relapse to pre-therapy levels of viremia. More than half (53%) of relapsing CAU patients were DRB1*03 (p = 0.027, Fisher). Prior to therapy most chronically infected patients had relatively poor in vitro T cell proliferative and interferon-gamma responses to HCV, compared to mitogen or tetanus toxoid (TT). Immune responses to HCV were significantly higher (p = 0.007) in patients that remained virus free 24 weeks after the end of therapy compared to patients in which virus was not eliminated or who had viral relapse. A very surprising finding was that T cell responses were significantly higher to tetanus toxoid (TT) than HCV proteins (p = 0.011) for non-DRB1*11 AA but not DRB1*11 AA (p = 0.307). This result suggests that HLA-DRB1 inheritance may have a remarkable impact on immune potential to HCV and may explain in part why DRB1*11 AA are more prone to chronic infection. This result may also implicate an influence of Treg in susceptibility to chronic HCV, a hypothesis currently under investigation. This research was supported by NIH grants NIAID U19AI066316 and NCRR R016949, RR022465, and M01 RR002011.

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1034 ADHESION OF IN VITRO GENERATED DENDRITIC CELLS TO HUMAN HEPATIC ENDOTHELIUM UNDER CONDITIONS OF SHEAR STRESS
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The incidence of hepatocellular carcinoma (HCC) is rising as a consequence of the increased prevalence of cirrhosis. Despite the presence of large numbers of tumour-infiltrating lymphocytes in HCC, effective killing of tumour cells is not achieved. Tumour-pulsed dendritic cells (DCs) can induce effective T cell responses in animals and early clinical trials are promising. However little is known about the mechanisms that regulate the recruitment of adoptively transferred DCs to the tumour. Aims: To determine the molecular regulation of DC trafficking to the human liver and hepatocellular carcinoma. Methods: Liver-derived DCs (LDC) were isolated from patients with HCC. Monocyte derived DCs (MoDC) were generated in vitro in the presence of GM-CSF and IL-4, before being matured with macrophage conditioned media. Primary hepatic sinusoidal endothelial cells (HSEC) isolated from human liver were cultured in glass capillary tubes before being perfused with DCs at physiologically relevant shear stress. Results: LDCs from livers containing HCC expressed maturation markers including CD86 and Class II but expressed higher levels of CCR5 than CCR7 suggesting only partial activation. Under conditions of fluid flow anti-CD40/FcR antibodies reduced adhesion of immature MoDCs to TNF-α stimulated HSEC by around 40%, though no significant variation was seen with mature MoDCs. Inhibition of CX3CCL1 on HSEC reduced total adhesion of both immature and mature MoDC by 60% and 40% respectively and transmigration by up to 70% in both cases. Similar inhibition of adhesion and transmigration was seen when pertussis toxin was used to block GI protein linked signalling. Conclusion: These data demonstrate that MoDCs use a unique combination of adhesion molecules comprising CX3CCL1, ICAM-1 and VCAM-1 to bind HSEC from flow. This data has allowed us to set up a small clinical study to visualise MoDC homing to the human liver following administration peripherally or via the hepatic artery, the results of which we will present at this meeting.

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1035 INTRAHEPATIC REGULATORY T CELLS DIFFER PHENOTYPICALLY FROM THEIR CIRCULATING COUNTERPARTS IN CHRONIC THERAPY NAIVE HCV PATIENTS
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BACKGROUND: Regulatory T cells (Treg) play a significant role in the hampered immune response against HBV, as we have shown previously. Similar findings have been suggested for Treg in chronic HCV infection. This may explain the weak HCV specific T cell response in these patients. The immune response at the primary site of infection, the liver, is likely to play a critical role in HCV persistence. METHOD: Minimally invasive aspiration biopsies enabled us to repeatedly study intrahepatic and peripheral Treg in parallel. RESULTS: In 20 chronic HCV therapy naive patients, CD8+ lymphocytes were more frequent in the liver than in blood (22.8% and 13.6% respectively, p<0.001). In contrast, the proportion of CD4+ lymphocytes was lower intrahepatically than peripherally (26.34% and 31.57% respectively, p<0.05). Careful analysis of CD4+CD25hi cells and FoxP3+CD4+CD25+ cells revealed a smaller proportion of Treg in the liver (8.1% against 9.6%). Preliminary data suggest that Treg in liver and in blood predominantly consist of a memory phenotype (a.o. CD45RO). Our findings indicate that a significant proportion of CD4+ lymphocytes infiltrating the liver are Treg. However, the ratio Treg to total CD4+ lymphocytes and total CD8+ lymphocytes was lower than in peripheral blood. CONCLUSIONS: Our data
indicate that the chronicity of HCV can not be solely explained by an increased ratio of Treg to effector T cells in the liver. We are currently further investigating phenotypical and functional differences between liver and blood Treg. This could delineate the contribution and possible mechanism of intrahepatic Treg to the attenuated HCV immunity.

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1036 THE ROLE OF NANOBACTERIA IN CHOLECYSTITIS AND CHOLELITHIASIS

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Background: Recently discovered sub-microscopic blood particles known as nanobacteria, have a special characteristic no other blood particles have been known to possess: they form calcium phosphate shells. They have been found in human blood, arterial wall plaques, psammoma bodies of ovarian tumors and nephrolithiasis. The clinical trials demonstrated that the eradication of nanobacteria reduced the calculations in coronary arteries. The aim of this study is to access the relation between nanobacteria and cholecystitis.

Materials & Methods: Twenty-six surgically resected gallbladders with cholecystitis were tested for the presence of nanobacteria by immunohistochemical staining using primary antibody (NanoVision Antibody 8D10, Nanobac OY, Finland) and super sensitive link-label immunohistochemical detection system (QA000-5L, BioGenex, San Ramos, CA). The formalin fixed tissue link-label immunohistochemical staining system Antibody 8D10, Nanobac OY, Finland) and super sensitive immunohistochemical staining using primary antibody (NanoVision Antibody 8D10, Nanobac OY, Finland) and super sensitive link-label immunohistochemical detection system (QA000-5L, BioGenex, San Ramos, CA). The formalin fixed tissues were imbedded in paraffin, sectioned at 50 microns and stained. The control slides (provided by Nanobac OY, Finland) included cell cultures with and without nanobacteria inoculation. The stained tissue slides were reviewed blindly and graded from 0 to 4+ in staining intensity and location of staining. The results were then compared to the clinical, gross pathology and histological findings. Results: The initial evaluation revealed that 25 out of 26 cases were stained positively for nanobacteria. Following intensity of staining was observed: 0 (1 case), 1+ (5 cases), 2+ (13 cases), 3+ (6 cases) and 4+ (1 case). The one case that did not stain was a gangrenous gallbladder with no normal epithelium remaining for evaluation. No staining was observed when the primary antibody was omitted. Six out of 13 cases with gross lithiasis, have staining intensity 3+ or more compared to one out of 13 without lithiases, suggesting a strong association between nanobacteria and cholelithiasis. Nanobacteria were detected within the gallbladder epithelium, inflammatory debris, calculi, surface sludge and focal arterioles. The stain was present in the arteriole intima and was not observed in the venules within the same slide. Conclusions: Nanobacteria were found in 25 out of 26 gallbladders with cholecystitis. It was present within the epithelium, inflammatory debris, stones and sludge of cholecystitis with and without lithiasis. The presence and intensity of staining were associated with well-formed gross lithiasis. This observation suggests the contributing role of nanobacteria in gallstones formation. It is conceivable that a specific therapy for nanobacteria may prevent cholecystitis and reduce the need for surgical interventions. Further assessment of additional specimens is underway.

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The following people have nothing to disclose: Somaia Shehab Eldeen, Linda k. Green, Vladimir Khaoustov, Boris Yoffe

1037 CRITICAL ROLE OF CD44 IN HEPATOTOXIN-MEDIATED LIVER INJURY

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Purpose. Blocking of adhesion molecules is considered to be one of the therapeutic strategies for inflammatory diseases, although it remains unclear whether this strategy is beneficial. Here, we evaluated role of CD44, which is one of the adhesion molecules against liver injury using carbon tetrachloride (CCL4). Methods. We injected CCI4 into CD44 deficient mice (CD44KO) or wild type mice and sacrificed mice at several time points after injection and analyzed serum ALT activity and cell number in intrahepatic leukocytes by FACS and cytokine, chemokine expression in the liver by RNAase protection assay. To determine which cell subpopulation produce cytokine we analyzed intrahepatic leukocytes by intracellular cytokine stain.

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The following people have nothing to disclose: Kiminori Kimura, Masahito Nagaki, Kazuhiro Kakimi, Hisataka Moriwaki
1038 INCREASED ACETYLATION OF ALPHA-TUBULIN AND DECREASED EXPRESSION OF FIBROCYSTIN ARE SENSITIVE MARKERS OF CELLULAR DAMAGE AND ACTIVATION IN INFLAMMATORY LIVER DISEASE

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Background. Primary cilia are highly conserved chemosensory organelles present in normal renal and hepatic ductular epithelium that determine cell polarisation, proliferation and activation. Alpha-tubulin is a constitutive structural component of the cytoskeleton, basal body and axoneme of primary cilia. Acetylation of alpha-tubulin (acAT) is necessary for microtubule stabilisation during primary ciliary formation, cellular proliferation and activation, however, hyper-acetylation of AT has been associated with hepatocyte damage in ethanol fed mice. Fibrocystin is a receptor protein, of unknown function, that also localizes to primary cilia. Mutations of fibrocystin cause a structural dysfunction of primary cilia associated with polycystic kidney and liver disease. Aims. To investigate acAT and fibrocystin expression in human liver tissue during cellular activation associated with inflammatory/malignant disease. Methods. Routine paraffin embedded liver tissue subjected to low temperature epitope retrieval was probed with a commercially available mouse monoclonal antibody to acAT or an antibody raised to the c–terminus of wild type human fibrocystin. Antigen localisation was visualised using a ChemMate Envision detection system (Dako). The cohorts were:- Adult polycystic liver disease (PLD); PBC; PSC; Allograft Rejection (AR) Autoimmune Hepatitis (AIH); HCV; Chronic Alcoholic Liver Disease (ALD); alpha-1-AT deficiency (AAT); Biliary Atresia (BA); Hepatocellular Carcinoma (HCC). Normal liver tissue (NL) surplus to transplantation was visualised using a ChemMate Envision detection system (Dako). The cohorts were:- Adult polycystic liver disease (PLD); PBC; PSC; Allograft Rejection (AR) Autoimmune Hepatitis (AIH); HCV; Chronic Alcoholic Liver Disease (ALD); alpha-1-AT deficiency (AAT); Biliary Atresia (BA); Hepatocellular Carcinoma (HCC). Normal liver tissue (NL) surplus to transplantation required was used as a control. (n=5 for each group). Results. acAT- normal donor liver was consistently negative. All other disease groups showed very strong acAT staining of bile ducts particularly in perisepal regions of ductular reactivity. Periseptal/periportal hepatocytes and bile ducts were very strongly +ve in ALD, HCV and HCC. Staining of cystic epithelium in PLD was variable and frequently +ve whereas tumour cells in HCC were frequently +ve. acAT staining was very strong in perivenular hepatocytes and surrounding inflammatory cells in AR. Fibrotic and cirrhotic lesions contained strongly positive inflammatory and stromal cells. By contrast, most bile ducts in normal tissue were strongly +ve for fibrocystin whereas staining was frequently low or absent in all diseased liver tissue. Conclusions. Acetylation of alpha tubulin is a sensitive marker of cellular activation in most common inflammatory liver diseases and the absence of fibrocystin staining reflects ongoing damage to the intrahepatic biliary tree, rather than a phenomenon specific to the so called ciliopathies.

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The following people have nothing to disclose: Michele T. Pritchard, Sanjoy Roychowdhury, Megan R. McMullen, Luping Guo, Gavin E. Arteel, Laura Nagy

1039 CRITICAL ROLE OF EARLY GROWTH RESPONSE-1 TO GALACTOSAMINE / LIPOPOLYSACCHARIDE-INDUCED ACUTE LIVER INJURY IN MICE

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Early growth response-1 (Egr-1) is a transcription factor that functions as a master regulator of gene expression in response to proinflammatory signals and cellular stress and plays a critical role in the pathology of ischemia/reperfusion injury, cholestasis, atherosclerosis, pancreatitis and ethanol-induced fatty liver in mouse models. The purpose of this study was to test the hypothesis that Egr-1 is also involved in the development of galactosamine/lipopolysaccharide (GalN/LPS)-induced acute liver injury. GalN/LPS exposure bi-phasically increased hepatic Egr-1 mRNA accumulation at 1h and then at 4-5.5h after treatment. The effects of GalN/LPS were next compared in wild type and egr-1 -/- mice. Within 4-5.5h after GalN/LPS exposure, wild type mice exhibited evidence of hepatocyte injury, cell death, and extensive areas of hemorrhage; these parameters were attenuated egr-1 -/- mice. Liver to body weight ratios and ALTs were lower in egr-1 -/- mice 5.5h post GalN/LPS administration compared to wild type mice. The initial proinflammatory response to GalN/LPS administration in egr-1 -/- mice was similar to wild type, with no differences in TNFα, IL-1β, IL-8, MCP-1 or ICAM-1 mRNA or protein between genotypes at 1h. However, expression of these genes was lower in the livers of egr-1 -/- mice at subsequent time points. The observed decrease in the hepatic inflammatory milieu was associated with a decrease in neutrophil extravasation from hepatic sinusoids into the liver parenchyma in egr-1 -/- mice, compared to wild type mice, 4h after GalN/LPS. In addition, there were fewer TUNEL positive cells in livers from egr-1 -/- mice 5.5h post GalN/LPS. Finally, the decreases in inflammatory and chemo tactic responses in egr-1 -/- mice were associated with increased survival in egr-1 -/- mice after GalN/LPS. In conclusion, these data demonstrate, for the first time, that Egr-1 plays a critical role during later phases of GalN/LPS-induced acute liver injury. Supported by NIH grants AA015833 to MTP, AA003624 to GEA and AA0138868 to LEN

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1040 INHIBITION OF THE KUPFFER CELL INCREASES SERUM HMGB1 LEVELS AND THE MORTALITY IN A RAT SEPTIC PERITONITIS MODEL

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High-mobility group protein 1 (HMGB1) is a nonhistone nuclear protein whose function depends on cellular location. Inside the cell, HMGB1 modulates a variety of important cellular processes, including transcription, whereas outside the cell, HMGB1 acts as an inflammatory cytokine and mediator sepsis in animal models. In in vitro studies, proinflammatory molecules can induce HMGB1 release from macrophages. Since the Kupffer cell (KC), the resident hepatic macrophage, is a predominant population of macrophage lineage, the specific purpose of this study was to investigate whether inhibition of the KC affect the expression of HMGB1 in sepsis. Rats were given saline or
gadolinium chloride (GdCl₃), a KC toxicant, 24 hours before cecal ligation and puncture (CLP). Survival was assessed for 7 days after CLP. Liver tissues and blood samples were harvested, and the expression of inflammatory mediators was assessed. Inhibition of the KC increased significantly the mortality 7 days after CLP. Plasma endotoxin and serum TNF-α levels were significantly greater in the GdCl₃ than in the control group 12 hours after CLP. Furthermore, serum HMGB1 levels were significantly greater in the GdCl₃ group than the control group. To assess the expression of HMGB1 in the liver after CLP, immunohistochemical staining for HMGB1 was performed. The expression of HMGB1 was detected on cells in the hepatic sinusoid 12 hours after CLP. These cells recognized morphologically as KCs. Furthermore, the number of the HMGB1-positive KCs was significantly greater in the GdCl₃ group than the control group. The mRNA expression of HMGB1 in the liver was assessed by RT-PCR each time after CLP. The expression was significantly greater in the GdCl₃ group compared with the control group after CLP. It was reported that GdCl₃ eliminates ED1- and ED2-positive macrophages, which were morphologically large KCs, and ED1-positive and ED2-negative KCs were still observed after treatment of GdCl₃. Therefore, the mRNA expression of HMGB1 was assessed in each subpopulation of the KC. The Kupffer cells were isolated by a counterflow centrifugal elutriation to separate cells based on cell size and density. The expression of HMGB1 was greatest in the small KCs, which were ED1-positive and ED2-negative cells assessed by flow cytometry, compared with large KCs, which were ED1- and ED2-positive cells. Thus, the large KC plays a beneficial role on the host defense during sepsis. Furthermore, the small KC, which may include infiltrating cells into the liver, is a predominant source of HMGB1 in the liver.

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MRNA EXPRESSION ANALYSIS OF IMMUNOREGULATION, APOPTOSIS AND FIBROSIS IN LIVER DISEASES
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Background: T-regulatory cells (Tregs) are important mediators of immune suppression. Their presence prevents self-immune reactions by inducing regulatory signals to APCs and/or effector T-cells. In previous studies the persistence of viral infection in liver has been associated with the expansion of Tregs in the peripheral blood of the affected patients. Our aim was to study, at the transcriptional level, the presence of Tregs in liver tissues of patients with various diseases. Patients and methods: Liver biopsies from 20 patients (7 with chronic HBV hepatitis and/or cirrhosis, 4 with chronic HCV hepatitis and/or cirrhosis, 6 with NASH, 2 with metotrexate related toxicity and 1 with autoimmune cirrhosis) were studied. Total RNA was extracted and cDNA was synthesized using standard protocols. Quantitative real-time PCR analysis was performed for the detection of mRNA expression of: Foxp3 (determinant of the presence of natural Tregs), TGF-β1 and IL-10 (cytokine determinants of inducible Tregs – Th3 and Tr1, respectively), TGFβRII, TGFβRI, Smad2, Smad3, Smad4, Smad7 (receptors and mediators of TGF-β signaling) and TRAIL, Fas, FasL (mediators of apoptosis). REST software was used for the statistical analysis. Results: Irrespective of the type of liver damage (viruses, drugs, NASH, autoimmune) an expansion of natural Tregs was present, evident through the dramatic increase of Foxp3 expression (for HBV OR: 28.025, 95%CI: 3.01 - 255.78, p<0.002, for HCV OR: 16.11, 95%CI: 3.837 - 96.625, p<0.025, for NASH OR: 20.194, 95%CI: 4.603 - 165.843, p<0.01), for methotrexate-related toxicity OR: 28.1, 95%CI: 9.391 - 107.627, p<0.001, for autoimmune cirrhosis 25.4 fold) accompanied by a significant increase in mediators of apoptosis (Fas-FasL, TRAIL), compared with normal controls (p<0.01). Furthermore, an increase in the expression of Smad3 (indicator of increased TGF-β signaling) was observed in patients with cirrhosis, compared with normal controls (OR:2.392, 95%CI: 1.604 - 3.575, p=0.012) Conclusions: Our results suggest that acute or chronic tissue damage, irrespective aetiology, results in increased apoptosis, followed by increased phagocytosis and possibly in self-antigen presentation by APCs. Eventually, this could lead in an expansion of natural Tregs that would prevent catastrophic autoimmunity in the damaged tissue. Elucidation of the pathways mediating this potential model could increase our understanding of the role that Tregs play in disease propagation and, for the first time, could have wider implications for the manipulation of Tregs in a therapeutic setting.

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1042
EXPRESSION OF NOTCH SIGNALING AND ANTIGEN PROCESSING MOLECULES IN PROGRESSIVE PATHOGENIC BIOPSIES OF CHRONIC HEPATITIS B INFECTION
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Background: The Notch signaling plays a key role in cell-fate determination and differentiation, functioning in a cell- and tissue-specific manner. Both upregulated and downregulated Notch1 signalling is seen in carcinogenesis of different tissues. Whereas, in stem cells, notch activity inhibits differentiation and promotes carcinogenesis but in primary epithelial cells high notch activity leads to exit from the cell cycle and commitment to differentiation. Notch1 exerts specific protective effects against HPV-virus and down-modulation of Notch1 was observed in the late stages of HPV-induced carcinogenesis. Aim: To study notch signalling in different stages of HBV induced liver disease and its role in liver disease progression. Patients and Methods: PBMCs of chronic hepatitis B (CHB) (n=10) (age 29.4±13.2 yr., M:F:8:2) patients and healthy controls (n=10) (age 27.9±6.4 yr., M:F:7:3) and liver biopsies from normal liver – 3 (age 49±3.8 yr., M:F:2:1), CHB patients (age 39±12.8, M:F:3:1) HBV cirrhosis –4 (age 36.2±20.0, M:F:3:1) and HBV related HCC –3 (age 55.5±7.2 yr., M:F:3:0) were studied. Total RNA was extracted from PBMCs and biopsies and Notch1, Hes1, Jagged1, and NFKB genes were analyzed by quantitative RT-PCR. Results: Compared to PBMCs of CHB patients, the liver biopsies showed significantly lower expression of Notch1 (p=0.034), Jagged 1 (p=0.021) but not Hes 1 (Table 1). The reduction in expression of these genes was significantly greater in HCC patients compared to CHB and controls. In comparison to control biopsies and PBMCs in CHB, cirrhotic tissue showed significantly lower expression of NFKB (p<0.02). Conclusions: In different stages
of liver disease due to HBV, the PBMCs show higher expression of notch signaling molecules, but in the target liver tissue, the expression of these molecules is significantly inhibited, maximum being in CHB and HCC. This expression pattern of notch1 signaling suggests that this protein may play a permissive or tumor-promoting function in chronic HBV infection.

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Disclosures:
The following people have nothing to disclose: Nirupma T. Pali, Sujoy Bose, Sukriti Baveja, Hissar Syed, Shiv K. Sarin

1043 INTRAHEPATIC STATUS OF REGULATORY T CELLS IN AUTOIMMUNE HEPATITIS, PRIMARY BILIARY CIRRHOSIS, CHRONIC HEPATITIS C, AND CHRONIC HEPATITIS B
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Background/Aims: Regulatory T cells (Tregs) maintain immunological tolerance and suppress autoreactive immune responses. We evaluated the intrahepatic status of Tregs in patients with autoimmune hepatitis (AIH), primary biliary cirrhosis (PBC), chronic hepatitis C (CH-C), or chronic hepatitis B (CH-B).

Methods: We analyzed 85 patients (20 AIH, 22 PBC, 27 CH-C, and 16 CH-B) and 14 controls. Using liver tissue samples obtained by needle biopsy or from marginal parts of resected metastatic liver tumors in controls, immunohistochemical analyses of forkhead box P3+ (Forkhead box P3+), which is a specific marker for Tregs, CD4+, and CD8+ cells were performed. Results: Intrahepatic Tregs were significantly infiltrated in patients with liver diseases than in controls. There were significantly fewer intrahepatic Tregs in AIH patients than in PBC patients (p=0.037). Patients with a low frequency of intrahepatic Tregs were detected significantly less in the AIH and CH-B groups than in the PBC and CH-C groups. We found significantly less infiltration of CD4+ T cells in AIH than in other diseases (p<0.05). Liver-infiltrating CD8+ T cells were detected more frequently in the CH-B group than in other groups (p<0.003). Conclusions: Intrahepatic Tregs were increased in both patients with autoimmune liver diseases and those with viral hepatitis. In autoimmune liver diseases, intrahepatic Tregs in AIH patients were fewer than in PBC patients.

1044 SPECIFIC CONTRIBUTION OF NADPH-OXIDASE IN DISTINCT CELL TYPES DURING HEPATIC FIBROSIS.
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Background: NADPH-oxidase is a multi-protein complex that produces differential amount of reactive oxygen species. In addition to its main activity in host defence, NADPH-oxidase plays a central role in the regulation of hepatic fibrosis via induction by angiotensin II, leptin and PDGF. NADPH-oxidase is functionally expressed in several cell types involved in hepatic fibrogenesis, such as hepatic stellate cells (HSC), Kupffer cells (KC) and hepatocytes. However the contribution of this complex in distinct cell types during hepatic fibrosis is unclear. Aims: To determine the role of NADPH-oxidase in different cell populations during hepatic fibrogenesis.

Methods: Hepatic fibrosis was induced by bile duct ligation (BDL) for 21 days or by methionine-choline deficient (MCD) diet for 10 weeks in wild-type (WT) and mice deficient in p47phox, a central component of both phagocytic and non-phagocytic NADPH-oxidase. Hepatic fibrosis was evaluated by Sirius red staining and mRNA expression of collagen α1(I), αSMA, TGFβ and TIMP-1 by quantitative real time PCR. Hepatic steatosis was determined by oil red-O staining. Hepatocellular injury was assessed by serum ALT and AST levels.

Results: Upon BDL, the increase of hepatic collagen and hydroxyproline content was lower in p47phox-/-. mice compared with WT mice. The chimeric mice that contained p47phox-/- BM into WT recipients and vice versa.

Conclusions: The expression pattern of NADPH-oxidase in different cell populations during hepatic fibrosis is consistent with the role of this protein in the development of hepatic fibrosis.

Disclosures:
The following people have nothing to disclose: Masashi Sakaki, Kazumasa Hiroishi, Toshiyuki Baba, Miki Kushima, Michio Imawari

The values are means ± SD.

* p = 0.037 (AIH vs PBC); ** p < 0.001 (Patients vs Control) p = 0.007, 0.045, 0.001 (AIH vs PBC, CH-C, CH-B); 1p = 0.026 (CH-C vs CH-B); 2p = 0.013 (CH-B vs Control); 3p = 0.003, 0.002, 0.001, 0.001 (CH-B vs AIH, PBC, CH-C, Control).
1045

TLR4/MYD88/NF-KB-MEDIATES HEPATIC STELLATE CELLS ACTIVATION BY THE REGULATION OF TGF-B PSEUDORECEPTOR BAMBI IN HEPATIC FIBROSIS

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Background: The gram-negative bacterial cell wall component lipopolysaccharide (LPS) is elevated in the plasma of patients with liver cirrhosis by increased intestinal permeability. Toll-like receptor (TLR) 4 recognizes LPS and results in NF-kB activation via MyD88-dependent pathway. TLR4 signal also activates IRF-3 via TRIF-dependent pathway. It was assumed that increased LPS level in portal may activate TLR4 on Kupffer cells (KCs), subsequently activating hepatic stellate cells (HSCs) by indirect or direct action of Kcs, resulting in promoting liver fibrosis. Surprisingly, TLR4 on HSCs, but not Kcs, are critical in hepatic fibrosis as we have previously shown using TLR4-chimeric mice. In addition, TLR4 signal activates HSCs by downregulation of Bambi, an inhibitor of TGF-b signal. However, the precise mechanism of Bambi regulation is unknown. Aim: To define the mechanism by which TLR4 signal regulates Bambi expression and HSC activation. Methods: Hepatic fibrosis was induced by bile duct ligation (BDL) on WT, MyD88-/- and TRIF-/- mice. To evaluate liver fibrosis, we measured collagena I (I), aSMA, TGF-b1 and TIMP-1 mRNA expression by real time PCR (qPCR). Collagen promoter activity was assessed in HSCs isolated from collagen-GFP transgenic mice following infection with control or Iksr super-repressor (sr) adenovirus after stimulation with TGF-b plus LPS. Bambi, KC, and IP-10 mRNA expressions in HSCs infected with control or Iksr adenovirus or in HSCs isolated from WT, MyD88-/- and TRIF-/- mice were assessed by qPCR. Results: Upon LPS stimulation, Bambi mRNA expression was downregulated in control virus-infected HSCs, but not in the HSCs infected with Iksr virus. Control, but not Iksr virus infected-HSCs have an increase in collagen promoter activity after TGF-b plus LPS stimulation. In addition, Bambi mRNA expression was downregulated in WT and TRIF-/- HSCs, but not in MyD88-/- HSCs. The MyD88-dependent inducible gene KC was upregulated in WT or TRIF-/- HSCs, but not in MyD88-/- or Iksr-expressing HSCs. In contrast, the MyD88-independent inducible gene, IP-10 was upregulated in WT, MyD88-/- or TRIF-/- HSCs, but not in Iksr-expressing HSCs. Analysis of profibrogenic gene expression showed upregulation of collagen I (I), aSMA, TGF-b1 and TIMP-1 in WT and TRIF-/- BDL liver. In contrast, profibrogenic gene expressions were less increased in MyD88-/- BDL liver. Conclusion: Bambi mRNA expression is controlled in a TLR4/ MyD88/NF-KB-dependent pathway. Consistent with Bambi regulation, the initial event of hepatic fibrogenesis is regulated by the TLR4/MyD88/NF-KB axis. In addition, our results suggested that TLR does not play roles in TLR4 signal in HSCs.

Disclosures:
The following people have nothing to disclose: Ekihiro Seki, Samuele De Minicis, David A. Brenner, Robert F. Schwabe

1046

GLYCATED FIBRONECtin STIMULATES HEPATIC STELLATE CELL (HSC) ACTIVATION AND TYPE I COLLAGEN PRODUCTION IN PRIMARY CULTURES

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INTRODUCTION: Type II diabetes increases the risk of chronic liver disease and hepatocellular carcinoma. Diabetes has been also found to be an independent predictor of fibrosis in nonalcoholic steatohepatitis (NASH). Metabolic milieu of type II diabetes is characterized by hyperglycemia and high insulin levels due to the insulin resistance. We have previously reported that high insulin levels stimulate type I collagen synthesis by HSC. As this effect was more potent in a high glucose media, in this study we examined the role of nonenzymatic glycation of matrix component fibronectin in HSC activation and fibrogenesis. MATERIAL AND METHODS: HSC were isolated from transgenic mice harboring transgene with Green fluorescent protein fused to COL1A1 gene promoter, using a collagenase pronase digestion followed by density gradient centrifugation. HSC were plated on plastic in low and high glucose media and treated with insulin. Additionally, HSC were initially plated on plastic (to ensure purer population of HSC), than replated at day 3 on plastic, native fibronectin and glycated fibronectin. Fibronectin was glycated for 30 days in 0.5M solution of glucose 6-phosphate at 37°C. HSC were imaged at day 6 and 7 for GFP expression and harvested at day 7 for protein analysis. Type I collagen was detected by Western blot of total cell extract at culture day 7. Adhesion and proliferation assays were performed at day 6 of culture. RESULTS: Insulin treatment of primary HSC cultures stimulated type I collagen expression only in high glucose conditions. HSC plated on glycated fibronectin showed increased proliferation and type I collagen synthesis when compared with cells plated on native fibronectin and plastic. In addition, significantly more HSC plated on glycated fibronectin expressed GFP transgene in culture on both day 6 and 7. Adhesion assay showed significantly more HSCs attached on glycated fibronectin than unaltered fibronectin, indicating possible role of integrins in this effect, a concept supported by our previous studies, demonstrating the importance of fibronectin receptor, alpha5beta1 integrin, in early stages of HSC activation. We detected higher levels of the focal adhesion kinase in HSC plated on glycated fibronectin, suggesting that integrin signaling may be involved in exaggerated HSC activation on glycated matrix. CONCLUSION: Glycated fibronectin stimulates activation and type I collagen synthesis in primary HSC cultures. Glycation of extracellular matrix in the liver of diabetic patients, may be a factor in stimulating fibrogenesis and contributing to a higher rate of fibrosis in these patients.

Disclosures:
The following people have nothing to disclose: Milan Dodig, Min Li, Srinivasan Dasarathy, Arthur J. McCullough

1047

BENEFICIAL EFFECT OF ANGIOTENSIN-BLOCKING AGENTS ON LIVER FIBROSIS IN PATIENTS WITH HEPATITIS C

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Background: The morbidity and mortality related to chronic Hepatitis C viral (HCV) infection is related to liver fibrosis progression. Current treatments for HCV are limited to interferon and ribavirin, which have unproven effectiveness and limited tolerability as long-term agents in nonresponders. Rational approaches are needed to identify viable antifibrotic agents. Several lines of evidence have implicated angiotensin II as a driver of liver fibrosis. Various animal and clinical studies have
shown that angiotensin-blocking agents attenuate liver fibrosis, but none has evaluated histological data in patients infected with HCV. In this study we evaluated the histological effect of angiotensin-blocking agents in patients with chronic HCV.

Methods: This was a retrospective chart review of 234 patients from our institution during the years 2001-2006 with chronic HCV and concomitant hypertension (HTN), and an available liver biopsy. 143 patients [Group I] received angiotensin-blocking agents (angiotensin-converting enzyme (ACE) inhibitors and angiotensin II receptor blocking agents (ARBs) to treat HTN and 91 patients [Group II] received other anti-hypertensive agents (including beta-adrenergic blocking agents, calcium antagonists, alpha-adrenergic blocking agents and diuretics). All available biopsies were reassessed and restaged by a single, blinded pathologist. Results: The two groups were similar with respect to age, sex, genotype, HCV RNA levels and estimated duration of disease, but the two groups varied significantly with regards to the total proportion of diabetic patients [Group I: 43/143 (30%); Group II: 10/91 (11%), p value 0.0007], a result of the recommended management of hypertension diabetic with ACE inhibitors. There was a significant difference in Ishak fibrosis score [Group I: 3.20; Group II: 3.73, p value 0.04]. Subgroup analysis of non-diabetics demonstrated an even more significant difference between the two groups in Ishak fibrosis score. [Group I: 3.07; Group II 3.69, p value 0.023]. Conclusion: The administration of angiotensin-blocking agents in patients with HCV infection and HTN was associated with histological evidence of decreased fibrosis. In patients without diabetes, this difference was even more pronounced. These data provide support for prospective evaluation of ACE inhibitors or ARBs as possible hepatic fibrosis inhibitors in patients with HCV.

Disclosures:
The following people have nothing to disclose: Nirali Shah, Hui Zheng, Joseph Misrahi, Raymond T. Chung

1048 ROLE OF P75 SIGNALLING IN THE PATHOGENESIS OF LIVER FIBROSIS IS CONTEXT AND LIGAND STRUCTURE DEPENDENT

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Hepatic myofibroblasts (MFB) express p75, the low-affinity nerve growth factor (NGF) receptor, and sortilin, a putative receptor for proNGF. We propose mature NGF is pro-apoptotic whilst proNGF is both protective from serum-deprivation induced apoptosis and proliferative. During resolution of experimental fibrosis or partially reversible cirrhosis levels of proNGF fall relative to mature NGF. We describe functions of p75 signalling in a murine model of liver fibrosis. Wild-type and p75NTR/exonIII-/- mice that do not express the ligand binding domain but express the intracellular domain were injured with carbon tetrachloride and allowed to recover. At peak fibrosis livers from p75-/- animals contained fewer α-smooth muscle actin (α-sma) positive cells, a marker of MFB, compared with wild-type animals. Wild-type livers had 6.9x more non-parenchymal cells staining positively for a proliferation marker (Ki-67) than p75-/- animals. In recovery there was significantly retarded histological resolution in the livers of p75-/- animals, determined by both semi-quantitative scoring and morphometric image analysis of Sirius red stained sections. There was also a significantly delayed loss of α-sma positive cells. The percentage of MFB undergoing apoptosis, determined by caspase 3 activation, was significantly reduced (day 7, 34% v 75%, p<0.05) in the livers of p75-/- animals relative to wild-type animals indicating that the persistence of MFB is likely to be due to reduced apoptosis. Our findings are broadly supported by the determination of the expression of procollagen-1, TIMP-1 and α-sma mRNA in injured liver by real-time PCR. Deletion of p75NTR/exonIII-/- does not influence the activation of hepatic MFB or the expression of procollagen-1, TIMP-1 and α-sma in vitro, determined by real-time PCR and immunofluorescence. However, MFB isolated from p75-/- mice and activated by culture on plastic significantly fail to respond to mitogens (fetal calf serum and PDGF), consistent with our in vivo findings in livers of p75-/- animals and suggesting a tonic proliferative role for p75 in hepatic MFB. We have demonstrated a dual role for p75 signalling in liver fibrogenesis. During injury p75 has a profibrotic role via proNGF mediation of MFB proliferation, and protection from apoptosis. In contrast, during recovery from liver fibrosis when proNGF levels fall and mature NGF dominates, p75 has anti-fibrotic effects by mediating MFB apoptosis.

Disclosures:
The following people have nothing to disclose: Timothy J. Kendall, R Christopher Benyon, John Iredale
red stained sections, examined by morphometry, indicated that portal tract collagen-I remained elevated in neutrophil depleted animals (7.72% area versus 3.52% in saline controls; p<0.05) despite normal reductions in bilirubin and liver enzymes. Quantitative real time PCR results for neutrophil depleted livers demonstrated dramatic increases and a loss of regulation of expression of the neutrophil chemoattractant CINC1, matrix metalloproteinases and tissue inhibitors of metalloproteinases. CONCLUSIONS: We conclude that functioning neutrophils are critical to liver repair following cholestatic injury. Further, neutrophil migration into repairing liver provides inhibitory feedback for normal chemokine and matrix metalloproteinase expression during cholestatic injury and repair.

Disclosures:
The following people have nothing to disclose: Mark Harty, Elaine F. Papa, Grant A. Ramn, Stephen H. Gregory, Thomas F. Tracy

1050 EVIDENCE OF SNAIL1 TRANSCRIPTION FACTOR INVOLVEMENT IN HEPATIC STELLATE CELLS ACTIVATION PROCESS
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Background: Following liver injury, hepatic stellate cells (HSC) undergo a process of activation from the quiescent fat-storing phenotype to a highly proliferative myofibroblast-like phenotype. Snail1 is a transcription factor best known for its ability to trigger epithelial-mesenchymal transition (EMT), to influence mesoderm formation during embryonic development and to favour cell survival. Recent studies showed that the aberrant activation of Snail1 is sufficient to trigger renal fibrogenesis and that is expressed in fibroblasts in vivo during the healing process. We therefore hypothesised that Snail1 could be involved in HSC transdifferentiation process. Methods: Primary HSC were isolated from livers of healthy mice and cultured on plastic up to 10 days (in vitro activation model) or from livers of CCl4-treated mice (8 weeks, twice-weekly, i.p.) and cultured 1 day (in vivo activation model). Activation status of HSC was monitored by assessing mRNA level of HSC activation-related genes: α-smooth muscle actin (αSMA), α(1)I collagen (COL1A1), tissue inhibitor of metalloproteinase-1 (TIMP1) and fibronectin (FN). Snail1 expression in HSC and liver was screened by immunostaining and qRT Real Time PCR, whereas Snail1 activity was checked indirectly quantifying mRNA of Snail1-target genes: E-cadherin (E-cad), desmoplakin (DSP) and metalloproteinase-9 (MMP9) by qRT Real Time PCR. Results: We observed a significant increase in Snail1 mRNA level in livers of mice treated with CCl4 for 8 weeks in comparison with livers of healthy mice. These data were confirmed by immunohistochemistry studies which evidenced Snail1 positive cells in fibrotic livers. In vitro studies showed that Snail1 is expressed by HSC and that its transcription is augmented in culture activated (10 days culture) and in vivo activated HSC as compared to quiescent HSC (1 day culture). At protein level we could observe the nuclear localization of Snail1 after the fourth/fifth day of culture. We next analysed the expression of genes which have been shown to be directly (E-cad, DSP) and indirectly (MMP9, FN) regulated by Snail1. Their expression resulted substantially unchanged until the fourth day of culture, afterwards E-cad and DSP expression significantly decreased while FN and MMP9 expression increased. Summary and conclusion: In activated HSC Snail1 is up-regulated and shows a nuclear localization and the expression of some target genes results significantly modified. Our data support a role for Snail1 transcription factor in liver fibrogenesis and its involvement in HSC transdifferentiation process.

Disclosures:
The following people have nothing to disclose: Melania Scarpa, Alessia Grillo, Paola Brun, Federica Ditadi, Giorgio Palù, Ignazio Castagliuolo, Diego Martines

1051 INCREASED MORTALITY, ENHANCED LIVER FIBROSIS AND DIMINISHED ACUTE-PHASE RESPONSE AFTER BILE-DUCT LIGATION IN HEPATOCYTE SPECIFIC C-MET/GP130 DEFICIENT MICE
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Background: C-Met (HGF receptor) and gp130 (IL6 signal transducer) mediate essential signals known to be involved in proliferation and survival of liver cells. However, a detailed knowledge about their role in hepatocytes during the progression of liver fibrosis is lacking. Material and Methods: Conditional c-Met (Δc-Met) and gp130 (Δgp130) knockout mice (Cre-loxP system) were subjected to bile duct ligation (BDL). Expression of Cre-recombinase was regulated by a hepatocyte-specific pre- and postnatally activated albumin promoter. Results: While generating conditional hepatocyte specific Δc-Met mice using the prenatal (d10.5) active Cre-recombinase resulted in embryonic lethality. In contrast the postnatally activated promoter produced vital offsprings of mice lacking c-Met in hepatocytes. After BDL we observed reduced survival rates in Δc-Met (75%) and Δc-Met/Δgp130 (25%) mice. Both conditional knockout mice had higher transaminases and a significantly enhanced apoptosis rate compared to controls (p<0.05). The knockout mice also displayed more necrotic areas and an enhanced periporal infiltration and proliferation. In contrast, hepatocyte proliferation determined by BrdU uptake was significantly reduced in Δc-Met (p>0.05) and Δc-Met/Δgp130 (p>0.05) compared to wt. Analysis of the Acute-Phase-Response (APR) in Δc-Met/Δgp130 mice revealed a complete lack in SAA, SOCS3 and STAT3 induction. Surprisingly, Δc-Met mice also displayed a significantly reduced induction of APR-gen genes, while preserving the activation of STAT3. In regards to the development of liver fibrosis conditional knockout mice showed a significantly stronger activation of hepatic stellate cells 48h after BDL (control>Δc-Met<Δc-Met/Δgp130). Our results demonstrate the essential protective role of the HGF/IL6 signalling after BDL. Abolished APR, higher apoptosis and reduced hepatocyte proliferation rates together with a stronger activation of hepatic stellate cells ultimately leads to a higher degree of liver fibrosis in mice lacking c-Met and gp130.

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The following people have nothing to disclose: Arne Giebeler, Nikolaus Gasser, Małgorzata Borowiak, Carmen Birchmeier, Christian Trautwein, Konrad L. Streitze
1052
AMELIORATION OF BILARY FIBROSIS BY AN ALPHA V-BETA 6 INTEGRIN ANTAGONIST IS MEDIATED BY INHIBITION OF CHOLONGIOTIC ADHESION TO FIBRONECTIN AND CHOLANGIOCYTE TGFBIETA1 ACTIVATION

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Background and aims: AlphaV beta6 is an epithelial integrin expressed during tissue development, inflammation, wound healing and carcinogenesis. It acts as a receptor for fibronectin and tenascin, and possibly as co-receptor for the activation of latent TGFbeta1. Mice lacking this integrin display resistance to the induction of pulmonary fibrosis and alphaVbeta6 inhibition can ameliorate rat biliary fibrosis (Patsenker et al, AASLD 2006). We therefore examined the molecular mechanisms underlying the antifibrotic effect of alphaVbeta6 inhibition. Methods: Adhesion assays were performed on fibronectin using the human cholangiocyte cell line TFK-1. TGFbeta1 activation was measured by human TGFbeta1 ELISA. Rats subject to bile duct ligation (BDL) for 6 weeks and MDR2-/- mice at age 4 weeks were treated i.p. with the specific alphaVbeta6 antagonist EMDS572040 at 20 or 60 mg/kg/day for 4 and 3 weeks, respectively. Beta6 expression was determined by immunohistology, fibrosis was measured as hepatic hydroxyproline content and fibrosis-related transcripts were determined by quantitative RT-PCR. Bile duct proliferation was quantified by double immunohistochemistry for CK19 and Ki-67 using a point counting technique. Results: AlphaVbeta6 was absent in normal livers, but highly expressed on proliferating bile duct epithelia in fibrosis, with 100-fold increased transcript levels. Its inhibition in vitro reduced cholangiocyte adhesion to fibronectin and endogenous activation of TGFbeta1 by 50%. The alphaVbeta6 antagonist prevented peribiliary collagen accumulation in fibrotic animals by 40-50%. This was paralleled by downregulation of profibrogenic and upregulation of fibrolytic genes, improved liver architecture and function. Conclusions: alphaVbeta6 integrin is strongly upregulated in proliferating bile duct epithelia and drives fibrogenesis via adhesion to fibronectin and auto/paracrine cholangiocyte TGFbeta1 activation. Its pharmacological inhibition blocks the progression of primary and secondary biliary fibrosis.

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1053
TOLL-LIKE RECEPTOR-9 ACTIVATION AMELIORATES HEPATIC FIBROSIS ASSOCIATED WITH LYMPHOCYTE INTERACTIONS

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Background: CD8 subsets mediate hepatic fibrosis and NK cells have an anti fibrotic effect. Oligodeoxynucleotides (ODNs) with immunomodulatory motifs (CpG) are acting through TLR9 to stimulate NK cells. We hypothesized that TLR9 would generate an anti fibrotic response. Methods: (I) Hepatic fibrosis was induced by CCl4 administration X2/wk for 6 wks in WT and TLR9-/- mice and was compared to naive. (II) To isolate the effect of TLR9 in lymphocytes, we transferred splenocytes from WT or TLR9-/- donors to irradiated WT recipients. Then fibrosis was induced in recipients by CCl4. (III) CCl4 was induced in WT male mice (groups A to D) for 6 wks. During the last 2 wks of CCl4 administration, mice were weekly treated by hydrodynamic based transfection with either saline (group A), CpG ODN (gr. B), pGEM7 (gr. C) and were compared to control CpG ODN injected mice (gr. D) and naive controls (Group E). Different routes of ODN administrations were also examined. (IV) As TLR9 activation is mediated by IL-6 and to further evaluate if the ODN effect on fibrosis is TLR9-mediated we used the IL-6/- mice. Thus, a similar design in (III) was used in IL-6/- mice and compared to WT response. Results: (I) Compared to WT mice, TLR9-/- mice showed a significant increase of fibrosis. While NK cells were unchanged in TLR9-/- groups compared to the WT, both CD4 and CD8 distributions were reduced in TLR9-/- groups. Post fibrosis; CD4 remains unchanged but CD8 increased. (II) Irradiated WT recipients with TLR9-/- lymphocytes also showed increased fibrosis. While NK cells were unchanged, both CD4 and CD8 distributions were increased in recipients with TLR9-/- lymphocytes and keeping CD8 predominance. (III) Control groups A/D displayed fibrosis levels higher than the ODN treated groups (B/C). Serum ALT levels were elevated in all CCl4 groups but were decreased in the CpG and pGEM7 treated groups. CD8 increased in control groups A/D but was alleviated in treated groups B/C. CD4 cells were decreased and Tregs increased in all fibrotic groups, but without remarkable differences. NK cells decreased following fibrosis in control groups A/D, however, both treated groups B/C showed an increase. Different routes of ODN administration showed similar anti fibrictic effects. (IV) Administration of ODNs to fibrotic IL-6/- mice, did not affect fibrosis or lymphocyte distribution, supporting that ODNs plays a role in fibrosis alleviation via TLR9 activation. Conclusions: TLR9 activation is involved in immune modulation of fibrosis. CpG and pDNA displayed significant anti-fibrotic activity, accompanied by CD8 suppression and increased NK cells.

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ANGIOTENSIN 1-7 REDUCES BILE DUCT PROLIFERATION AND HEPATIC FIBROSIS IN THE BILE DUCT LIGATION RAT

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Introduction: Angiotensin 1-7 (Ang-(1-7)) is a biologically active peptide of the renin angiotensin system (RAS) which opposes the deleterious actions of angiotensin II. The effects of Ang-(1-7) infusion on the progression of liver disease have never previously been described. We have used the bile duct ligation (BDL) model to determine the impact of Ang-(1-7) on the development of hepatic fibrosis, generation of growth factors and feedback mechanisms within the RAS. Methods: Male Sprague-Dawley rats underwent sham operation (n=11) or BDL (n=22). Animals were studied in three groups. Sham operated rats and 11 BDL rats were implanted with intraperitoneal osmotic minipumps delivering normal saline. A further 11 BDL rats were implanted with pumps delivering Ang-(1-7) (28µg/kg/hr i.p.). Animals were killed after 2 weeks and plasma and liver tissue collected for analyses. Results: Ang-(1-7) significantly improved plasma...
biline and GGT concentrations and reduced hepatic hydroxyproline content and collagen 1A1 gene expression (P<0.05). Alpha smooth muscle actin (α-SMA) immunohistochemistry showed significant attenuation in the Ang-(1-7) treatment group (P<0.05). Similarly, quantitative PCR demonstrated a reduction in α-SMA gene expression (P<0.05). These findings were supported by morphometric analysis of picrosirius staining and bile duct proliferation assessment in liver sections. Bile duct ligation resulted in significant gene up-regulation of key components of the RAS, including ACE, ACE2, AT1 and the putative Ang(1-7) receptor, Mas (P<0.01). However, infusion of Ang-(1-7), resulted in a down regulation of ACE and Mas gene expression (P<0.05). The ACE gene data was further supported by ACE autoradiography of liver tissue which showed a significant suppression of ACE protein in the Ang-(1-7) treated group (P<0.001). The gene expression of growth factors CTGF and VEGF were both attenuated by Ang-(1-7) infusion (P<0.05). Data were analyzed by analysis of variance (ANOVA) with Newman-Keuls Multiple Comparison Test. Conclusions: The RAS is upregulated following bile duct ligation. Ang-(1-7) infusion reduces hepatic fibrosis and bile duct proliferation. This is associated with down-regulation of key components of the RAS (ACE and Mas) as well as critical growth factors (CTGF and VEGF). Reduced gene and protein expression of SMA indicate that Ang-(1-7) may attenuate hepatic stellate cell activation. These novel findings indicate that the ACE2/Ang-(1-7)/Mas axis is activated in chronic liver injury and may represent a potential target for antifibrotic therapy.

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ROLE OF C-C CHEMOKINE RECEPTOR 2 (CCR2) DURING CONSTITUTION AND RESOLUTION OF FIBROSIS INDUCED BY CARBON TETRACHLORIDE IN MICE
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Inflammatory reaction is part of the fibrogenic process and it is accompanied by an infiltration of leukocytes that is regulated by chemokines. The C-C chemokine receptor, CCR2, has been identified as the primary receptor that mediates monocyte chemoattractant protein-1 (MCP-1) responses. MCP-1 and CCR-2 are up-regulated during the progression of fibrosis in humans. During liver injury, MCP-1 is expressed by hepatocytes, endothelial, Kupffer cells and stellate cells (HSC). In contrast, CCR-2 expression is mainly associated with macrophages and a subpopulation of lymphocytes. These cells have been shown to play a major role in both the constitution and the resolution of liver fibrosis induced by carbon tetrachloride (CCl4) in mice. However, the role of CCR2 in these processes has never been evaluated. We used CCR2 knockout mice (CCR2/-) to determine whether CCR2 is involved both in the establishment and/or the resolution of fibrosis. CCR2/- mutant (n=18) and wild type (n=18) male mice of the same Balb/c Ola background were injected twice weekly during 6 weeks with CCl4. Animals were killed either 48 hours (peak of fibrosis) or 7 days (partial resolution of fibrosis) after the end of the injury. We observed a significant lower level of the fibrotic scar at the peak of fibrosis in mutant animals compared to their control littermates as assessed by red sirius quantification. This effect was correlated with a reduction in the proportion of activated macrophages present in the lesion as defined by quantification of CD68 positive macrophages. In contrast, 7 days after the end of the fibrogenic challenge, whereas fibrosis was diminished in CCR2+/+ mice, CCR2/- mice experienced no regression of their liver injury, and presented marked regions of calcification. This persistence of hepatic injury in mutant animals was correlated with a maintain in TIMP1 mRNA expression. In conclusion, these findings demonstrate that CCR2 stimulates liver fibrogenesis by inducing macrophage infiltration that will subsequently activate HSC and confirm the critical role of macrophages and inflammatory cells for the fibrogenic process. In addition, CCR2 is also involved in the resolution of the fibrotic scar, with an opposite role allowing macrophages to eliminate injured hepatocytes.

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INCREASED SUSCEPTIBILITY TO HEPATIC STEATOSIS, INFLAMMATION AND ADVANCED FIBROSIS IN APOLIPROTEIN-E DEFICIENT MICE
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The contribution of hyperlipidemia, one of the most important metabolic risk factors, to the progression of liver disease is not established. In this study, we employed apolipoprotein E-deficient (ApoE-) mice, a mouse model of hypercholesterolemia, to assess the relationship between the dysregulation of lipid metabolism and the progression of liperoxidative-induced liver damage. To this end, ApoE-/- and wild-type mice were exposed to carbon tetrachloride for 4, 6 and 8 weeks. Two additional groups received only olive oil. As compared to wild-type, ApoE-/- mice had increased hepatic steatosis as revealed by oil red-O staining. Moreover, the number of F4/80-positive cells, indicative of macrophage infiltration, as well as necroinflammation were increased in liver sections from ApoE-/- mice. At a more advanced stage, ApoE-/- mice exhibited exacerbated fibrosis, assessed by Sirius red staining and α-SMA immunostaining. Interestingly, the number of non-parenchymal cells with a TUNEL-positive nucleus was significantly reduced in ApoE-/- mice. Moreover, liver injury (i.e. ALT and AST) was more severe in ApoE-/- than in wild-type mice. Importantly, significant direct correlations were identified between serum cholesterol and oxysterols and oxidized LDL. Conversely, the contribution of hepatic injury as determined by liver injury parameters (ALT and AST), was more severe in ApoE-/- than in wild-type mice. In addition, ApoE-/- mice exhibited exacerbated hepatic steatosis as assessed by oil red-O staining. Moreover, the number of F4/80-positive cells, indicative of macrophage infiltration, as well as necroinflammation were increased in liver sections from ApoE-/- mice. At a more advanced stage, ApoE-/- mice exhibited exacerbated fibrosis, assessed by Sirius red staining and α-SMA immunostaining. Interestingly, the number of non-parenchymal cells with a TUNEL-positive nucleus was significantly reduced in ApoE-/- mice. Moreover, liver injury (i.e. ALT and AST) was more severe in ApoE-/- than in wild-type mice. Importantly, significant direct correlations were identified between serum cholesterol and oxidized LDL. Conversely, the contribution of hepatic injury as determined by liver injury parameters (ALT and AST), was more severe in ApoE-/- than in wild-type mice.
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DELETION OF THE THROMBIN RECEPTOR GENE; PAR 1, AMELIORATES LIVER FIBROSIS AND DECREASES TGFβ EXPRESSION BY HEPATIC STELLATE CELLS IN A MURINE MODEL OF CIRRHOSIS

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Thrombin, a pleuripotent serine protease, is principally known for modulating hemostasis but can promote extracellular matrix (ECM) production and has been implicated in liver fibrogenesis. Profibrotic effects of thrombin are thought to occur through protease activated receptor 1 (PAR1) signaling. A previous study has shown that PAR 1 antagonism ameliorates liver fibrosis in a rat bile duct ligation model (Fiorucci 2004). The aim of this study was to investigate whether deletion of the PAR 1 gene protected against liver fibrosis in a model of hepatocyte inflammation and fibrosis and to identify potential mechanisms. Methods: Twice weekly intraperitoneal carbon tetrachloride injections for 5 and 8 weeks were used to induce liver fibrosis in 9 week old wild type and PAR 1 knock-out(-/-) C57/BL6 mice. Liver sections were stained with Sirius red and fibrosis was quantified by computer assisted morphometry. Hepatic collagen content was quantified by hydroxyproline assay. α-SMA Smooth muscle actin(α SMA) expression was detected by Western blot. Hepatic stellate cells (HSC) were isolated from wild type and PAR 1 -/- mice and cultured on plastic in M199 media supplemented with 10% fetal bovine serum. TGF wild type and PAR 1 -/- mice and cultured on plastic in M199 Western blot. Hepatic stellate cells (HSC) were isolated from in a necroinflammatory model of liver fibrosis. The protective effect is magnified with longer duration of liver injury. HSC isolated from PAR1 knockout animals express less TGF β and less α SMA than wildtype animals, suggesting less HSC activation and thus a potential mechanism for the antifibrotic effect. The observation that fibrosis was not completely ameliorated with PAR 1 gene deletion suggests a multifactorial pathway for hepatic fibrogenesis.

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HUMAN DISCOIDIN DOMAIN RECEPTOR 1: INTRAHEPATIC FORMS AND IN VITRO FUNCTIONS

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Discoidin Domain Receptor 1 (DDR1) is a tyrosine kinase receptor that binds and is activated by collagens. Being a receptor of collagen, DDR1 expression needs to be tightly regulated. Transcriptional profiling of human cirrhosis with DNA array and quantitative real-time RT-PCR detected elevated mRNA expression of DDR1 compared to non-diseased liver (Shackel NA, et Am J Pathol. 2002;160:641). The study aims to characterize DDR1 expression in cirrhotic and non-diseased liver and to examine the cellular effects of DDR1 expression. Immunoblots of 10 cirrhotic and 5 non-diseased livers used the anti-DDR1 (C-20 peptide) antibody (Santa Cruz). In situ hybridisation (ISH) used a Digoxigenin (DIG)-labeled (Roche) human DDR1 566 bp riboprobe. Immunostains included cytokeratin 18 (CK18), DDR1 (C-20), CD45, CK19 and CK7. Green fluorescent protein (GFP): DDR1 isoform A fusion protein was transiently expressed in the Huh7 cell line for cell adhesion and transwell migration assays on collagen-I, fibronectin and matrigel. The actin cytoskeleton was stained using phalloidin-594. Two molecular forms of DDR1 protein were differentially expressed in cirrhotic versus non-diseased liver: A 132 kDa form of DDR1 in non-diseased liver but not cirrhotic liver and an 85kDa form present only in cirrhotic liver. As DDR1 is subject to protease-mediated cleavage after collagen-induced activation, this differential expression may indicate more intense activation of DDR1 protein in cirrhotic liver. To determine the cellular localization of DDR1, ISH and immunofluorescence were performed. Intense DDR1 mRNA expression was detected in hepatocytes at the portal/parenchymal interface and the reactive bile duct cells in the septum. DDR1 protein was predominantly in leucocytes, the luminal aspect of biliary epithelium and the bile canalicular domain of hepatocytes, indicating the presence of DDR1 on the apical domain of these epithelial cells. DDR1 overexpression increased Huh7 cell adhesion to collagen-I but decreased adhesion to fibronectin and matrigel. DDR1 overexpressing Huh7 cells showed decreased migration on collagen-I, fibronectin and matrigel. As DDR1 is known to be involved in cellular morphogenesis, the actin cytoskeleton was visualised but was unaltered in DDR1 overexpressing cells. Flow cytometry analysis indicated that DDR1 overexpression did not regulate the expression of matrix metalloproteinase (MMP) 1, 2, 3 or 9. These findings suggest that increased DDR1 expression and activation in cirrhotic liver may have an important role in the pathogenesis of cirrhosis by influencing extracellular matrix – hepatocyte interactions including adhesion and migration.

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BXR RECOMBINANT INBRED MOUSE LINES - A GENETIC REFERENCE POPULATION FOR DISSECTION OF THE COMPLEX GENETICS OF LIVER FIBROSIS

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Background: Previously we have shown that quantitative trait locus (QTL) analysis can detect susceptibility loci for liver fibro-
sis, based on genome-wide linkage analysis in experimental crosses of inbred mouse strains (Hillebrandt et al. Gastroenterology 2002;123:2041-51). In previous studies we identified multiple QTLs and a quantitative trait gene (C5) in a large F2 intercross of fibrosis-susceptible and resistant strains of inbred mice (Hillebrandt et al. Nat Genet 2005;37:835-43). Our aim now was to establish a murine genetic reference population for integrated and replicated analyses of the pathobiology of liver fibrogenesis. Methods: We availed of BXD recombinant inbred lines generated by (i) crossing the two inbred strains C57BL/6J and DBA/2J, and (ii) inbreeding the F2 progeny by brother×sister matings for at least 20 generations. Each of the 80 lines shows a unique mosaic gene set of the two founder strains and has been genotyped for 13377 single nucleotide polymorphisms (SNPs). We conducted whole-genome association scans in the BXD panel for integrated analysis of profibrogenic susceptibility genes. Conclusions: The observed differences in susceptibility to liver fibrosis among the BXD lines demonstrate that this set provides a genetic reference panel for integrated analysis of profibrogenic susceptibility genes. We conclude that the use of such a panel, coupled with transcriptome profiling, will lead to novel insights into the complexity of the genetic control of liver fibrogenesis and allow modeling of gene networks during chronic liver injury. Supported by the German Network for Systems Genetics.

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GENETIC ASSOCIATION STUDY OF PROFIBROGENIC GENE VARIANTS USING TRANSIENT ELASTOGRAPHY FOR PHENOTYPIC CHARACTERIZATION OF LIVER FIBROSIS

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Introduction: Transient elastography (TE) has been employed to determine the stage of liver fibrosis in patients with chronic liver diseases with close correlation to liver histology. Recently based on a genome-wide scan, a novel “gene signature” consisting of 7 single nucleotide polymorphisms (SNPs) has been proposed to identify the risk of progressive fibrosis in patients with chronic hepatitis C virus (HCV) infection (Huang et al. Hepatology 2007; epub). Our aim now was to evaluate this gene signature in a large cohort of patients with chronic liver diseases who underwent TE for assessment of liver fibrosis.

Patients and methods: We enrolled 516 patients with chronic liver diseases, including a subset of 256 patients with chronic HCV infection. Liver stiffness measurements (LSM) were performed by TE; in addition we included patients with unequivocal signs of liver cirrhosis in whom TE failed (e.g. because of ascites). We stratified patients in a severe fibrosis group (LSM >9.5 kPa, or signs of liver cirrhosis) and a no-fibrosis group (LSM <7.5 kPa). Patients were genotyped for the 7 SNPs identified by Huang et al. using PCR-based Taqman assays.

Results: The genetic analyses revealed that carriers of the common allele of a SNP in the ornithine decarboxylase antizyme inhibitor (AZIN1) gene are at increased risk for severe liver fibrosis (OR = 1.75; 95% CI = 1.01 - 3.04; p < 0.05). This association was even more pronounced in the group of patients with chronic HCV infection (OR = 2.89; CI = 1.28 - 6.52; p < 0.01). Overall, there was also a trend (p = 0.069) for an association with the common allele of a SNP in the 5’ region of the adaptor-related protein complex 3 (AP3S2) gene (rs2290351), which showed a significant association in the HCV subgroup (OR = 6.56; p < 0.05). However, inverse risk alleles and no associations between LSM and the other SNPs included in the gene signature in our cohort or in the HCV subgroup indicate that additional studies are needed to validate the predictive value of the profibrogenic gene signature.

Conclusions: Our study demonstrates that TE qualifies for non-invasive phenotyping of fibrosis patients in genetic association studies. Furthermore, some of the recently identified cirrhosis risk variants are also associated with progressive liver fibrosis in our cohorts of patients with chronic HCV infection in particular and chronic fibro-proliferative liver diseases in general.

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SYNERGISTIC ANTI-FIBROTIC EFFICACY OF STATIN AND PROTEIN KINASE C INHIBITOR IN HEPATIC FIBROSIS

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Background & Aims: Activated hepatic stellate cells (HSCs) are major participants in hepatic fibrosis, and thus, the suppression of HSC activation or survival has been proposed as anti-fibrotic therapies. Statin has anti-fibrotic efficacy in human fibrosing diseases, such as pulmonary and renal fibrosis, and therefore, is implicated in hepatic fibrosis. However, statin can also activate protein kinase C (PKC), which augments hepatic fibrogenesis, and thereby, is likely to reduce statin’s anti-fibrotic efficacy. Therefore, we hypothesized that the simultaneous treatment of statin and PKC inhibitor may synergistically enhance anti-fibrotic efficacy in hepatic fibrosis. Methods: LX-2 cells (an immortalized human HSC line), pravastatin and enzastaurin (PKC inhibitor) were used in this study. Hepatic fibrosis model was established in BALB/c mouse by injecting carbon tetrachloride intraperitoneally twice per week for 6 weeks. Statin and PKC inhibitor were administered by gavages 5 days per week for 5 weeks. Cellular apoptosis was explored using DAPI or TUNEL staining and immunoblot analysis. Hepatic fibrosis and HSC activation were assessed by morphometric analysis of histological findings and immunohistochemistry for α-smooth muscle actin. Results: Statin induced LX-2 cell apoptosis through activating mitochondrial apoptotic signals. While PKC inhibitor did not induce LX-2 cell apoptosis, the addition of this inhibitor significantly increased statin-induced apoptosis, and this was due to enhanced caspase 9 activation in these cells. The per-
centages of TUNEL-positive HSCs were significantly increased in mice treated with statin+PKC inhibitor as compared to control or single compound-treated mice. The extent of collagen deposition and the percentage of area occupied by activated HSCs were significantly decreased in mice treated with statin+PKC inhibitor as compared to control or single compound-treated mice. Conclusions: These results demonstrate that the simultaneous treatment of statin and PKC inhibitor synergistically enhanced anti-fibrotic efficacy in in vitro and in vivo model of hepatic fibrosis. Therefore, this combination strategy may have therapeutic implication in reducing hepatic fibrosis.

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TGFβ1 STIMULATES THE HUMAN α1(I) COLLAGEN PROMOTER BY COOPERATION BETWEEN SMAD2/4 AND SP1 TRANSLATING FACTORS

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Hepatic fibrosis and cirrhosis result from the excessive deposition of predominantly type I collagen, composed of two α1 chains and one α2 chain. TGFβ1, a principal pro-fibrogenic cytokine, activates the human α2(I) collagen promoter via Smads (2, 4). However, the role of Smads in activation of the human α1(I) collagen promoter remains unknown. Sp1, an ubiquitous zinc-finger family transcription factor, binds to numerous sites along the length of the human α1(I) collagen promoter. The enhancement of expression of distinct genes by cooperation of Sp1 and Smad proteins has been described previously. The aim of this study was to determine the roles of Smads and Sp1 in mediating the effect of TGFβ1 signaling on activation of the human α1(I) collagen promoter. The binding of Smads and Sp1 to the proximal collagen promoter was determined by EMSA and their specific sites of binding were determined by sequential 2 bp mutations of the promoter. Transient transfection experiments were performed with cultured human LX-2 stellate cells. Pre-designed shRNA plasmids were used to knockdown Smads and Sp1. Smads and Sp1 separately bound to adjacent sites of the proximal - 174 α1(I) collagen promoter, and expression vectors of Smad2 and Sp1 separately as well as their combination activated the collagen promoter. Smad2 or Sp1 knockdown decreased activation of the promoter with or without the presence of TGFβ1. Smad4 knockdown alone had no effect in either case, but simultaneous knockdown of Smad4 and Sp1 again effectively decreased activation of the promoter in cells not treated with TGFβ1, but not in TGFβ1-treated cells. However, simultaneous knockdown of Smad2 and Sp1 effectively decreased activation of the promoter with or without the presence of TGFβ1. Smad4 knockdown alone had no effect in either case, but simultaneous knockdown of Smad4 and Sp1 again effectively decreased activation of the promoter in either TGFβ1-treated or untreated cells. The knockdowns were confirmed by Western blot and/or mRNA determined by real time PCR. In conclusion, Smad2, Smad4 and Sp1 cooperate in enhancing human α1(I) collagen promoter activity and type I collagen production in human hepatic stellate cells.

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shown to reduce fibrosis severity in animal models of disease. Since liver macrophages may be essential for effective fibrosis recovery, a means to specifically target myofibroblasts with a therapeutic that promotes apoptosis has been examined. A human recombinant antibody (termed C1-3) was generated to a peptide sequence present on an extracellular domain of synaptofysin, a plasma membrane protein expressed in activated hepatic stellate cells and myofibroblasts. FITC-labelled C1-3 antibody bound to human and mouse liver myofibroblasts in vitro and was taken up into cells by endocytosis. FITC-labelled C1-3 antibody was then injected i.p. into control mice or mice with liver fibrosis (treatment with CCl4). Antibody appeared in circulating blood within 20 minutes and concentrations peaked at 2 hours. Tissue analysis indicated that the antibody was localised to the fibrotic liver and was not detected in brain, kidney spleen or muscle. Fibrotic liver levels of antibody were maximal after 6 hours and binding was to cells within the scar region as determined by immunohistochemistry or fluorescence microscopy in liver sections. Co-staining for α-smooth muscle actin indicated that the antibody localised specifically only to myofibroblasts. The antibody was not detected in livers from control mice injected with the antibody. In rats, gliotoxin reverses fibrosis through the stimulation of myofibroblast apoptosis, although hepatocyte and Kupffer cell apoptosis also occur. To examine whether targeting gliotoxin to liver myofibroblasts with the C1-3 antibody may be more effective, mice with liver fibrosis were injected with either free gliotoxin; an equivalent dose of gliotoxin conjugated to C1-3 or an equivalent dose of gliotoxin conjugated to an unrelated antibody that does not target any liver cells. Both free gliotoxin and C1-3-gliotoxin stimulated an increase in myofibroblast apoptosis and reduced the numbers of α-smooth muscle actin positive cells in liver sections. However, C1-3-gliotoxin was significantly more effective than free gliotoxin and prevented the increase in hepatocyte and Kupffer cell apoptosis seen with free gliotoxin. The C1-3 antibody is therefore able to selectively target myofibroblasts in a mouse model of liver fibrosis. Antibody conjugation did not prevent antibody targeting or therapeutic activity.

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1065 ENHANCED IL-6 PRODUCTION BY HEPATIC STELLATE CELLS IN RESPONSE TO IMATINIB MESYLATED STI571 - (GLEEVEC ®), A NOVEL LINK BETWEEN RECEPTOR TYROSINE KINASE INHIBITION AND HEPATIC REGENERATION

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Background: The hepatic stellate cell (HSC) is a key target of antifibrotic therapies. Gleevec® (imatinib mesylate), is among a new class of small molecule receptor tyrosine kinase (RTK) inhibitors, and is indicated for treatment of CML and GI stromal tumors. Because Gleevec inhibits β-PDGFR signaling, a major pathway of HSC proliferation, we assessed its activity in culture and in vivo, and examined downstream targets of its activity in an immortalized human stellate cell line (LX-2) using cDNA microarray. Methods and Results: Gleevec dose-dependently inhibited LX-2 cell proliferation (0.5 µM – 2.0 µM) with no effect on viability at 12h and 24h. This was associated with progressive inhibition of β-PDGFR phosphorylation as assessed by Western Blot. Mitochondrial activity and superoxide anion production were decreased in response to Gleevec, as assessed by Mito-tracker and FACS. To identify HSC genes altered by Gleevec treatment, cDNA microarray (Affymetrix U133 Chip) was performed on cells with or without Gleevec (2 µM) treated for 12 hours. There was a significant up-regulation of 28 genes involved in cell growth, metabolism or apoptosis, of which 7 were validated by qRT-PCR. This included Interleukin-6 (IL-6), which was elevated 4-fold (P<0.05), and correlated with progressive IL-6 secretion in LX-2 supernatants. Gleevec decreased gene expression in LX-2 of collagen α1(I), α-SMA, β-PDGFR, TGFβ1 receptor type 1, MMP2 and tissue inhibitor of metalloproteinase II (TIMP II). In vivo, Gleevec administered to rats (5 d/wk) beginning 4 weeks after 3d per week IP dosing of thioacetamide, led to reduced lobular inflammation, and collagen content as assessed by pathological scoring and/or morphometry, with significant reductions in portal pressure and downregulation of key fibrogenic genes in whole liver. Importantly, hepatic IL-6 mRNA levels were significantly (1.4 to 2-fold) increased in TAA-treated animals receiving Gleevec compared to animals receiving TAA and vehicle. Conclusions: These findings uncover an unexpected link between inhibition of HSC activation by Gleevec and enhanced secretion of IL-6, a key regenerative cytokine. Impaired IL-6 production during liver injury could be related to up-regulation of one or more RTK targets inhibited by Gleevec. Clarification of specific RTK pathways in HSCs that are linked to IL-6 secretion will broaden our understanding of this agent’s effects and refine anti-fibrotic and regenerative therapies in humans.

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The following people have nothing to disclose: Carlos E. Alvarez, Youngchul Kim, Efsevia Albanis, Maria Isabel Fiel

1066 CD147 REGULATION OF HEPATOCYTE DERIVED MATRIX METALLOPROTEINASES: A NOVEL PATHWAY INVOLVED IN LIVER FIBROGENESIS

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Introduction: We have previously shown CD147 is upregulated in the human cirrhotic liver and expressed predominantly on hepatocytes. CD147 is an abundant glycoprotein and a potent inducer of matrix metalloproteases (MMPs-1, 2, 3 & 9) in malignant cells and fibroblasts. The progression of liver fibrosis is characterised by the dysregulation of extracellular matrix turnover, primarily mediated by MMPs. Therefore, we hypothesize that CD147 regulates hepatocyte-derived MMP expression which is capable of matrix remodelling. Methods: Cellular localisation of CD147 and MMPs was determined by immunohistochemistry and immunofluorescence in human cirrhotic liver explants. Progressive changes in CD147 expression were analysed in a rat bile duct ligated model of injury (0, 1, 2, 3 & 4 weeks). MMP and tissue inhibitors of metalloproteinase (TIMP) activity was analysed in primary rat hepatocytes (with and without CD147 blocking antibody) and hepatocelllular hepatic stellate cells (HSCs) by zymography and reverse zymography respectively. In-vivo functional studies of CD147 were performed in mice treated with carbon tetrachloride for 4 weeks using an anti-CD147 blocking antibody (mAb R737.2). Injury was assessed by histological analysis and severity of fibrosis quantitated by hydroxyproline estimation. Results: CD147 mRNA
and protein were increased in progressive injury leading to cirrhosis in humans and in the rat bile duct ligated injury model. Localisation studies showed CD147 and MMPs-1, 2 & 9 are expressed predominantly on hepatocytes. In-vitro studies of MMP from rat primary hepatocytes showed significantly increased MMP-2 & 9 comparable to either rat primary quiescent or in-vivo activated HSCs. Further, significant TIMP-1 & 2 activity was found in media from primary HSCs but not in media from hepatocytes. Importantly, MMP-2 & 9 activity was reduced following administration of a non-CD147 blocking antibody. In-vivo antibody treatment blocking CD147 was associated with a significant reduction in hepatic inflammation, the preservation of hepatic architecture and increased in collagen I deposition (p<0.01). Conclusion: Our studies demonstrate that CD147 regulates hepatocyte derived MMP-2 & 9 production, and that this pathway has a distinct role in remodelling of extracellular matrix in liver injury and fibrogenesis.

Disclosures: The following people have nothing to disclose: Sarah R. Richardson, Fiona J. Warner, Geoff W. McCaughan, Mark D. Gorrell, Rosa Lam, Nicholas A. Shackel

1067 DETECTION AND PREVENTION OF HEPATIC FIBROSIS TARGETING TGF-β ACTIVATION REACTION
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Transforming growth factor (TGF-β), playing a pivotal role in the pathogenesis of liver diseases, is produced as a high molecular weight latent form, and thus must be activated before exerting its biological activities. TGF-β activation is the reaction, by which 25 kD active TGF-β molecule is released from the latent complex. We previously showed that TGF-β is activated by plasmin (PLN) and plasma kallikrein (PLK) during pathogenesis of liver fibrosis and impaired liver regeneration, respectively, and that blockage of these activation reactions with low molecular weight protease inhibitors prevented the development of the diseases in animal models. Now, we determined that PLN and PLK cleaved between K56-L57 and R58-L59 in LAP portion of human latent TGF-β1, respectively, and produced antibodies that specifically recognize the cut ends of each cleavage site. In Western blotting, the antibodies against the PLK-cut ends (anti-R58 and anti-L59 antibodies) recognized the degradation products produced during PLK-mediated activation of latent TGF-β, but did not recognized uncleaved latent TGF-β and only weakly recognized PLN-cleaved latent TGF-β degradates. A similar result was obtained with the antibodies against the PLN-cut ends (anti-K56 and anti-L57 antibodies). The anti-R58 antibodies strongly stained liver sections from patients with fulminant hepatitis compared to normal liver sections from patients that died from pulmonary embolism. On the other hand, the anti-L59 antibodies were successively used to establish ELISA to quantitate LAP degradates released into and circulating in the serum in rat fibrosis models. In bile duct ligation models, we found that serum LAP degradates concentration in fibrotic animals was 7-fold higher compared with those in the control animals (no treatment or sham operation), and showing a good correlation to hepatic contents of hydroxyproline and serum GOT/GPT. Furthermore, we produced the peptide containing these cleavage sites as well as its decoy peptide, in which cleavage site amino acids were all mutated to A. These peptides efficiently suppressed the TGF-β activation reaction, prevented the activation of hepatic stellate cells in culture, and prevented impaired liver regeneration observed in LPS-pre-treated partially hepatectomized mice. These results suggest a key role for PLN/PLK in the generation of active TGF-β, thereby addressing a potential use for PLN/PLK inhibitors and decoy peptides in hepatitis/fibrosis therapy. These results also suggest a potential usage of a LAP degradate as a biomarker for liver fibrosis.

Disclosures: Shinji Ogawa - Employee: Pfizer
Katsuhiko Shinjo - Employee: Pfizer
The following people have nothing to disclose: Soichi Kojima, Ayako Arai, Ryutaro Teraoka

1068 C-REACTIVE PROTEIN AMELIORATES HEPATIC FIBROGENESIS THROUGH INHIBITION OF HEPATIC STELLATE CELL TRANSACTIVATION
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Recently, minimal increment in serum C-reactive protein (CRP) levels has been recognized as a sensitive marker of micro-inflammation in atherosclerotic lesions and hepatic inflammation in non-alcoholic steatohepatitis (NASH). Although it is well-known that CRP participates in regulation of inflammatory responses involving complement activation, the role of CRP in hepatic fibrogenesis has not been well elucidated. In the present study, therefore, we investigated the effect of CRP on transactivation of isolated hepatic stellate cells (HSCs), and experimental hepatic fibrosis in the rat. Methods: HSCs were isolated from male Wistar rats, and cultured in DMEM supplemented with 10% heat-inactivated FBS up to 7 days prior to experiments. Proliferative responses in cultured HSCs were evaluated by BrdU incorporation, and phosphorylation of ERK1/2 and Akt was detected by Western blotting. For experiments in vivo, hepatic fibrosis was induced in male Wistar rats by repeated intraperitoneal injection of thioacetamide (TAA, 200 mg/kg, 3 times/week) for 6 weeks. Some animals were given rat CRP (1 mg/kg, i.p.) simultaneously starting from the third week for 4 weeks. The degree of hepatic fibrosis was assessed by picro-sirus red staining, and expression of smooth muscle α-actin (αSMA) in the liver was detected by immunohistochemistry. Results: In day-3 cultured HSCs, PDGF-BB (5 ng/ml)-induced increases in BrdU uptake were blunted in the presence of human CRP in a dose-dependent manner, reaching 57% inhibition at a concentration of 1 mg/dl (p<0.05, vs. PDGF alone). The similar tendency was observed in 7 day-cultured, activated HSCs. More importantly, this anti-proliferative effect of CRP was observed not only in human CRP but also in rat CRP, which lacks complementary activation capability. PDGF-induced phosphorylation of ERK1/2 was also inhibited by CRP; however, phosphorylation of Akt was not decreased even in the presence of CRP. On the other hand, TAA-induced hepatic fibrosis was ameliorated markedly when CRP was given simultaneously for 4 weeks. Indeed, induction of αSMA in the liver was prevented remarkably, indicating that CRP also inhibited transactivation of HSCs in vivo. Conclusions: These findings clearly indicated that CRP has an anti-fibrogenic property including direct inhibitory effect on HSC transactivation. The anti-proliferative effect of CRP on HSCs appears to be independent on complement activation, and most likely involves inhibition of the MAP kinase pathway. It is therefore postulated that CRP produced from hepatocytes plays a regulatory role in progression of hepatic fibrosis in NASH.

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THE FUNCTIONAL CONTRIBUTION OF BONE MARROW- Derived CELLS TO LIVER FIBROSIS IN A MOUSE MODEL OF CHRONIC LIVER INJURY

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Aims: A significant proportion of the hepatic fibrogenic cell population in chronic liver disease in humans and animal models is of bone marrow (BM) origin. The purpose of this study was to 1) evaluate the relative contribution of the BM-derived (exogeneous) and intra-hepatic (endogeneous) fibrogenic cell compartments to hepatic fibrosis in a carbon tetrachloride (CCL4) model of injury; 2) to determine whether the BM can influence the liver’s fibrotic response to injury by manipulation of BM genotype. Methods: r/r collagen transgenic mice (r/r) have an exaggerated fibrotic response to liver injury compared to C57B6 wild-type (Wt) equivalents due to constitutive resistance of collagen I to degradation. Recipient female mice were myelo-ablated and received BM transplantation from male donors according to the following four experimental groups: 1) Wt BM into Wt recipient, 2) r/r BM into Wt recipient, 3) Wt BM into r/r recipient, 4) r/r BM into r/r recipient. Liver fibrosis was induced by 8 weeks of intraperitoneal CCL4. Liver tissue was harvested immediately post injury (day 1) and into recovery (day 9) and fibrosis quantified by digital image analysis of histological sections. (groups of n=6) Results: r/r collagen mice display increased hepatic fibrosis to CCL4 injury over controls, a phenotype enhanced into recovery after liver injury (4.63% vs 3.22% p=0.038, and 4.06% vs 2.40% p=0.004, respectively). Wt mice receiving BM transplantation from r/r donors have an increased hepatic fibrotic response and r/r transgenic mice receiving BM transplantation from Wt donors have a diminished fibrotic response to injury compared to controls, particularly into the recovery phase (2.80% vs 2.40% p=0.013, and 2.78% vs 4.06% p=0.008, respectively at day 9). The degree of liver fibrosis in the two mismatch BM transplanted groups was approximately equivalent (3.70% & 3.63% at day 1, 2.80% & 2.78% at day 9, in the r/r BM into Wt recipient and Wt BM into r/r recipient groups respectively) and levels of fibrosis were intermediate between wholly wild-type or r/r mice indicating that BM-derived cells and endogeneous intra-hepatic fibrogenic cell populations contribute equally to the pathogenesis of liver fibrosis. [results expressed as mean % surface area collagen] Conclusions: In this in vivo functional assay of liver fibrosis, both circulating BM-derived cells and intra-hepatic derived fibrogenic cell populations contribute equally to the development of liver fibrosis. Moreover, manipulation of the fibrogenic propensity of the BM can influence the ensuing fibrotic response of the liver to a chronic injurious stimulus. 

Disclosures: The following people have nothing to disclose: Yiannis N. Kallis, Rob Goldin, John Iredale, Stuart Forbes

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ACCELERATED ORTHOTOPIC HCC GROWTH IS LINKED WITH INCREASED PRO-ANGIOGENIC AND PRO-METASTATIC FACTORS IN MURINE LIVER FIBROSIS

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Background & Aims: Most experimental therapy studies are performed in mice that bear subcutaneous or orthotopic hepatomas but are otherwise healthy and non-fibrotic. However, the majority of hepatocellular carcinoma develops in patients suffering from pre-existing liver fibrosis. Therefore, we wanted to investigate tumor establishment and development after tumor cell implantation into mice with pre-existing liver fibrosis. Methods: Liver fibrosis was induced by thioacetamide and ethanol co-administration in female C3H/He mice. Orthotopic HCC was established by subcapsular intrahepatic injection of 105 HCC cells (Hepa129) into the left liver lobe. Fibrosis induction was verified by measuring hepatic microcirculation (HM) using laser doppler flowmetry one week before implantation and collagen staining. Ten day post implantation tissue samples were harvested for mRNA/cDNA preparation. Transcript levels of VEGF-A, -C, -D and their receptors VEGFR-1/2 and -3 were determined besides MMP-2 and MMP-9 by semiquantitative real time PCR (LightCycler, Roche Diagnostics, Mannheim, Germany). Results: The combination of TAA/EtOH induced liver damage resulting in 64% fibrosis as demonstrated by collagen staining. Accordingly, functional relevance was demonstrated by a relative decline (about 50%) in HM in fibrotic mice liver. Tumor growth was considerably accelerated in fibrotic mice compared to the non-treated controls ten days after tumor cell implantation. Mean tumor volume in fibrotic and non-fibrotic mice reached 956 [mm3] and 260 [mm3] (p=0.0073), respectively. Additionally, metastatic spots were mainly observed in fibrotic but not in non-fibrotic mice (27 vs. 3.1, p<0.0001). Advanced tumor stage was associated with elevated transcription levels VEGFR-1, -2 and high VEGFR-3 values were observed in tumors from fibrotic mice. VEGF-C/D levels were even higher elevated by pre-existing fibrosis. Furthermore, intratumoral MMP-2 and MMP-9 transcription was also increased in liver fibrotic mice. In contrast to VEGF-C and VEGF-D, VEGF-A transcription itself was not affected by pre-existing fibrosis at the time point of examination. Conclusion: Our data show, that tumor growth and metastasis were considerably accelerated in fibrotic livers in mice and that advanced tumor stage was associated with elevated intratumoral Flk-1 and Flt-1 and increased hepatic MMP-2 and MMP-9 transcription levels. Elevated intratumoral VEGFR-3 receptor status and increased intratumoral VEGF-C/D transcription levels had been linked with advanced metastasis. This work was supported by a Deutsche Krebshilfee grant.

Disclosures: The following people have nothing to disclose: Miroslaw Kornek, Esther Raskopf, Rene Tolba, Tilman Sauverbruch, Volker Schmitz

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CONCORDANCE BETWEEN FIBROTEST® (FT) AND FIBROSCAN® (FS): A NEW NON-INVASIVE METHODOLoGY FOR IMPROVING ACCURACY IN A WORLD WITHOUT A GOLD STANDARD

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Background. Liver stiffness measurements (LSM) using FS, and FT, are widely used as non invasive fibrosis markers. The aim was to identify factors associated with FS accuracy using FT as a non-invasive endpoint and vice versa. Methods. The proof of concordance between FT and FS was established in patients suffering from pre-existing liver fibrosis. Therefore, we wanted to investigate tumor establishment and development after tumor cell implantation into mice with pre-existing liver fibrosis. Methods: Liver fibrosis was induced by thioacetamide and ethanol co-administration in female C3H/He mice. Ortho-
Background / Aim: The role of nitric oxide (NO) on liver injury and fibrosis is unclear. The purpose of this study was to determine the effects of inducible NO synthase deficiency (iNOS-/-) on liver injury and fibrosis produced in mice by chronic CCl4 administration. METHODS: Wild type (wt) or mice with iNOS deficiency were subjected to biweekly CCl4 injections over 8 weeks, while controls were given isovolumetric injections of olive oil. RESULTS: Serum AST and ALT levels were lower after CCl4 in the iNOS-/− than in the wt animals, which correlated with decreased necrosis on liver histology. Also, there were a lower number of stellate cells (α-SMA immunostaining), a lesser degree of fibrosis (morphometric determination of sirius stained slides), and twice as many apoptotic cells (TUNEL assay) after CCl4 in the iNOS-/− as compared to wt animals. In addition, immunostaining for ssDNA, another measure of apoptosis, was significantly higher in the iNOS-/− animals. α1(I) collagen mRNA, determined by real time PCR, was markedly increased after CCl4 in the wt and to a significantly lesser extent in the iNOS-/− mice. Tissue inhibitor metalloproteinase 1 (TIMP-1) mRNA, matrix metalloproteinase 2 (MMP-2) mRNA and MMP-9 mRNA were increased after CCl4 in the wt but not in the iNOS-/− mice. Conclusion: NO protects against CCl4 induced apoptosis. In the absence of iNOS, there is decreased necrosis, increased apoptosis, and reduced liver fibrosis.

Disclosures: The following people have nothing to disclose: Ghazaleh Aram, James J. Potter, Xiaopu Liu, Esteban Mezey.
1074 TREATMENT OF ESTABLISHED CIRRHOSIS WITH SV40 VECTORS ENCODING INSULIN-LIKE GROWTH FACTOR 1
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Liver transplantation is the only curative treatment for advanced liver cirrhosis. Therapies aimed at halting the progression of the disease are urgently needed. Previous studies have shown that the administration of recombinant insulin like growth factor-1 (IGF-I) induces hepatoprotective effects in experimental cirrhosis. However, the necessity of using high daily doses of the protein for a long period of time makes this therapy very costly hampering their clinical application. As an alternative therapeutic approach, we have evaluated whether sustained IGF-I expression within the liver from viral vectors based on the Simian Virus 40 (rSVIGF-I) could exert therapeutic effects in liver cirrhosis. We have previously shown that intraportal injection of rSVIGF-I in rats prior to the development of liver cirrhosis induces the expression of hepatocyte growth factor (HGF) in the liver and delays the progression of the disease. In the present study we have tested the effect of the vector in rats that had already developed advanced liver cirrhosis. Liver cirrhosis was induced in Sprague-Dawley male rats by intragastric administration of CCl4 for 12 weeks and then a single dose of rSVIGF-I (1011 vp/rat) was administered by different routes. The following administration routes were tested: intraportal (IP), intrahepatic (IH), intraarterial (IA) and intrabiliar (IB). At the end of the study, cirrhotic rats treated with rSVIGF-I showed reduced serum bilirubin, transaminases and liver fibrosis scores as well as increased expression of serum albumin as compared to mock-infected cirrhotic animals. The effects were more prominent in rats treated by IP or IA injection, suggesting that these routes could allow better access of the vector to hepatocytes in fibrotic livers. IGF-I expression correlated with the increase of mRNA levels of IGF-I binding protein 3 (whose synthesis is induced by IGF-I) and with upregulation of HGF, a factor that seems to mediate hepatoprotective effects of IGF-I. These results indicate that established cirrhosis can be reverted by IGF-I and also, that rSVIGF-I are promising vectors for the treatment of this disease.

Disclosures: The following people have nothing to disclose: Luciano Sobrevals, Nerea Razquin, Gloria Gonzalez-Aseguinolaza, Carlos Rodriguez, Puri Fortes, Jesus Prieto

1075 MULTIPLE GENETIC MARKERS IN TLR4 REGION ARE ASSOCIATED WITH CIRRHOSIS RISK IN CHC PATIENTS
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BACKGROUND: We have recently developed a 7 gene Cirrhosis Risk Score (CRS) signature that can predict the risk of developing cirrhosis in patients with chronic hepatitis C (CHC) (Hepatology, 2007, in press, E-pub April 26). Among them T399I is located in the Toll like receptor 4 (TLR4) gene, which has an identified role in hepatic fibrosis. AIM: To determine the role of other genetic markers in or near TLR4 in cirrhosis risk.

METHODS: We developed assays for an additional 157 single nucleotide polymorphisms (SNPs), covering the region from 98 kb upstream to 161 kb downstream from the original T399I SNP. These 157 SNPs were genotyped in 1,020 CHC patients (708 Caucasians) enrolled from 2 US centers. All patients had liver biopsy prior to treatment and clinical data. In all statistical analyses, Cases were defined as subjects with Bridging fibrosis/Cirrhosis (F3-4) while Controls were No-fibrosis (F0) on liver biopsy. We first performed univariate analysis to identify individual markers significantly associated with cirrhosis risk. We then used the Haplo.Score program to determine significant haplotypes in this region. Last, logistic regression was used to determine the relative importance of each SNP conditional on the effect of other SNPs for all possible pairs in the region.

RESULTS: Of 157 SNPs in the TLR4 region, nine were significantly associated with cirrhosis risk with p<0.005. Their odds ratios (OR=1.68-2.96) were comparable to that of the original T399I SNP. The frequency of the risk alleles is 23.8-94.3%. Among the nine SNPs, one missense (D299G), three intronic and four intergenic SNPs were located in or near the TLR gene; one intronic SNP was in an uncharacterized gene (47kb from TLR4). The two missense TLR4 SNPs T399I and D299G are in nearly complete linkage disequilibrium (R²=0.934). In addition, a 2-SNP haplotype containing the risk allele of the original T399I SNP showed a 24-fold stronger association with cirrhosis risk than T399I by itself (p value 1.37E-05 vs. 3.34E-04). Analysis of the relative importance of the SNPs in the entire region identified one intronic SNP in TLR4 and one intronic SNP in the nearby uncharacterized gene that were associated with cirrhosis risk independent of T399I, suggesting these 3 SNPs may be responsible for the cirrhosis risk in this region.

CONCLUSIONS: We found additional SNPs and haplotypes associated with cirrhosis risk at an equivalent or more significant level than the original T399I. Identification of additional markers in the remaining six gene regions of CRS is ongoing. These findings could lead to a more predictive signature for cirrhosis risk and improve the management of CHC patients.

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1076 BMP-7: THE KEY ANTAGONIST OF TGF-β1 IN LIVER FIBROSIS?
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Background: Transforming growth factor-β1 (TGF-β1) has been accepted as the master cytokine not only in the fibrotic response in the liver. Bone morphogenetic protein-7 (BMP-7), a member of the same superfamility of cytokines, antagonizes profibrogenic functions of TGF-β1 in diverse cellular systems. In the liver a regenerative function of BMP-7 was described but the expression of BMP-7 in isolated liver cells as well as its antifibrotic potential is controversially discussed. Results: First of all we could detect BMP-7 in isolated liver cells. Although hepatic stellate cells (HSC) and hepatocytes express all necessary BMP-7 receptors, only hepatocytes express BMP-7 while HSC do not. This was shown by RT-PCR and immunohistochemistry. In vitro we could show that HSC are responsive to BMP-7 by western blot analysis of phosphorylated Smad1/5 and an increased luciferase activity when using the artificial BMP-reporter (BRE)2-
luciferase. When applied in parallel we could clearly demonstrate that BMP-7 antagonizes the profibrogenic actions of TGF-β1, in that the TGF-β1-induced collagen I and PAI-1 synthesis was completely blocked. Furthermore, we could show by using the (CAGA)12-MLP luc reporter that the basis for this antagonism is most likely a reduced activation of Smad3. Although hepatocytes show a similar sensitivity towards BMP-7 compared to HSC, TGF-β1 induced (CAGA)12-MLP luc activity was not influenced by BMP-7. Nevertheless BMP-7 was able to reduce the LDH concentration in TGF-β1 treated hepatocytes, implying an anti-apoptotic effect. Finally we could demonstrate, in a model of liver fibrosis induced by bile duct ligation (BDL), an increase in BMP-7 expression confirmed by an adenoviral (BRE)2-luc reporter activated in vivo and RT-PCR. Conclusion: We conclude that BMP-7 is a cytokine which is involved in the maintenance of normal liver function. We figured out two different aspects of BMP-7 function: 1) The upregulation of BMP-7 during liver injury protects hepatocytes from TGF-β-mediated apoptosis. 2) BMP-7 directly antagonizes TGF-β-induced extracellular matrix synthesis regulated by Smad3.

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The following people have nothing to disclose: Olaf Scherner, Wanda N. Vreden, Steffen K. Meurer, Ralf Weiskirchen, Axel M. Gressner

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KUPFFER CELL-DERIVED CONNECTIVE TISSUE GROWTH FACTOR (CTGF) AND TRANSFORMING GROWTH FACTOR ALPHA (TGFα) MEDIATE THE PROFIBROGENIC EFFECTS OF LEPTIN IN VITRO

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Introduction The mechanism[s] by which leptin exerts its profibrogenic effects are currently unclear. Some data suggest that leptin acts directly on hepatic stellate cells (HSCs) to trigger fibrogenic gene expression. We hypothesized that in vitro cell culture models will help elucidate the mechanisms whereby leptin activates profibrogenic gene expression in HSCs. Methods Non-parenchymal liver cells (HSCs, KCs and SECs) were isolated from wt or leptin-resistant fatty (fa/fa) Zucker rat and cultured for various times from 2 to 6 days. Recombinant rat leptin (10 nM, 100 nM) was subsequently added. Gene expression of Type I collagen, TIMP1, TGFβ1 and CTGF were assessed by real time PCR. HSC α-smooth muscle actin (α-SMA) and JAK/STAT3, MAPK and PI-3K signalling pathways were assessed by immunoblot. The WST-1 assay was used for HSC proliferation. Immunocytochemistry was performed to assess CTGF protein expression. Electrophoretic mobility shift assays (EMSA) were performed for AP-1 and NF-kB DNA binding ability. Results Leptin treatment of HSCs in culture failed to potentiate profibrogenic effects. In KCs treated with leptin, TGFβ1-induced collagen I and CTGF were in part responsible for mediating these effects. In KCs treated with leptin compared to controls. We were unable to demonstrate any increase in TGFβ1 protein in KC lysates or in KC-conditioned medium by ELISA or immunoblotting. Since CTGF expression is stimulated by TGFβ, we next inhibited TGFβ production in KCs treated with or without leptin using a soluble TGFβ type II receptor. Kupffer cells treated with the soluble receptor but not a control immunoglobulin demonstrated down regulation of CTGF protein. Finally, leptin potentiated pSTAT3, pAKT and pERK1/2 in KCs as well as enhanced AP-1 and NF-kB DNA binding activities in KCs. Conclusion: Leptin and its functional receptors play a crucial role in HSC activation and liver fibrosis principally through indirect effects via soluble mediators released from KC. Increased expression of CTGF and TGFβ are in part responsible for mediating these effects.

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The following people have nothing to disclose: Jianhua Wang, Isabelle Leclercq, Joanne Brymora, Mehdi Ramezani-Moghadam, Roslyn M. London, Kumar Subramaniam, Jacob George

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LOSARTAN REDUCES THE EXPRESSION OF PROFIBROGENIC GENES AND INFLAMMATION IN PATIENTS WITH CHRONIC HEPATITIS C

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Background & Aims: There are no antibiotic therapies for patients with chronic hepatitis C in whom viral infection cannot be cured. Angiotensin II type 1 receptor (AT1) blockers have been shown to reduce liver fibrogenesis in experimental models of liver disease, but their effects on liver fibrogenesis in humans is unknown. We investigated the effect of losartan, an AT1 blocker, on liver fibrogenesis in patients with chronic hepatitis C who did not respond to antiviral therapy. Methods: Forty patients with chronic hepatitis C and significant liver fibrosis (F2-F4) received oral losartan (50 mg/day) for 18 months. Two paired liver biopsies were performed before and after treatment. The degree of fibrosis and inflammation were evaluated by histological analysis (METAVIR). Collagen content, the amount of myofibroblasts and inflammatory cells were estimated by measuring the percentage of the whole biopsy area stained with Sirius Red, anti-α-SMA and anti-CD43, respectively. Liver fibrogenesis was assessed by quantifying mRNA expression of key genes encoding extracellular matrix proteins, fibrogenic mediators, inflammatory cytokines, pro-oxidant proteins and components of the renin-angiotensin system through real-time PCR. Results: Oral losartan was well tolerated in all patients. Treatment with losartan was associated with a decrease in inflammatory activity in 8 patients (57%) and a decrease in the fibrosis stage decreased in 7 patients (50%). Treatment with losartan was associated with a significant reduction in the expression of key genes involved in liver fibrogenesis. The mean reduction in the expression of procollagen α1(I), procollagen α2(I), utPA, MMP2 and Rac1 was 30%, 23%, 40%, 27% and 21%, respectively. Patients with reduced inflammatory activity showed a pronounced down-regulation of procollagen α1(I) (44%) and additional down-regulation of TIMP-1 and inflammatory cytokines (MCP-1 and Gro-α). Patients with improved fibrosis showed a reduction in collagen content and in the amount of myofibroblasts compared to patients without fibrosis improvement. Patients with improved inflammation showed a significant decrease in the CD43-positive area compared to patients in whom inflammation did not improve. Losartan treatment did not affect renal function, serum liver tests or viral load. Conclusions: Oral losartan for 18 months decreases the expression of genes involved in liver fibrogenesis and
reduces the inflammatory activity in patients with chronic hepatitis C. The effects of AT1 blockers in chronic hepatitis C should be further investigated in randomized controlled studies.

Disclosures:
The following people have nothing to disclose: Jordi Colmenero, Ramón Bataller, Pau Sancho-Bru, Xavier Forns, Miquel Brugera, Marlene Dominguez, Montserrat Moreno, Vicente Arroyo, David Brenner, Pere Gines

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ATORVASTATIN ATTENUATES ANGIOTENSIN-II INDUCED INFLAMMATORY AND FIBROGENIC ACTIONS IN THE LIVER
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Background & Aims: Statins, besides their lipid lowering properties, exert beneficial effects in chronically damaged tissues. Angiotensin II (Ang II) plays an important role in liver fibrogenesis by inducing oxidative stress, promoting hepatic inflammation and stimulating TGFβ1 and collagen gene expression. We investigate whether atorvastatin modulates Ang II-induced pathogenic effects in the liver. Methods: Male Wistar rats were infused with saline or Ang II (50 ng/kg/min) for 4 weeks through a subcutaneous osmotic pump. Rats received either vehicle or atorvastatin (40 mg/kg/day) by gavage. H&E liver specimens were blindly scored by an expert pathologist. The degree of hepatic inflammation, oxidative stress as well as the expression of genes involved in the hepatic wound healing response to injury were assessed by CD43 immunostaining, 4-HNE immunostaining and quantitative PCR, respectively. The effect of atorvastatin on Ang II-pathogenic effects were also assessed in cultured hepatic stellate cells (HSC), a major fibrogenic cell type. Cell proliferation (3H-thymidine incorporation), secretion of interleukin-8 (ELISA) and gene expression of procollagen a1(I) were studied cultured primary HSC. Ang II (10-8M) stimulated cell proliferation (3H-thymidine incorporation), secretion of interleukin-8 (ELISA) and gene expression of procollagen a1(I) and TGFβ1. All these effects were reduced in the presence of atorvastatin. In addition, atorvastatin reduced Ang II–induced procollagen a1(I) and TGFβ1 gene expression in the rat livers. These results were markedly blunted by atorvastatin attenuates the inflammatory and fibrogenic effects of Ang II in the liver. Therefore, statins could have beneficial effects in conditions characterized by hepatic inflammation and fibrogenesis.

Disclosures:
The following people have nothing to disclose: Leandra N. Ramalho, Montserrat Moreno, Pau Sancho-Bru, Marta Ruiz-Ortega, Fernando Ramalho, Juan González-Abraldes, Jordi Colmenero, Marlene Dominguez, Jesus Egido, Vicente Arroyo, David A. Brenner, Pere Gines, Ramón Bataller

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T CELL DERIVED MEMBRANE MICROPARTICLES CONTAINING EMMPRIN INDUCE PROFIBROLYTIC ACTIVATION IN HEPATIC STELLATE CELLS
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BACKGROUND: T cell interactions with hepatic stellate cells (HSC) may be a determinant of liver fibrosis progression. Microparticles (MP) are small membrane vesicles shed from various cell types during activation and apoptosis. In most chronic liver diseases, such as viral and autoimmune hepatitis, activated T cells are driving force of disease progression and their turnover is greatly increased at the sites of active tissue remodeling. Here we studied and analyzed the effect of T-cell derived MP on the profibrogenic activation of HSC. METHODS: MP were generated from Jurkat T cells subjected to apoptosis induction with staurosporine, and purified from cell-free supernatant by differential ultracentrifugation. Characterization and quantification of MP was done by electron microscopy FACS. MP proteins were analyzed by MALDI-TOF mass spectrometry. Human LX-2 HSC (gift of Dr. S.L. Friedman) were incubated with increasing numbers of MP for up to 36h. Profibrogenic and fibrolytic transcripts were quantified by quantitative RT-PCR. To study membrane interaction of MP with HSC, tracking experiments were performed using PKH26 fluorescent-labeled MP. RESULTS: Apoptosis induction in Jurkat cells lead to 7-9 fold increase of MP production compared to untreated cells. Electron microscopy revealed that preparations of MP contained round empty vesicles with a lipid bilayer and sizes between 50 and 700 nm. Incubation of freshly isolated MP with HSC greatly induced fibrolytic MMP-1 and 3 mRNA transcripts (3- and 30-fold, respectively), while profibrogenic TIMP-1 mRNA, was unchaged and procollagen a1(I) was moderately downregulated. Tracking experiments revealed rapid transfer of fluorescent label from MP to HSC membranes already after 30 min, being retained for at least 12h. MALDI-TOF mass spectrometry of purified MP revealed high levels of CD147/Emmprin/Basigin, an MMP inducer that is putatively responsible for the observed effects. CONCLUSIONS: 1. T cell derived MP potently induce fibrolytic activation of HSC; 2. This suggest a novel cytokine-independent mechanism of fibrogenesis modulation by activated/apoptotic T cells in chronic liver diseases. 3. EMMPRIN is a prime candidate to mediate the fibrolytic switch.

Disclosures:
The following people have nothing to disclose: Yury Popov, Jessica Zaks, Franck Grall, Tawia Liebermann, Nezam H. Afdhal, Detlef Schuppan
Background/Aim: We previously reported that celecoxib potentiates hepatic fibrosis in CCl4-treated rat. However, Hui et al. reported that celecoxib potentiates hepatic fibrosis in bile duct ligation (BDL)- and thioacetamide (TAA)-induced hepatic fibrosis models in rat. Methods: We used immortal human HSC established by functional expression of the telomerase catalytic subunit. We treated HSC with celecoxib (50 µg/ml) after infection of adenovirus expressing constitutively active Akt (Ad5myrAkt) or adenovirus expressing GFP (Ad5GFP). Hepatic fibrosis was induced in Wistar rats by BDL for 4 weeks or by peritoneal TAA injection for 5 weeks. Celecoxib (20mg/kg/day) was orally administered from the beginning of liver injuries. Untreated BDL- and TAA-rats served as controls. Extracellular matrix (ECM) was Trichrome stained and quantified by morphometry. Hydroxyproline content in liver tissue was measured. Expression of α-SMA was assessed by immunohistochemistry. Hepatic mRNA levels of COX2, α-SMA, TGF-β1, collagen α1(I) was quantified by real-time RT-PCR. Results: Celecoxib induces HSC apoptosis and inhibits Akt phosphorylation in HSC. Celecoxib-induced HSC apoptosis is significantly attenuated in HSC infected with Ad5myrAkt but not in those infected with Ad5GFP. AST, ALT, and alkaline phosphatase levels are decreased in celecoxib treated group in TAA-rats (p<0.01) but not in BDL-rats. Liver tissue morphometry after Trichrome staining shows that celecoxib decreases hepatic ECM deposition in both BDL-rats (BDL only vs. BDL-celecoxib 52.8% vs. 37.9%, p=0.035) and TAA-rats (TAA only vs. TAA-celecoxib 17.9% vs. 10.8%, p<0.001). Hepatic hydroxyproline content and α-SMA expression are significantly decreased by celecoxib in both BDL- and TAA-rats. In both BDL-rats and TAA-rats, COX2, α-SMA, TGF-β1, and collagen α1(I) mRNA is upregulated. Celecoxib treated rats shows significantly decreased mRNA expression of COX2, α-SMA, TGF-β1, and collagen α1(I) compared with untreated rats in both BDL- and TAA-induced hepatic fibrosis models. Conclusions: Celecoxib shows anti-fibrogenic effects in both BDL- and TAA-rats suggesting celecoxib as a potential anti-fibrotic agent for hepatic fibrosis. Celecoxib-induced inhibition of Akt activation in hepatic stellate cells at least partially contributes to the anti-fibrotic effect of celecoxib.

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1081 CELECOXIB SHOWS ANTI-FIBROTIC EFFECT IN HEPATIC FIBROSIS MODELS IN RAT AND INDUCES APOPTOSIS OF HEPATIC STELLATE CELLS THROUGH INHIBITION OF AKT ACTIVATION

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Objective: Nonalcoholic steatohepatitis (NASH) may develop into fibrosis, cirrhosis and hepatocellular carcinoma. The rennin-angiotensin system is related to liver fibrosis and hepatic stellate cells (HSCs) play an important role in the liver fibrogenesis. Telmisartan, an angiotensin II type 1 receptor (AT1) antagonist, can function as an agonist of peroxisome proliferator-activated receptor γ. So we hypothesized that telmisartan could improve NASH-related fibrosis. Materials and Methods: The effect of telmisartan was studied by in vivo and in vitro. In vivo, HSCs were isolated from rat liver, and the cells were treated with telmisartan at the dose of 0 to 25µM. Using Western blotting, we checked HSC activation marker of α-SMA and how HSCs express AT1 and ANG II. Using real-time PCR, we determined fibrotic gene expression. In vitro, we used choline-deficient L-amino acid–defined diet induced rat liver fibrotic model, which is an established model of NASH. The total study periods were 10 weeks. The rats were treated with telmisartan at the dose of 0, 0.5, 1.0 and 2.5mg/kg body weight/day. The liver fibrosis index was measured by Azan and Sirius red stain and immunohistochemistry of α-SMA. The liver tissue expression of fibrotic gene and the serum biochemical marker were also determined. Furthermore, the preneoplastic lesion of placental glutathione S-transferase (GST-P) positive lesions was analyzed. Results: In vitro, telmisartan inhibited HSCs expression of α-SMA and decreased the mRNA expression of type 1 procollagen and TIMP-1, 2 in a dose-dependent manner. HSCs expressed AT1 and ANG II and the expression increased as the cells undergoing activation. In BrdU incorporation assay, we found telmisartan blocked ANG II induced HSCs proliferation. In vivo, telmisartan (0.5, 1.0 and 2.5mg/kg) treated rats showed decreased extracellular matrix accumulation by 25%, 41% and 65% of control (p<0.01 respectively); simultaneously, telmisartan reduced the area of α-SMA positive cells by 27%, 35% and 49% of the control (p<0.01 respectively). The development of GST-P-positive preneoplastic lesions in each group revealed in area as (125.1±42.9, 87.0±21.3, 58.8±20.4, 45.9±17.4 µm²/section) and the number as (4±3.0, 2±1.8, 2±1±1.4, 0.8±0.6/section), which were showed significant reduced by telmisartan (p<0.01 respectively). The mRNA expression of type 1 procollagen and the serum hyaluronic acid, total bile acid were significantly decreased by telmisartan. Conclusions: Telmisartan prevented liver fibrosis and attenuated enzyme-altered preneoplastic lesions in NASH related liver fibrosis via inhibition of HSCs activation. Telmisartan will be a candidate drug for NASH patients.

Disclosures: The following people have nothing to disclose: Haiyan Jin, Naoki Yamamoto, Shuji Terai, Fuyu Murakami, Isao Sakaida.
1083 Natriuretic peptide improved liver fibrosis in rat model using new treatment system

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[Aim/Background]: The natriuretic peptide (NP) are used as the acute heart failure treatment in clinical and it is reported that the suppression of fibrosis in the heart and the lung recently. In this study, we made the in vivo model that can estimate the effect of new drugs on liver fibrosis by new injection system using 24 hour continuous infusion pump system. Using the in vivo model, we checked the effect of atrial natriuretic peptide (ANP) or C-type natriuretic peptide (CNP) on liver fibrosis. [Methods]: Wistar rats were injected with dimethyltinrosamine (DMN) twice a week via interperitoneal (i.p.) for 4 weeks to make the liver fibrosis. Fibrotic rats were assigned to three groups (each group n=6): DMN only, DMN+ANP (1.0 µg/kg/min), DMN+CNP (1.0 µg/kg/min). Each group was given by continuous intravenous dosage system used 24 hour infusion pump for 3 weeks after 1 week of DMN administration. The liver fibrosis index was measured by H-E, Azan, Sirius Red staining. The state of hepatic stellate cell was analyzed by α-SMA staining. Laboratory data was also analyzed. Collagen type 1, TIMP-1, TIMP-2 and MMP2 mRNA expression was analyzed by real time RT-PCR system. [Results]: The NP treated groups showed decrease of serum AST (mean value: ANP 81.8 vs CNP 95.4 vs Control 2000 IU/l P<0.01) ALT (mean value: ANP 63.8 vs CNP 44.8 vs Control 2000 IU/l P<0.01). Liver fibrosis was suppressed by ANP group or CNP group more than control group in both Azan and Sirius Red Stain (P<0.01). In Western blot analysis, ANP or CNP group inhibited α-SMA expression (P<0.01). ANP or CNP also significantly inhibited Type 1 procollagen, TIMP-1, TIMP-2, MMP9 and MMP2 mRNA expression (all of P<0.01). [Conclusion]: The natriuretic peptide (NP) inhibited liver fibrosis in our in vivo model. The NP will be a candidate drug for liver cirrhosis patients.

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1084 Transient elastography (FibroScan®) in patients with chronic hepatitis C virus infection and haemophilia A

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Introduction: In patients with bleeding disorders and chronic hepatitis C virus (HCV) infection liver biopsy for assessment of fibrosis stage is generally avoided because of potential hazardous complications, high costs, and low patients' acceptance. Thus, non-invasive methods for fibrosis staging are warranted. Methods and patients: We recruited 83 patients with chronic HCV infection and haemophilia A (HCV-A) and 297 HCV patients without haemophilia A (HCV-wo); a significant proportion of patients was co-infected with HIV (40% and 12%, respectively). In addition, we included 29 HCV patients and sustained virological response to interferon therapy (HCV-SVR). We determined serum surrogate markers for fibrosis (AST/platelets ratio index [APRI], Forns index) and performed transient elastography (FibroScan®) in all patients, and recorded histological stages of liver fibrosis if available. Correlation of serum markers and liver stiffness was tested using Pearson’s correlation coefficient and linear regression analysis. Results: In the HCV-wo group, histological stages of liver fibrosis show a highly significant linear correlation with all non-invasive fibrosis tests (transient elastography, APRI and Forns index (n=88; p<0.001). HCV-A patients display more advanced liver fibrosis, as indicated by differences in liver stiffness (15.4 vs. 11.4 kPa; p=0.011) and Forns index (8.98 vs. 8.27; p<0.05), albeit there are younger than HCV-wo patients (42 vs. 55 yrs; p<0.001). In HCV-A patients, elastography results correlate with serum markers tests (p<0.001), but this correlation is not present in HCV-A patients with pronounced fibrosis in elastography (> 12.5 kPa). Of note, responders to interferon therapy display lower liver stiffness than non-responders or naive patients (7.2 vs. 14.0 kPa; p<0.001; 7.2 vs. 10.9 kPa; p<0.01), whereas Forns index and APRI show no differences. Conclusions: Despite younger age, haemophilic patients present with more advanced fibrosis, most likely due to coinfection with HIV. Responders to interferon therapy show lower liver stiffness than patients with ongoing HCV infection. We conclude that transient elastography and serum surrogate markers help to identify haemophilic patients at risk for rapid progression of fibrosis. Moreover, transient elastography might be useful for monitoring regression of fibrosis after resolution of HCV infection.

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1085 Inhibition of angiogenesis by a vitronectin receptor antagonist worsens liver fibrosis

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Background and aims: Hepatic fibrogenesis is dependent on neoangiogenesis. However, the impact and role of angiogenesis in liver fibrosis progression are ill defined. The vitronectin receptor (integrin alpha V beta 3) is central to angiogenesis by mediating migration, proliferation and tubulogenesis of endothelial cells, but also drives fibrogenic activation of hepatic stellate cells (Xu et al, JBC 2004; Patsenker et al, J Hepatol 2007). We therefore studied the effect of alphaVbeta3 inhibition in models of liver fibrosis in vivo. Methods: Liver fibrosis in rats was induced by bile duct ligation (BDL) for 6 weeks or by i.p. thioacetamide (TAA) for 12 weeks. The alphaVbeta3 inhibitor EMD121974 (cilengitide) was given i.p. twice daily at 15 mg/kg from week 2-6 after BDL and for 8 weeks after discontinuation of TAA. Liver collagen was determined as hydroxyproline content, and fibrosis related gene expression was measured by quantitative RT-PCR. Angiogenesis was assessed by immunostaining with CD31 antibody and vessel counting using a point counting technique. Results: Treatment of both BDL and TAA fibrotic rats with cilengitide resulted in a further significant increase of hepatic collagen accumulation by 30%, upregulation of profibrogenic gene expression, and was accompanied by an increase of GGT and bilirubin (4.7- and 1.3-fold vs. untreated fibrotic controls, respectively) in rats with BDL, while liver enzymes and bilirubin remained unchanged in TAA-treated rats that received cilengitide. In both fibrosis mod-
HYPERDYNAMIC CIRCULATION IN INFANTS WITH BILARY ATRESIA AND PORTAL HYPERTENSION

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The hyperdynamic circulation (HC) is a well described phenomenon in adults with cirrhosis, yet it has not been carefully examined in children. Infants with biliary atresia (BA) often develop cirrhosis and prominent portal hypertension (PH) with its complications, ascites and variceal bleeding. Goal: To evaluate the presence of HC in infants with BA and PH. Methods: Chart review of patients < 1 year with BA and PH that underwent pretransplant cardiac evaluation between 1/1994 to 12/2006. PH was defined by: 1. presence of endoscopic evidence of esophageal / gastric varices, or 2. Splenomegaly or ascites by clinical examination and/or imaging, and radiologic evidence of esophageal /gastric varices or hypersplenism. Evaluation included vital signs and echocardiographic assessment including measurement of left ventricular systolic and diastolic dimensions, septal and posterior wall thickness, quantification of shortening fraction, detailed diastolic function assessment (mitral valve inflow Doppler) and assessment of longitudinal function (Doppler Tissue, DTI), derived indices of both systolic and diastolic function. Patients with congenital heart disease or receiving medications that can affect HR or BP were excluded. Twice as many healthy aged matched infants that underwent cardiac evaluation served as controls. Results: 9 patients (5M) mean age 7±1.8 months and 18 controls (12M) mean age 6.52±2.2 months (p=0.76) were included. 8/9 patients underwent prior portoenterostomy; 7/9 had one or more episodes of variceal bleeding. Mean Hb level at echocardiography was 10 ±1.5 gr/dl. There was no statistical difference in the HR, Pulse Pressure (Table 1) and DTI parameters between the groups. Shortening fraction, systolic and diastolic BP were significantly higher in infants with BA.

Conclusion: Circulatory differences were observed in infants with biliary atresia and PH. Resting heart rate is relatively unchanged and blood pressure is increased. The hyperdynamic circulation is instead manifest by evidence of increased contractility. These novel findings have important implications for the management of portal hypertension in young children.

Table 1

<table>
<thead>
<tr>
<th></th>
<th>Patients (n=9) Mean ±SD</th>
<th>Controls (n=18) Mean ±SD</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean resting HR</td>
<td>139 ± 27</td>
<td>126 ± 13</td>
<td>0.11</td>
</tr>
<tr>
<td>Mean Systolic BP (mmHg)</td>
<td>95 ± 14</td>
<td>84 ± 9</td>
<td>0.02</td>
</tr>
<tr>
<td>Mean Diastolic BP (mmHg)</td>
<td>55 ± 8</td>
<td>44 ± 7</td>
<td>0.002</td>
</tr>
<tr>
<td>Pulse pressure (mmHg)</td>
<td>39 ± 7</td>
<td>40 ± 9</td>
<td>0.9</td>
</tr>
<tr>
<td>Mean left Ventricular End Systolic Septal Thickness (cm)</td>
<td>0.89±0.12</td>
<td>0.62±0.06</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Mean left Ventricular End Diastolic Septal Thickness (cm)</td>
<td>0.60±0.09</td>
<td>0.41±0.06</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Mean Shortening Fraction (%)</td>
<td>42.58±4.5</td>
<td>37.60±3.2</td>
<td>0.02</td>
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</tbody>
</table>

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The following people have nothing to disclose: Eleonora Patsenker, Yury Popov, Felix Stickel, Gerald Niedobitek, Detlef Schuppan

ADAPTIVE CHANGES OF HEPATOCYTE TRANSPORTERS AND NUCLEAR RECEPTORS IN PEDIATRIC PATIENTS WITH EARLY AND LATE-STAGE CHOLESTASIS

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The adaptive changes of hepatocyte transport proteins and nuclear receptors during obstructive cholestasis in humans are important yet remain unclear. To investigate the early-stage and late-stage adaptive responses, we investigated liver samples from children with chronic obstructive liver disease, including patients with biliary atresia during Kasai operation (n=8, 64+/−17 days), and patients who progressed to late-stage cholestasis underwent orthotopic liver transplantation (n=8, 10.50+/−3.12 months). Expression levels of the sinusoidal and canalicular transporters, nuclear receptors, and CYP enzymes were analyzed by real-time RT-PCR and immunofluorescent staining in comparison to age-matched pediatric controls and neonatal intrahepatic cholestasis. At early-stage cholestasis, most canalicular transporters and sinusoidal uptake transporters were down-regulated, including BSEP, MDR3, MR2P, NTCP, and OATPβ; except that OStα/β were up-regulated. At late-stage obstructive cholestasis, BSEP returned to normal, MDR3 and MDR1 were up-regulated, and sinusoidal exporter OStα/β and MRP4 were up-regulated. FXR was down-regulated at early-stage cholestasis, returned toward normal levels at late-stage cholestasis, and the expression level of PXR was decreased profoundly in late-stage obstructive cholestasis, while SHP and CAR were unchanged. Immunofluorescent staining showed unchanged BSEP, increased MDR3, MDR1, and decreased MR2P signals well-targeted to the canalicular membrane at late-stage obstructive cholestasis, with higher expressions in zone 1 and 2 than zone 3. The expression levels of multiple genes were directly related to FXR levels, confirming FXR as the main regulator of bile acid transport. In conclusion, different adaptive changes occurred in early-stage and late-stage cholestasis in human. FXR-BSEP remained unchanged at late-stage cholestasis to maintain bile acid homeostasis. PXR was markedly down-regulated at the progress of late-stage obstructive cholestasis, and could be a potential target for therapy.

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The following people have nothing to disclose: Huey-Ling Chen, Yu-Jung Liu, Hui-Ling Chen, Yan-Hsuan Ni, Shan-Shin Wu, Ming-Chih Ho, Hong-Yuan Hsu, Mei-Hwei Chang
THE ROLE OF ENDOSCOPIC RETROGRADE CHOLANGIOPANCREATOGRAPHY IN DIAGNOSIS OF BILIARY ATRESIA IN INFANTS
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Aim: To investigate role and safety of endoscopic retrograde cholangiopancreatography (ERCP) in diagnosing biliary atresia (BA) in infants with prolonged conjugated jaundice, where standard clinical work up including laboratory tests, ultrasound and liver biopsy have failed to produce a definite diagnosis. Patients and Methods: We retrospectively analysed our database for infants younger than 100 days who underwent ERCP for diagnosis of BA from June 1997 to May 2007. Results: Amongst 2079 infants investigated for prolonged jaundice, 224 (10.7%) was diagnosed with BA over the observation period. ERCP was performed in 48 (2.3%) infants (24 male; median age: 58.5 days, range 19 – 98 days; median weight: 4 kg, range, 2.07- 5.3 kg). All infants had acholic stools, including 3 with associated alpha-1-antitrypsin PiZZ deficiency and 2 with bile salt export pump deficiency (PFC-2). Forty-seven infants had liver biopsy, while the remaining one had haemophilia C. Liver histology showed non-specific cholestasis (n=18, 38%), giant cell hepatitis (n=12, 26%), large bile duct obstruction (n=10, 21%) and mixed cholestatic and hepatic features (n=7, 15%). Cannulation of papilla was unsuccessful in 3 (6.25%) infants. These three infants were eventually diagnosed with BA on laparotomy. Of the remaining 45 children, ERCP demonstrated intrahepatic biliary tree in 20 (42%), while 25 (52%) proceeded to laparotomy. Of those, BA was confirmed macroscopically and histologically in 22 (46%). Three remaining infants who underwent explorative laparotomy, but not Kasai portoenterostomy had CMV hepatitis, neonatal sclerosing cholangitis, 1 (5%) Caroli disease and 9 (45%) normal biliary tree. After ERCP 3 infants (6.25%) had obstruction (n=10, 21%) and mixed cholestatic and hepatic features (n=7, 15%). Cannulation of papilla was unsuccessful in 3 (6.25%) infants. These three infants were eventually diagnosed with BA on laparotomy. Of the remaining 45 children, ERCP demonstrated intrahepatic biliary tree in 20 (42%), while 25 (52%) proceeded to laparotomy. Of those, BA was confirmed macroscopically and histologically in 22 (46%). Three remaining infants who underwent explorative laparotomy, but not Kasai portoenterostomy had CMV hepatitis, neonatal cholestasis and right and left hepatic duct hypoplasia. Amongst the 20 children in whom ERCP ruled out BA, 10 (50%) had neonatal sclerosing cholangitis, 1 (5%) Caroli disease and 9 (45%) normal biliary tree. After ERCP 3 infants (6.25%) had slight intraabdominal extravasation of the contrast, but none had clinical pancreatitis or perforation. Conclusion: ERCP is a safe procedure for diagnosing BA even in small infants with the positive predictive value of 88% and negative predictive value of 100%. In specialized centres this procedure could prevent unnecessary laparotomy in majority of infants with ambiguous clinical findings.

Disclosures: The following people have nothing to disclose: Naresh P. Shanmugam, P. Harrison, J. Devlin, N. Kumaran, Mark Davenport, Dino Hadzic.
parison. Using phylogenetic tree construction and direct sequence comparison, we analyzed the distinct features of HBV genomes from children with HCC. [Results]: Phylogenetic analysis indicated that the majority (71.4%) of HB from children with HCC contained recombinant sequences of both genotypes B and C and clustered in two very closely related clades that were clearly distinguishable from HBV genomes of patients (children and adults) with chronic HBV infection and adults with HCC. Sequence analysis identified a consensus 12-bp pre-S2 deletion in 57.1% of HBV genomes of children with HCC. Other characteristic mutations were also found throughout HBV genome. [Conclusion]: The majority of full-length sequences of HBV isolated from children with HCC belonged to recombinant strains of genotype B and C, which are clearly distinguishable from HBV genomes of patients with chronic HBV infection and adults with HCC. Those HBV strains from HCC children contained unique mutations, particularly a short pre-S2 deletion, which may be associated with early HCC development.

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INTRAFAMILIAL TRANSMISSION OF HEPATITIS C VIRUS IN CHILDREN: CORRELATION WITH ViroLOGICAL AND IMMUNOLOGICAL PARAMETERS
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Background: Intrafamilial transmission of hepatitis C virus (HCV) infection has been reported, however the extent of its role and the factors associated with it are poorly understood. Objectives: To determine risk factors of transmission in children potentially exposed to HCV when one or both parents are infected. Patients and Methods: We examined 20 families (total of 93 subjects: 40 adults, 53 children) where one or both parents were HCV infected. Children living with infected parent/s were enrolled, a questionnaire inquiring about HCV risk factors was completed, and serum was screened for HCV antibodies (ELISA). Positive samples were confirmed by polymerase chain reaction and tested for genotype, HCV RNA viral load, HVR1 sequence analysis (nucleotide positions 1156 to 1234), HCV-specific CD4+ and cytotoxic CD8+ T cell responses (ELSpot), natural killer cells, and cytokines. Generalized linear mixed effects models were used to analyze the data to account for correlation between children from the same family. Results: Fifteen children (28%) were HCV infected and none had risk factors other than contact with the infected parent/s. Boys were more likely to be infected than girls (Odds Ratio (OR)=2.3; 95% confidence interval (CI): 0.6, 8.3; p = 0.23). Genotype and nucleotide sequencing of the HVR1 region of HCV isolates infecting parents and children had > 95% homology. When both parents were HCV infected, 43% of children were infected compared to 9% when one parent was infected (OR = 8.0; 95% CI: 1.6, 40.6; p = 0.012). Among families with only one infected parent, children of infected mothers had 9.5 times greater odds of being infected than children of infected fathers (95% CI: 0.4, 217, p = 0.16). Although older parental age and higher maternal HCV viral load were associated with higher odds of child infection, only higher paternal viral load was significant (OR=1.02 per 10,000 copies, 95%CI:1.001, 1.04, p = 0.039). Those delivered vaginally had 2.5 higher odds of infection than those delivered via Cesarean section (95% CI: 0.3, 23.1, p = 0.41). Interestingly, 74% of children with no detectable viremia had HCV-specific CD4 + or CD8+ T cell responses of > 30 SFC x 106 (95% CI: 49%, 90%). Conclusion: Our data show that biparental HCV infection and high paternal HCV RNA levels were significantly associated with intrafamilial pediatric HCV infection. The observation that HCV-specific CD4+ and CD8+T-cell responses exist in HCV negative children may imply a protective potential for these responses and might have important implications for vaccine development and epidemiologic studies.

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HCV AUTOIMMUNITY IN A U.S. MULTI-CENTER COHORT OF TREATMENT NAIVE CHILDREN
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Background: Auto-antibodies are described frequently in adults with chronic HCV, with thyroid antibodies present in 14% and autoimmune hepatitis antibodies in up to 90%. These antibodies have prevented some adults from undergoing or successfully completing treatment with interferon. Although children with HCV generally tolerate treatment better than adults and have a higher response rate, little is known about the frequency with which autoantibodies are present. Understanding this baseline feature may be important in developing recommendations for treatment of pediatric patients with chronic HCV. Aim: Our aim was to assess the incidence and describe the correlates of positive antibodies, thyroid peroxidase (TPO), anti-nuclear antibody (ANA), anti-smooth muscle antibody (ASMA) and anti-Liver Kidney Microsomal antibody (LKM) in treatment naive children (ages 5 to 18 years) with chronic HCV. Methods: From 12/2004 to 6/2006, 128 children were screened at entry to the multi-center, NIH funded Peds-C Network clinical trial. Labs were determined centrally and a single pathologist scored all biopsy slides. Assays for ANA, ASMA, ALKM, and TPO were measured at baseline. Results: Subjects had a mean age of 11.2 years (56% male), 77% had vertically acquired HCV, 15% unknown, 7% transfusion, less than 1% drug or sex. 82% were Caucasian, 6% African American, 9% Hispanic,
4% Asian. Genotype 1a was present in 60%, 1a/1b in 1%, 1b in 22%. Genotype 2a in 5%, 2b in 1%, Genotype 3b in 10%, Genotype 6a in 1%. Mean ALT was 60.2 U/L. Liver histology demonstrated moderate inflammation in 38%/severe in 3%. Moderate fibrosis 5% / cirrhosis 2%. Of the 128 children screened, 18 (14%) had measurable auto-antibodies - 44% were ASMA positive (range 1:80-1:160), 33% ANA positive (range 1:80-1:320), 17% LKM positive (range 33.9-65.2 units, ULN20.1), and 6% TPO positive (108.8 IU/ml ULN2.1 IU/ml). Using Chi square and Fisher’s, no significant correlates of antibody positivity were found with age, gender, race/ethnicity, ALT, mode of acquisition, genotype, degree of liver fibrosis or inflammation scores. A trend toward older age was found for ANA positivity, with the three oldest patients having the highest titer (ages 15, 16 and 17 yr. ANA 1:320). Conclusion: This study demonstrates less frequent TPO, LKM, ASMA and ANA positivity in children with Chronic HCV compared to adults. We speculate that these autoantibodies may be of importance in the side-effect profile and treatment response in children treated with antivirals.

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1093 TREATMENT OF HEPATITIS B USING INTRON A VS PEGINTERFERON IN COMBINATION WITH LAMIVUDINE IN CHILDREN

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Aim: To assess the safety, tolerability and efficacy of 2 combination therapy regimens using interferon (IFN) and lamivudine (LAM) to treat E antigen (Ag) positive pediatric hepatitis B (HBV) infected patients. Methods: 34 patients (age 2-16 years) at a single center were assessed over a 5 years. Baseline labs included liver profile (LFT), complete blood count (CBC), thyroid function test (TFT), HBV E Ag, HBV E antibody (Ab) and HBV DNA quantitative PCR. All patients had a liver biopsy with the intent to treat to grade and stage disease. Exclusion criteria included HIV co-infection, stage 4 disease, severe thrombocytopenia, thyroid disease, uncontrolled depression and history of non-compliance. Two treatment regimens were used. Group A: 16/34 patients received Intron A, 5 million unit/m2 SQ daily (max dose 10 million units) for 5 months with LAM (staggered by one month) 4mg/kg/day orally (max dose 100mg) until E Ag seroconversion or resistance. Group B: 6/34 patients received Peginterferon Alfa 2b (Peg-IFN), 1.5mcg/kg/week for 24 weeks with LAM as above. Monthly assessments included vital signs, weights, LFT, CBC, TFT, HBV DNA, HBV E Ag, and HBV E Ab. Results: Twenty-four males and 10 females were assessed. Liver biopsies demonstrated Stage 0-3, Grade 0-3 disease. Group A demographics: 4 Asian, 2 Hispanic, 6 African American, 4 Caucasian with vertically transmitted disease in 13/16. At screening all had HBV DNA > 200 million copies, 6/16 had normal LFT. On therapy 15/16 had undetectable HBV DNA with 8/16 achieving E Ag seroconversion at mean of 44 weeks (range 22-69). Safety data: none developed thyroid dysfunction, 2/16 patients had neutropenia (1 treated with IFN dose reduction, 1 treated with Neupogen), 1 patient required an antidepressant, 1 patient stopped IFN due to seizure, and 2/16 required nutritional supplements for weight loss. One subject developed LAM resistance. Group B demographics: 3 Asian, 2 Hispanic, 1 African American all with vertical transmission. At screening all had HBV DNA > 200 million copies and normal LFT. No patient cleared DNA on therapy and no patient achieved E Ag seroconversion. Two of 6 required nutritional supplements for weight loss and no other adverse effects occurred. Conclusions: Combination therapy with IFN and LAM in a staggered manner is safe and effective in pediatric patients with chronic HBV infection. Combination therapy with Peg-IFN and LAM at the dosing assessed is not equally efficacious and should not be used in pediatric patients until further clarification of dosage and frequency.

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1094 HBEAG SEROCONVERSION CORRELATES WITH A HIGH NUMBER OF CORE GENE MUTATIONS AND WITH GENOTYPES B AND D IN PAEDIATRIC PATIENTS

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Background: The outcome of hepatitis B virus (HBV) infection results from the interaction between virus and host immune response. While effective immune recognition usually controls viral replication, escape mutations may arise from the immune system selective pressure, with consequent persistent infection. Aim: To investigate whether there is a relationship between presence of mutations within HBV core gene, HBV genotypes, HBV DNA viral load and HBeAg seroconversion in five different HBV infection settings. Patients: One hundred paediatric patients (median age 13.1 years, 54 males) were divided into 5 groups according to their HBeAg/HBsAg status and amino-transferases activity (Table 1). Methods: HBV DNA was isolated from serum samples. The presence of point mutations within HBV pre-core and core promoter regions and amino acid substitutions within immunodominant epitopes of HBV core gene was analysed by direct sequencing (mutations/patient). HBV genotypes were determined by direct sequencing. HBV DNA viral load was quantified by real-time PCR (log10 copies/ml). Results: A higher number of mutations within the HBV pre-core region and HBV core gene was detected in HBeAg negative patients than in those HBeAg positive (pre-core: 4.5 ± 0.2 vs. 2.6 ± 0.2, p = 0.001 and core: 5.5 ± 0.4 vs. 4.1 ± 0.3, p = 0.04). Genotypes B and D were more prevalent in HBeAg negative children than in those HBeAg positive (80% vs. 58%, p < 0.001). HBV-DNA viral load was significantly higher in groups A (mean ± SEM, 7.9 ± 0.2), B (6.8 ± 0.3), and D (7.5 ± 1.2) compared to C (3.4 ± 0.3) and E (1.0 ± 0.4) (p = 0.001). Among HBeAg negative children, those with genotypes B and D had a higher number of pre-core mutations and tended to have a lower number of core mutations than those with genotypes A, C and E (pre-core: 5.5 ± 0.4 vs. 4.1 ± 0.2, p = 0.04; 4.7 ± 0.3 vs. 5.7 ± 0.3, p = 0.1, respectively). Conclusions: Irrespective of genotype, emergence of HBV core gene mutations is associated with HBeAg seroconversion. In patients with genotypes B and D, that more frequently lose HBeAg, seroconversion is associated also to a high number of pre-core mutations. To what extent these mutations result from the selective pressure of the immune system or are virally determined remains to be clarified.
1095 GRAFT HISTOLOGY UP TO 10 YEARS AFTER PEDIATRIC LIVER TRANSPLANTATION: HIGH INCIDENCE OF SEVERE FIBROSIS, CORRELATED WITH TRANSPLANT RELATED FACTORS

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Introduction: Previously we showed that 31% of liver grafts have portal fibrosis at 1 year after pediatric liver transplantation (LTx; Transplantation 2000; 6:236). The presence of fibrosis correlated positively with transplant related factors, including cold ischemia time (CIT) and biliary complications. Aim of the study: To assess progress and development of fibrosis in liver grafts in the long term after pediatric LTx and to identify possible risk factors. Methods: We reviewed protocol biopsies obtained at 5 and 10 yrs after LTx from the originally included 77 patients/grafts. We analyzed the relationships between histology and parameters of transplantation and follow up. Severity of fibrosis was evaluated using a three point scale: 0 = no fibrosis, 1 = portal fibrosis without bridging (mild fibrosis), 2 = portal fibrosis with occasional bridging (moderate fibrosis), 3 = diffuse portal bridging (severe fibrosis/cirrhosis). Results: At 5 and 10 yrs after LTx, 66/77 (85%) and 55/77 (71%) biopsies were available, resp. Missing biopsies at 10 yrs included 77 deaths and 6 retransplants. Between 1 yr and 5 yrs after LTx, the prevalence of fibrosis increased from 31% to 67%, but remained stable thereafter (10 yrs: 65%). However, the percentage of patients with severe fibrosis continued to increase, from 10% (5 yrs) to 27% (10 yrs). The presence of fibrosis at 1 yr tended to correlate with re-transplantation (p=0.054). The incidence of severe fibrosis after 10 yrs was significantly higher in patients who had fibrosis at 1 yr after LTx, compared with those without fibrosis at 1 yr (p= 0.004). At 5 years, ASAT and yGT values were significantly higher in patients with fibrosis than in those without fibrosis (ALAT 23 ± 13 vs. 36 ± 28 U/l; GGT 30 ± 52 vs. 112 ± 149 U/l; resp., each p<0.05). At 10 yrs after LTx, corresponding values were not significantly different. Sixty four percent (64%) of patients without fibrosis at 1 yr after LTx had developed some degree of fibrosis at 10 yrs (25/39). The development of fibrosis starting after 1 yr was strongly related to CIT, donor/recipient ratio and type of preservation fluid (these factors were also strongly interrelated). The CIT of the grafts that developed late fibrosis was longer than that of grafts without fibrosis at 10 yr (11.4 ± 3.9 h vs. 7.2 ± 3.8 h; p = 0.004). Conclusion: At 5 and 10 yrs after pediatric LTx, the majority of grafts have fibrosis in protocol biopsies. At least 25% of the grafts, fibrosis progresses to cirrhosis in 10 yrs after pediatric LTx. Even fibrosis that only develops after the first year post LTx is strongly correlated with transplant related factors.

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1096 IMPROVED PREDICTIVE VALUE OF PELD WITH THE ADDITION OF SERUM SODIUM AND PLATELET COUNT

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Aim: To re-evaluate the prognostic value of the Paediatric End-stage Liver Disease (PELD) score with the inclusion of parameters that indicate portal hypertension reflecting on platelet count (Plt), aspartate aminotransferase (AST) to Platelet Ratio Index (APRI), spleen size and haemodynamic decompensation [serum sodium (Na)] at listing and at time of transplantation (LT). Methods: Medical records of 85 patients (38 male) with biliary atresia waiting for liver transplantation from 2001-2006, were reviewed retrospectively. 3 patients died and 5 patients were admitted in PICU requiring ventilation prior to transplantation. Median age at transplantation was 1 year [range, 0.39-13]. PELD score and above mentioned parameters were recorded at the time of listing and at the time immediately before liver transplantation. A multivariate and a univariate Cox regression analysis were performed for the endpoints death/PICU admission whilst on the list. Three models were evaluated for their predictive power for both endpoints. The area under the receiver operating characteristic curve (AUC ROC) was used to compare models. Results: The median time between listing and LT was 84 [range, 13-682] days. The median PELD score at the time of listing was 15 [range, –10 to 36], which significantly increased to 17 [range, –5 to 42] just before LT (p<0.001). At the time of listing the median values were for sodium 136mmol/L [range, 128-144], for platelet count 16×10^12/L [range, 29-577], and for APRI 2.7 [range, 0-27.84]. Paired statistics between existing PELD and our variables did not improve its prediction of endpoints. In a multivariate Cox regression models PELD+Plt, PELD+Na and PELD+Plt+Na (King’s PELD) all variables had a significant association (P<0.7). The regression coefficients of predictors were used as multiplicative factors to calculate the new severity scores. A univariate logistic regression analysis for death was performed (King’s PELD: odds ratio 2.72, 95% CI 1.01-7.32; p 0.05). The ROC analysis that was done to the King’s PELD showed that the best cut-off point to dichotomise was 48.84 to predict death (odds ratio 6.73, 95%CI 1.63-27.84; p 0.01). The new scoring system is: -0.42*Age(<1yr)-0.55*Sbili+2.34*INR-1.78*Alb+0.46*Growth+0.47*Plt+11.09*Na The median King’s PELD at the time of listing was 48 [range, 46-50] and prior to transplantation was 46 [range, 44-48]. Conclusions: The PELD does not reflect the severity of disease in our cohort. The new King’s PELD is a better predictor of endpoints on the waiting list. Further prospective analysis is in progress.

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1. APRI, spleen size and haemodynamic decompensation [serum sodium (Na)] at listing and at time of transplantation (LT).
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PEDIATRIC LIVER RETRANSPRANTATION: OUTCOMES AND A PROGNOSTIC SCORING TOOL

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Approximately 15% of all pediatric transplant recipients require retransplantation. No multi-centered study has evaluated the outcome of retransplantation or the predictors of transplant-free survival in children. Aim: To evaluate the outcome of liver retransplantation in children at multiple centers and develop a simple prognostic scoring system. Methods: Retrospective cohort study using the United Network for Organ Sharing (UNOS) transplantation database of patients who were ≤20 years of age when they received their primary transplant and who underwent at least one retransplantation (N=1,408). Using a random 2/3 of the subjects, we developed a prognostic scoring system by performing a multivariate Cox analysis using primary diagnostic category, reason for graft failure, age and whether the patient was on life support at the time of transplant as predictors. The scoring system was verified in the remaining 1/3 of the subjects. Results: Stratifying the verification group into terciles by prognostic score demonstrated its predictive value. Those in the low-risk category had similar survival to primary transplant recipients. Those in the high-risk category had 5.7 (95% CI: 4.0 – 8.0) times the risk of death or retransplantation as those in the low risk category (Figure 1). Discussion: This scoring system could assist practitioners in providing anticipatory guidance to families on expectations after retransplantation. Current donor allotment in pediatrics functions on an urgency basis, however directing liver donations towards those with better expected outcomes may bring higher utility from a limited resource. This tool could assist in developing an outcomes based allotment system.

Figure 1.

Pediatric Liver Replantation
Event Free Survival of Initial Replantation by Prognostic Score

Prognostic Score Stratification
Low Risk (N=157), Medium Risk (N=176), High Risk (N=134)

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LIPID-ENRICHED DIET INDUCES GROWTH RETARDATION AND LIVER STEATOSIS IN CYSTIC FIBROSIS MICE

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Background: There is evidence to indicate that, in cystic fibrosis (CF), the nutritional status influences lung and liver injury, which currently represent the two main causes of death in this disease. To better define the impact of nutrition in CF disease, we herein compared the phenotypic traits of CF mice fed either a liquid diet (Peptamen) or a standard chow together with a PolyEthylenGlycol (PEG) osmotic laxative, two strategies commonly used to prevent intestinal obstruction in CF mice. Peptamen, a diet designed to feed infants or young children who suffer malnutrition, contains more lipids than standard chow (3.7 vs 1.6 g/100 Kcal) and mostly medium-chain fatty acids (more than 95%), whereas standard chow contains predominantly long-chain fatty acids (70%). Methods: Male CF mice with a complete exon-10 knockout of the CFTR gene (CFtrtm1Unc) (n = 56) and their normal littermates (n = 35) were randomly assigned at weaning, to standard chow, standard chow with PEG or Peptamen. Survival, growth, liver and ventilatory status assessed by whole-body plethysmography, were determined in these animals, followed-up until 120 days. Results: The survival rate of CFtr-/- mice was similar in the groups under Peptamen feeding and PEG treatment (77% and 73%, respectively, after 60 days). At weaning, CFtr-/- mice were significantly smaller than normal littermates. While PEG did not affect growth in CFtr+/+ mice, Peptamen regimen caused growth delay in both CFtr-/- and CFtr+/+ mice during the pre-adult period (30-60 days). Growth catch-up occurred thereafter, but CFtr-/- mice remained smaller than CFtr+/+ mice, whatever the diet. Hepatomegaly was observed at 120 days in Peptamen fed CFtr-/- mice, liver/body weight ratio being significantly higher in this group than in all other groups. Liver steatosis was detected in all animals of this group and exceeded 10% in half of them. By comparison, steatosis exceeding 10% was detected in 1 out of 5 Peptamen fed CFtr+/+ animals and in no PEG-treated CFtr-/- animals. No bile duct lesion was observed. Minute ventilation was significantly lower in CFtr-/- mice than in CFtr+/+ mice, without any difference between Peptamen-fed and PEG-treated animals. Conclusions: These results provide evidence that CFTR genetic defect may cause growth retardation, hypoventilation, and predisposition to liver steatosis, the most common hepatic lesion in CF patients. They indicate that growth delay and steatosis in this setting, are influenced by regimen and are paradoxically aggravated by a lipid-enriched diet, designed for malnutrition (whereas bile duct lesions are likely to be influenced by genetic factors).

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CAN INSULIN-LIKE GROWTH FACTOR BINDING PROTEIN-1 (IGFBP-1) IDENTIFY OVERWEIGHT CHILDREN AT RISK OF DEVELOPING STEATOHEPATITIS?
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The prevalence of obesity in children and the high morbidity associated with metabolic syndrome emphasizes the importance of the early diagnosis of insulin resistance (IR). IR has been considered the main derangement in nonalcoholic fatty liver disease and steatohepatitis (NASH). NASH can progress to severe liver fibrosis. Recent data implicate hepatic IR as a culprit in accumulation of free fatty acids as triglycerides in hepatocytes. Markers of hepatic IR in children are lacking. Insulin is the main inhibitor of the hepatic production of IGFBP-1; this prompted us to study IGFBP-1 as an early marker of hepatic IR in overweight children. Oral glucose tolerance test was performed in 34 children and adolescents with BMI≥85th percentile and the insulin sensitivity index (ISI) was calculated. Glucose (G), insulin (I) and IGFBP-1 were measured in fasting serum samples. In order to characterize children at risk for IR a clinical-laboratory index (CLI) was calculated considering the following risk factors: BMI≥3 SDS, acanthosis nigricans, positive family history of IR, insulin levels≥20 μU/l, HOMA-R≥2 and G:I ratio<7. Considering CLI≥3 (3 or more risk factors) as a criterion to define IR, ISI<4.6 showed 87.5% of sensitivity and 94.5% of specificity in diagnosing IR. Based on this ISI value, patients were further classified as IR (ISI<4.6, n=15) and non-IR (ISI≥4.6, n=19). Fasting IGFBP-1 was lower in IR (median: 17 μg/l) than in non-IR (43 μg/l) group (P<0.01) and IGFBP-1<26 μg/l was observed in 11/15 (73%) IR but in only 5/19 (26%) non-IR patients (P<0.01). Four patients with IR had IGFBP-1>26 μg/l suggesting impairment of insulin action at hepatic level. In conclusion, ISI<4.6 seems to be a good indicator of peripheral IR in overweight children and adolescents, while IGFBP-1 determination may contribute to diagnose hepatic-IR among children and adolescents with peripheral IR and in this way identify those patients with higher risk of developing more severe liver injury.

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PREDICTORS FOR SEVERE COURSE OF CYSTIC FIBROSIS-RELATED LIVER DISEASE IN CHILDREN
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Cystic Fibrosis (CF) is associated with a specific form of liver disease in some patients. Cystic fibrosis-related liver disease (CFLD) is usually characterized by a relatively benign clinical course. A minority of CF patients, however, develop cirrhosis and portal hypertension. Currently, we cannot predict which CF patients develop a severe form of CFLD. We set out to determine which clinical, biochemical and ultrasound parameters were associated with the development of a severe course of CFLD in a cohort of CF patients. METHODS: We reviewed the clinical charts, including ultrasound and biochemical results, of all pediatric CF patients born between 1988 and 2006 seen in our institution. Severe CFLD was defined as multilobular cirrhosis on ultrasound and/or signs of portal hypertension (splenomegaly, varices). Patient characteristics, clinical details and ultrasound findings were recorded. RESULTS: Sixty-three children with CF were included. Multilobular cirrhosis and/or portal hypertension were found in 8 children (12.7%). The mean age at which severe CFLD was diagnosed was 7.8 years (range 3-12 years). No apparent biochemical or ultrasound abnormality was present one year before severe CFLD was diagnosed in 4 of the 8 children (50%). At 1 year before diagnosis, growth retardation (height < -2SD) and elevated γ-glutamyltransferase (γ-GT two times or more > 50 U/l in successive controls) were significantly associated with severe CFLD (p=0.045 and 0.011 respectively). No other biochemical serum parameters [alkaline phosphatase, ASAT, ALAT, bilirubin, bile acids], nor gender, history of meconium ileus, distal intestinal obstruction syndrome or neonatal cholestasis, were associated with the development of severe CFLD. Similarly, hepatomegaly, diffuse inhomogeneous parenchyma or increased hepatic echogenicity on ultrasound were not associated with a severe course of CFLD. CONCLUSIONS: In our retrospective cohort of 63 pediatric CF patients, 13% developed CFLD with multilobular cirrhosis and/or portal hypertension. At 1 year before the diagnosis of severe CFLD, growth retardation and elevated γ-GT levels were associated with subsequent development of severe CFLD. Ultrasound findings or biochemical parameters did not predict the development of severe CFLD. Half of the patients did not have any biochemical or liver ultrasound abnormality at 1 year before the diagnosis of severe CFLD.

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PATTERN OF DIAGNOSTIC EVALUATION FOR THE CAUSES OF PEDIATRIC ACUTE LIVER FAILURE (PALF): OPPORTUNITIES FOR QUALITY IMPROVEMENT
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PALF is a rare disease that leads to liver transplantation or death in up to 50% of affected children. We hypothesized that there would be variability in the diagnostic evaluation of PALF that might suggest opportunities for improved diagnosis of causes of PALF. Thus, the goal of this study is to describe the frequency of specific diagnostic evaluations for the common causes of PALF in the PALF Study Group Database of 611 patients to clarify current practice. Methods: PALF was defined as severe liver dysfunction occurring within 8 weeks of onset of illness, with no known underlying chronic liver disease in patients from birth through 17 years of age. Patients with coagulopathy corrected with Vitamin K are not included in the study. The database was queried for the diagnostic evaluation for the most common causes of PALF which, outside of indeterminates (48.4% of the cohort) were drug exposure including acetaminophen (16.2% of the cohort), metabolic disease (12.3% of the cohort), autoimmune hepatitis (5.9% of the cohort), infections (5.2% of the cohort). We examined the frequency of screening for these etiologies in patients labeled as indeterminate as final diagnosis by the investigator and clinical team. Of the 296 subjects cate-
orized as indeterminate, 48 were less than 6 months old. The number screened for the four most common etiologies are shown in the table. Of the 296 subjects categorized as indeterminate, only 2% had a complete evaluation for the most common etiologies. Conclusions: Current practice indicates that screening methods are not standardized for drug, metabolic and autoimmune causes of PALF and hence are inconsistent in patients ultimately given a diagnosis of indeterminate ALF. This offers an opportunity to improve the diagnosis and potential treatment options in children with PALF.

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<th>Clinical Significance of the Abernethy Malformations (Congenital Abse...</th>
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1012 CLINICAL SIGNIFICANCE OF THE ABERNETHY MALFORMATIONS (CONGENITAL ABSENCE OR HYPOPLASIA OF THE PORTAL VEIN)

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PURPOSE/METHODS: Congenital absence of the portal vein (Abernethy I) and congenital hypoplasia of the portal vein (Abernethy II) have been collectively classified as one clinical entity, congenital extrahepatic portosystemic shunt (CEPS). Anecdotal differences between Abernethy I and Abernethy II malformations have been noted. However, aggregate data do not exist as these rare malformations have been reportedly sporadically in case reports or small series. We performed a systematic literature review to elucidate the difference between the two types of Abernethy malformations. RESULTS: 109 CEPS cases were identified (70 Abernethy I and 39 Abernethy II). There was a significant predisposition for developing hepatic tumors among Abernethy I cases (50% Abernethy I vs. 10% Abernethy II, p<0.0001). Malignant tumors were more common among Abernethy I cases (11% Abernethy I vs. 2.5% Abernethy II, p=0.15). Hepatic encephalopathy (HE) was less frequent in Abernethy I cases (14% Abernethy I vs. 36% Abernethy II, p=0.017); however, an additional 14% of Abernethy I cases reported subtle neurologic and developmental findings without HE (i.e. mild mental retardation, developmental delays and radiologically evident lesions in the brain). When hyperammonemia was present, 26% of Abernethy I cases reported HE, with an additional 26% reporting subtle neurologic and developmental findings. In contrast, 70% of Abernethy II cases with hyperammonemia reported HE (p=0.006). Four of 10 Abernethy I cases with HE (40%) reported treatment via liver transplantation with a success rate of 100%. Thirteen of 14 Abernethy II cases with HE (93%) reported treatment via shunt ligation or banding with a success rate of 92%. Hepatopulmonary syndrome (HPS), an uncommon complication of end stage liver disease, occurred in both types of Abernethy malformations without hepatic dysfunction (5.7% Abernethy I vs. 2.5% Abernethy II). Successful liver transplantation was reported in a single Abernethy I case. Shunt ligation was successful in another Abernethy II case. CONCLUSION: Abernethy I patients should be screened for development of hepatic tumors, given 11% prevalence of hepatic malignancy. Hyperammonemia was a stronger predictor of HE among Abernethy II cases than among Abernethy I cases. However, rigorous neurologic testing for Abernethy I patients with hyperammonemia may be warranted, given 26% prevalence of subtle neurologic and developmental findings without HE. In Abernethy I cases with refractory HE, liver transplantation may be considered. Shunt ligation or banding is recommended for such Abernethy I cases.

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The following people have nothing to disclose: Michael Narkewicz, Dominic Dell Olio, Saul J. Karpen, Susan Krug, Kathleen B. Schwarz, Nada Yazigi, Sang Zhang, Steven H. Belle, For the Pediatric Acute Liver Failure Study Group.

1103 SCLEROSING CHOLANGITIS IN CHILDREN-RETROSPECTIVE SINGLE CENTER REVIEW

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Sclerosing Cholangitis (SC) is a rare progressive cholestatic disease in children with limited data on presentation and management in childhood. Charts of 47 patients younger than 21 years with cholangiography or histology proven SC, seen at Mount Sinai Hospital within the last 20 years were reviewed. 29 patients had both liver biopsy (Bx) and magnetic resonance cholangiopancreatography (MRCP), 8 also had endoscopic retrograde cholangiopancreatography (ERCP). 4 ERCP and Bx, 4 had only Bx and 2 patients only MRCP or ERCP. The mean age at diagnosis was 11 +/- 4.9 years (range 2-20 years), 62% were male. Signs and symptoms occurred in 91% with hepatomegaly, abdominal pain, diarrhea, fatigue and splenomegaly in decreasing prevalence. Inflammatory Bowel Disease (IBD) was found in 65% [Ulcerative Colitis, UC, 49% and Crohn's 16%], concurrent with SC in 59%, IBD first in 26% and SC first in the rest. Autoimmune hepatitis (overlap) occurred in 32% (16% with concurrent IBD). Peripheral anti-neutrophil cytoplasmic antibody was positive in 42% (50% of patients had IBD), anti smooth muscle antibody in 42%, anti-neutrophil cytoplasmic antibody was positive in 42% (50% of patients had IBD), anti smooth muscle antibody in 42%, anti-nuclear antibody in 26% and liver kidney microsomal antibody in none. Family history for autoimmune disease was found in 15%. In patients studied with MRCP, both extra and intrahepatic biliary disease were found in 40%, intrahepatic only in 14%, extrahepatic only in 10% and normal in 36%. Cholelithiasis was diagnosed in 12% and ERCP intervention (papillotomy, stent placement or balloon dilatation) was performed in 15%. Colonoscopy was performed in 60% of patients revealing pancolitis in 29%, rectal sparing in 29% and was normal in 18%. All patients were treated with ursodeoxycholic acid (UDCA), 9 patients with autoimmune overlap were treated with additional steroids and/or azathioprine, 2 patients were treated with methotrexate. Median pre/post-therapy laboratory values were gamma-glutamyl transpeptidase 317/42 U/L, alkaline phosphatase 566/193 U/L and alanine aminotransferase 143/41 U/L. Liver transplantation was performed in 8
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ROLE OF LIVER HISTOLOGY IN THE MANAGEMENT OF ACUTE LIVER FAILURE IN CHILDREN
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Aim: The aetiology of acute liver failure (ALF) remains unknown in many children (46% in this series). Liver biopsy may be perceived to be a useful tool in diagnosis and management of ALF, though risks increase with multisystem disease and coagulopathy leading to the transjugular approach in some centres. The aim of this study was to evaluate the role of liver histology in the diagnosis and management of paediatric ALF. Methods: 211 children presented to a single paediatric hepatology centre from 1989-2004 with ALF (INR>2 unresponsive to vitamin K with abnormal liver enzymes). History slides were available in 111 children (57 male, median age 5.15 years, range 0-17.4). These were examined by a single histopathologist, blinded to clinical details. 53 samples were from biopsies (50 percutaneous, 2 open, 1 transjugular) taken during the course of ALF with blood product support (8), immediately after death (19), or at recovery (26). 58 samples were slides from explanted livers taken at transplantation. The histology findings were compared with the final clinical diagnosis. Results: The median interval between admission and biopsy was 15 days (range 1-298). History did not contribute to diagnosis in 46/53 (87%). 6 samples showed massive hepatic necrosis, 3 severe acute hepatitis, 16 non-specific. In 11, histological diagnoses were suggested but these differed from the final clinical diagnosis. In 7, histology confirmed the clinical diagnosis. In all of these cases the clinical diagnosis was made and management decided before biopsy was performed. In 3, histology findings were suggestive of chronic liver disease, possibly autoimmune hepatitis, but the clinical diagnosis was indeterminate. In 2 of these autoantibodies were negative, immunoglobulins normal, and they remain without treatment and with normal blood test results after 2 years follow-up. In the 3rd case the child died on day 1 of admission. In 7, histology performed median 32 (range 5-298) days from admission showed normal liver. In the explant group, histology did not contribute to diagnosis in 44/58 (76%). 39 samples showed massive hepatic necrosis, 2 non-specific. In 3, histological diagnoses were suggested but these differed from the final clinical diagnosis. In all of these cases the clinical diagnosis was made and treatment instituted before transplantation. Conclusion: In our experience, histopathology from liver biopsy in children with ALF did not add to management, nor did explant histology further identify any diagnoses.

Disclosures:
The following people have nothing to disclose: Jonathan M. Hind, Alberto Quaglia, Rachel Taylor, Anil Dhawan

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BUDD CHIARI SYNDROME IN CHILDREN: A SINGLE CENTRE EXPERIENCE IN THE UNITED KINGDOM
Roshni Vara1, Natalie J. Bab1, John Karani2, Gholam J. Mutti2, Nigel Heaton3, Giorgia Mieli-Vergani1, Anil Dhawan1; 1Paediatric Liver Centre, King’s College Hospital NHS Foundation Trust, London, United Kingdom; 2Haematology Department, King’s College Hospital NHS Foundation Trust, London, United Kingdom; 3Liver Transplantation, King’s College Hospital NHS Foundation Trust, London, United Kingdom; 4Radiology Department, King’s College Hospital NHS Foundation Trust, London, United Kingdom

Methods A retrospective review of 13 children diagnosed with Budd Chiari Syndrome (BCS) between 1982-2006. Data was collected to study the clinical presentation, aetiology, management and outcome. Results 13 children (6 male), median age (range) at presentation was 10.68 years (1.62-16.31 years). Presenting features included hepatosplenomegaly in 13, ascites in 11, jaundice in 7, lethargy in 7 and gastrointestinal bleeding in 2. Laboratory investigations showed median (range) INR 1.44 (1.03-6.00), bilirubin 25umol/L (5.185-158 umol/L), aspartate transaminase 53 iu/L (8-510 iu/L), albumin 40 (25-51), serum sodium 138mmol/L (121-146mmol/L), haemoglobin 11.0g/dl (7.2-13.8) and platelet count 242x109/L (29-373). Investigations included ultrason, CT and angiography, in addition detailed procoagulant studies were carried out as and when they became available. Procoagulant states were identified in 9; paroxysmal nocturnal haemoglobinuria (PNH) in 3, antiphospholipid syndrome in 2 and factor V Leiden heterozygosity and protein C deficiency (40 u/dl) in each. Ultrasound was diagnostic of BCS in 12 (92%) whilst CT identified the abnormality in 1 and liver biopsy and angiography in 1. Sites of occlusion were main hepatic veins in 5 (38%), main hepatic veins with partial inferior vena cava (IVC) occlusion in 3 (23%), small hepatic venules in 3 (23%) and IVC thrombosis in 2 (15%), co-existing portal vein thrombosis in 2. Liver biopsy showed features of venous outflow obstruction in 9, cirrhosis in 2 and fibrosis in 2. Acute management included diuretics, anti-coagulation, paracentesis and endoscopy. Definitive management included liver transplantation (LT) in 4; indications being acute liver failure in 2 and decompensated cirrhosis in 2. Surgical shunts in 4, percutaneous venous dilatation in 2 and transjugular intrahepatic portosystemic shunt in 1 of the patients with PNH, followed by matched unrelated bone marrow transplantation. 5 (36%) died, median age 9.85 years (range 6.09-14.98 years), causes of death were; multisystemic complications of PNH in 1, hepatopulmonary syndrome in 1. 3 patients died after LT (1 of septicemia 3 months after LT, 1 of multiorgan failure in the immediate post operative period and the other died of an unexplained neurologic event 3 years after LT). 8 patients are alive and well at their last follow up, median 2.53 years (range 0.66-6.57 years). Conclusions Procoagulant states are the commonest cause of BCS in children. The management and outcome of this rare condition continues to be heterogeneous and carries a high mortality.

Disclosures:
The following people have nothing to disclose: Roshni Vara, Natalie J. Bab, John Karani, Gholam J. Mutti, Nigel Heaton, Giorgia Mieli-Vergani, Anil Dhawan
Non-alcoholic Fatty Liver Disease (NAFLD) is known to be related to predictors of coronary heart disease (CHD) such as insulin resistance, dyslipidemia, central obesity, and metabolic syndrome (MS). Recently, several reports suggested a possible role of NAFLD in the development of CHD. However, correlation between NAFLD and CHD in the general population is not well established. The aim of this study was to determine whether subjects with NAFLD in a general large population have an elevated 10yr risk of CHD, as estimated using the Framingham risk score (FRS). 21130 subjects enrolled in general health examination were recruited. NAFLD was diagnosed by typical sonographic findings and < 20 gram/day alcohol consumption, based on questionnaire. The risk of cardiac events in 10 yrs was estimated using Framingham equation: the score was computed based on age, total cholesterol, HDL-cholesterol, blood pressure, diabetes status and smoking status. Class of FRS was defined: low risk ≤5%, mild risk >6<10%, intermediate risk 10<20%, high risk: ≥20%. 3289 out of 21130 (15%) subjects were excluded because they had known causes of liver disease (1690 alcoholic, 975 HBV, 242 HCV, 193 other hepatitis history) and history of heart disease (189). NAFLD was diagnosed in 5888 (27.9%) subjects. Other normal 11953 subjects were enrolled in control group. The severity of NAFLD was graded into three group by ultrasonographic findings; 0 normal [n=11953], grade [gr] 1 mild fatty liver [n=3356], grade 2 moderate-severe fatty liver [n=2532]. Univariate analyses revealed that severity of NAFLD related to age, BMI, waist circumference, HT, AST, ALT, Total cholesterol, triglyceride (TG), HDL-cholesterol, LDL-cholesterol, glucose, HbA1c, uric acid, and FRS class (p<0.05). Multivariate regression analyses with confounding factors showed strong association of higher FRS with the severity of NAFLD, both among men (age<50: gr 1 OR 1.35 95% CI 1.13-1.62, gr2 OR 1.66 95% CI 1.36-2.02; age>50 gr1 OR 1.19 95% CI 1.03-1.38, gr2 OR 1.48 95% CI 1.25-1.74, p<0.01) and women (age<50: gr1 OR 3.18 95% CI 1.23-8.21, gr2 6.14 95% CI 2.11-17.89; age>50: gr1 1.84 95% CI 1.53-2.22, gr2 2.38 95% CI 1.89-2.99, p<0.01). ALT threshold for increased risk of CHD (FRS >10%) was lower in women (>18.5 IU/L) than in men (>24.5 IU/L). NAFLD itself represents an important risk factor of CHD regardless of other metabolic syndrome components. Also, we found that ALT level for increased risk of CHD was much lower than normal range. These associations are more prominent in women

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1108 INHERITED VARIANTS OF GENES INVOLVED IN INSULIN SIGNALING AFFECT THE RISK OF HEPATIC FIBROSIS IN NAFLD
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Background & Aims: Inherited factors play a major role in the pathogenesis of type 2 diabetes and the metabolic syndrome, whose hepatic expression is NAFLD, a leading cause of liver disease. Aim was to assess the role of well-characterized functional polymorphisms of genes involved in insulin signaling and lipogenesis on the risk of NAFLD progression to fibrosis. Patients and Methods: 235 pts with NAFLD, 150 with liver biopsy (57 with and 93 without fibrosis), and 135 controls. The Leu162Val PPARalpha, Pro12Ala PPARgamma2, Lys121Gln PC-1, Gly972Arg IRS-1, and Glu84Arg TRB3 polymorphisms were determined by restriction analysis. Data were compared by chi-square and t-Test, when appropriate. The risk of fibrosis was adjusted by logistic regression analysis considering as independent variables genetic polymorphisms, age, ALT, and ferritin levels. Results: By univariate analysis, the IRS-1 972Arg and the PPARalpha 162Val alleles, previously linked to insulin resistance, were associated with higher prevalence of diabetes/impaired glucose tolerance and higher HOMA insulin resistance index (p<0.05). The IRS-1 972Arg allele (prevalence 13% in NAFLD) was associated with higher prevalence of fibrosis in biopsied patients (55% in positive patients vs. 36% in negative ones; OR 2.5, 95% CI 0.99-7; p=0.06), whereas the gain of function 84Arg allele (prevalence 23% in NAFLD) of TRB3, which inhibits lipogenesis, was associated with lower fibrosis (28% in positive patients vs. 43% in negative ones; OR 0.39, 95% CI 0.15-0.95; p=0.04). Conclusions: Genetic factors affecting insulin signaling, which regulates metabolism and survival of hepatocytes, are associated with liver damage in NAFLD, thus supporting therapy with insulin sensitizing drugs. However, insulin resistance is not always associated with fibrosis, but the molecular mechanism and the genetic background may play a role.

1109 DAILY CANNABIS USE, A NOVEL RISK FACTOR OF STEATOSIS SEVERITY IN PATIENTS WITH CHRONIC HEPATITIS C
Christophe Hezode1,2, Elie-Serge Zafra2,3, Françoise Roudot-Thoraval1,2, Charlotte Costentin1, Ali Hessami1, Fatima Medkour1, Magali Bouvier-Alias4, Jean-Michel Pawlotsky4, Sophie Lotersztajn2, Ariane Mallat1,2; 1Hepatology, Hopital Henri Mondor, Paris XII University, Creteil, France; 2INSERM U841, Hopital Henri Mondor, Paris XII University, Creteil, France; 3Pathology, Hopital Henri Mondor, Paris XII University, Creteil, France; 4Virology, Hopital Henri Mondor, Creteil, France; 5Public Health, Hopital Henri Mondor, Paris XII University, Creteil, France

Cannabis Sativa binds two receptors CB1 and CB2. We recently reported a profibrogenic effect of CB1 receptors (Teixeira-Clerc, Nature Med 2006) and found a significant relationship between daily cannabis use and fibrosis severity in patients with chronic hepatitis C (CHC). Recent experimental evidence from others and our group indicates that both CB1 (Osei Hyaiman et al, J Clin Invest 2005) and CB2 receptors (Deveaux et al. AASLD 2007) promote liver steatogenesis. Therefore, the aim of this study was to evaluate the impact of cannabis use on steatosis severity in patients with CHC. 315 consecutive naïve patients were included. Several parameters were recorded at liver biopsy, including epidemiological data, intake of cannabis, alcohol, and maintenance treatment over the preceding semester, BMI, diabetes, serum fasting glucose, triglycerides and cholesterol levels, HCV genotype and viral load. Histology was assessed, without knowledge of clinical data, by two pathologists according to METAVIR (activity grade and fibrosis stage), and marked steatosis was defined as ≥30%. Patients (M/F: 223/92, mean age 45±11 yrs, 60 patients with steatosis ≥30%) were grouped according to cannabis smoking as none (200, 63.5%), occasional (<1 daily joint: 39, 12.4%, median/month =4) or daily users (≥1 daily joint: 76, 24.1%, median/month = 75). In unadjusted analysis, intake of cannabis, alcohol, and maintenance treatment significantly more frequent in daily cannabis users, compared to occasional and non users (p=0.03 and p=0.02, respectively). By logistic regression analysis, independent predictors of steatosis severity included daily cannabis use [OR: 2.1, [95%CI: 1.0-4.5], p=0.03], activity ≥A2 [OR: 2.1, [95%CI: 1.0-4.3], p=0.04], genotype 3 [OR: 5.4, [95%CI: 2.6-11.3], p=0.0001], hyperglycemia or diabetes [OR: 5.1, [95%CI: 1.8-15.0], p=0.003], BMI ≥27 [OR: 2.1, [95%CI: 1.0-4.3], p=0.05] and high viral load [OR: 1.7, [95%CI: 1.0-2.9], p=0.05]. Upon adjustment on viral genotype (3 vs non 3) or alcohol intake (< vs ≥30g/d), marked steatosis was significantly more frequent in daily cannabis users, compared to occasional and non users (p=0.03 and p=0.02, respectively). In summary, our study discloses a strong link between daily cannabis use and steatosis severity in patients with CHC, in keeping with the known steatogenic effect of CB1 receptors and with our recent experimental data indicating that CB2 also promotes steatogenesis. Our findings further indicate that patients with untreated CHC should be advised to refrain from daily cannabis use.

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PIGMENT EPITHELIUM-DERIVED FACTOR (PEDF) REGULATES HEPATOCYTE LIPID CONTENT THROUGH ITS INTERACTION WITH ADIPOSE TRIGLYCERIDE LIPASE (ATGL)

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Background: PEDF is a multifunctional protein with neurotrophic, antiangiogenic and anti-tumor activity. A recent report indicated that the receptor for PEDF is ATGL, a newly discovered lipase that mediates the first step in triglyceride (TG) breakdown. Our studies identify PEDF—ATGL binding in murine hepatocytes and HCC cell lines, and demonstrate that loss of PEDF leads to fat accumulation in the liver. Methods: Wildtype (WT) and PEDF null hepatocytes were isolated and used 24-48h after isolation. Immunoprecipitation (IP) of ATGL with recombinant PEDF (rPEDF) was done using WT and PEDF null hepatocytes, and HCC cell lines. Immunofluorescent colocalization of PEDF and ATGL was characterized. Qualitative and quantitative TG content was assessed with Oil Red O staining and enzymatic determination of lipid content. TG content was normalized per µg protein or body weight. A liver/body weight ratio was calculated for WT and PEDF null animals.

Results: ATGL from PEDF null hepatocyte lysates was preferentially bound by rPEDF compared to WT lysates suggesting tight native binding interactions. Immunoblotting of the post-IP supernatant showed that rPEDF binds the majority of ATGL in HCC lysates. Immunofluorescence showed PEDF and ATGL localization around adiposomes. TG content was significantly greater in PEDF null hepatocytes than WT, 141.3 ± 16.1 vs 74.7 ± 12.1 mg/dl/µg, (p<0.05). Restoration of rPEDF to PEDF null hepatocytes showed a trend toward decreased TG content from 141.3 ± 16.1 to 110.2 ± 5.7 mg/dl/µg (p=0.06) but not in WT hepatocytes, 74.7 ± 12.1 vs 61.4 ± 4.3 mg/dl/µg (p=NS). The TG content was similarly increased in PEDF null livers compared to WT. The liver/body weight ratio in PEDF null livers was significantly greater than WT livers (4.62 ± 0.11 vs 4.09 ± 0.08, p<0.001). Sections of PEDF null livers showed increased cell proliferation compared to WT.

Conclusion: PEDF regulates hepatocyte lipid content through its interaction with ATGL.

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ALCOHOL CONSUMPTION IN SEVERELY OBESE PATIENTS WITH NONALCOHOLIC FATTY LIVER DISEASE: RELATIONSHIP WITH HEPATIC FIBROSIS

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Background: Moderate alcohol consumption has been associated with improved insulin activity, a lower prevalence of type II diabetes and a favorable vascular risk in severely obese. This study aimed to examine the association between light-to-moderate alcohol consumption and severity of nonalcoholic fatty liver disease (NAFLD) in the obese patient. Method: A cross-sectional study was performed in severely obese subjects, who underwent liver biopsy during bariatric surgery from October 2004 to April 2005. The patients were classified into 3 groups according to alcohol consumption: G1: alcohol intake > 20g/day and < 40g/day; G2: alcohol intake= 20g/day; G3: no alcohol intake. Alcohol consumption was determined by clinical interviews and questionnaire. Insulin resistance (IR) was calculated by homeostasis model assessment (HOMA). IR was considered when HOMA was > 3. Biopsies were scored and classified as isolated steatosis (Type I); steatosis + inflammation (Type II); steatohepatitis (steatosis, ballooning of hepatocytes and inflammation) (Type III); and steatohepatitis with fibrosis and cirrhosis (Type IV). The study was approved by the Ethics Committee for Medical Research (CPqGM - FIOCRUZ, Bahia).

Results: There were 132 subjects (83 female and 49 male) mean age 37.27 +/- 11.06 (18 to 65) years with a body mass index of 43.9 +/- 5.6 kg/m². G1, G2 and G3 included 19, 56 and 57 patients respectively. Histological diagnoses by levels of alcohol consumption were: G1 (moderate alcohol): 2 (10.5%) had normal liver and 89.5% (17) had NAFLD, Type III or IV, G2 (light alcohol): 6 (10.7%) had normal liver and 1.8% (1) had Type 1 or 2 and 87.5% (49) had Type III or IV of NAFLD, and G3 (no alcohol intake): 6 (10.5%) had normal liver, 3.5% (2) had Type 1 or 2 and 86% (49) had Type III or IV. One of these had cirrhosis. IR was evaluated in 102 patients. It was correlated with moderate and no alcohol intake in 81.3% and 78.7% cases respectively (p<0.05). In patients with light alcohol intake 54.8% did not present IR. Conclusions: Light-to-moderate alcohol consumption was not associated with the severity of NAFLD in obese patients. Steatohepatitis with fibrosis had a similar prevalence in patients with or without history of alcohol intake. However, light alcohol intake may protect the severely obese patients from insulin resistance. Dissociation of the effect on IR versus liver histology is consistent with a complex and as yet incompletely understood interaction between alcohol and obesity-related liver disease.

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ARTERIALIZATION OF CENTRAL ZONES IN NONALCOHOLIC STEATOHEPATITIS (NASH)

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Background. Injury with fibrosis in the central zone (CZ) is a typical diagnostic feature of NASH. We have reported small arteries within CZ scars in alcoholic steatohepatitis, and noted in this setting that the arteries appear to extend from portal areas to CZ in serial sections. Since that study, we have anecdotally also noted arterial ingrowth into the CZ in NASH, a finding that in either entity can lead to mistaken identification of a scarred CZ as a portal tract (and in particular, a portal tract with ductopenia), and so results in errors in the pathologic diagnosis. This study examines for the presence of these arteries in NASH and at what stage they are noted. Methods. 100 biopsies (BX) of NASH with fibrosis stage > 1a from the NASH Clinical Research Network participants from Feb 2005 through Aug 2006 were randomly selected for evaluation of H&E and trichrome stains. Parameters examined included: prevalence of arteries graded as 0 (none), 1 (1-2/BX), 2 (<50% of CZs), 3 (>50% of CZs) and ductular metaplasia in CZ as + or -. The NASH activity score (NAS), stage of fibrosis, and size of liver needle BX determined by central review were obtained from the database. All BXs reviewed had at least 5 CZ and were deemed adequate by central review. Results. Arteries were present in 40/100 (40% of cases), determined as rare in 18%, and common (grade 2-3) in 22% (See Table). The arterIALIZATION of CZs was increased in the higher stage lesions (68%, or 29/47 cases in stage 3-4) as compared to stage 1-2 (21%, or 11/53 cases), with increased prevalence correlating directly with higher stage. Ductular metaplasia was a common associated finding (53%, or 22/40 cases with arteries). Conclusions. Arteries in the scarred CZ of NASH are a common finding in late stage disease, but can rarely be seen in this location as early as stage 1b. Such arteries are likely due to the remodeling of the vascular architecture as scarring progresses in NASH. The presence of this aberrant artery, particularly in samples with ductular metaplasia in the same scarred CZ, can lead to the erroneous determination of the CZ as a portal tract, and thus result in failure to diagnose NASH by a pathologist who may not be aware of this finding. (Supported by Research-Grant DK61738 from the NIDDK.)

NASH fibrosis stage* versus presence of CZ arteries (grade)

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Disclosures: The following people have nothing to disclose: Linda D. Ferrell, Patricia Belt, Nathan M. Bass, Clinical Research Network for the NASH

1114  
MODERATE LIQUOR CONSUMPTION IS ASSOCIATED WITH A DECREASED PREVALENCE OF SUSPECTED NONALCOHOLIC FATTY LIVER DISEASE

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Background: Moderate liquor consumption has been shown to protect against coronary heart disease (CHD). Subjects at risk for coronary heart disease (CHD) are often at risk for nonalcoholic fatty liver disease (NAFLD). The effect of modest wine consumption on NAFLD has not been studied and the recommendation of wine for patients at risk for both diseases is controversial. Despite the potential for liver injury, wine has been shown to ameliorate metabolic risk factors common to NAFLD and CHD. Therefore the aim of this study is to test the hypothesis that modest wine consumption is associated with decreased prevalence of NAFLD. Methods: We included subjects in the Third National Health and Nutrition Examination Survey who either reported no alcohol consumption or preferentially drinking wine with total alcohol consumption up to 10g per day. Suspected NAFLD was based on unexplained ALT elevation over two sets of cut points: the cut point of the reference laboratory (ALT > 43) and the cut point based on the 95th percentile of healthy subjects (ALT > 30 for men, ALT > 19 for women). Alternate explanations of abnormal ALT were excluded (excessive alcohol, viral hepatitis, iron overload, and medications). Multivariate analysis adjusted for age, gender, race, neighborhood population density, income, education, caffeine intake and physical activity level. Result: 7211 non-drinkers and 945 modest wine drinkers comprised the study sample. Based on the reference laboratory cut point, suspected NAFLD was observed in 3.2% of nondrinkers and 0.4% of modest wine drinkers. The adjusted odds ratio was 0.13 (95% CI 0.05 – 0.37). Using the healthy subject cut point, suspected NAFLD was observed in 14.3% of nondrinkers and 8.6% of wine drinkers. The adjusted odds ratio was 0.48 (95% CI 0.31 – 0.74). Conclusion: Modest wine consumption is associated with reduced prevalence of abnormal ALT from sus-
The following people have nothing to disclose: Winston Dunn, Ronghui Xu, Jeffrey Schwimmer

1115 ANALYSIS OF HEPATIC EXPRESSION OF GENES INVOLVED IN LIPID AND IRON METABOLISM IN NON-ALCOHOLIC FATTY LIVER DISEASE

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Aims: Oxidative stress is considered critical in the development of nonalcoholic fatty liver disease (NAFLD). Hepatic steatosis results in reactive oxygen species (ROS) generation through oxidation of fatty acid and/or iron overload, thereby progressing to steatohepatitis. Although the important molecules involved in these metabolisms are well defined, their roles in the natural course of disease remain unknown. Therefore, we investigated hepatic genes involved in oxidation and synthesis of fatty acid and iron accumulation in patients with NAFLD. Methods: Liver biopsies were undertaken in 74 consecutive patients with NAFLD (simple steatosis / steatohepatitis: 33 / 41). Histological staging followed that of Brunt. Hepatic levels of thioredoxin (Trx), a ROS-inducible antioxidant, were used as markers of oxidative stress. The following genes were quantified from biopsy specimens using real-time PCR and normalized by β-actin: Trx, peroxisome proliferators-activated receptor-α (PPARα), acyl-CoA dehydrogenase C4-12 (ACADM), cytochrome P450 2E1 (CYP2E1), acyl-CoA oxidase (ACOX), sterol regulatory element binding proteins-1c (SREBP1c), fatty acid synthase (FASN), transferrin receptors 1 and 2 (TFR1 and TFR2), and hepcidin (HAMP). Twelve normal livers were used as controls. Results: Hepatic levels of Trx were significantly higher in livers of patients with NAFLD than in normal livers (p < 0.0001). The level of Trx increased as the stage progressed (p = 0.074) and significantly correlated with that of PPARα, ACADM, CYP2E1, and ACOX (p < 0.0001, each). However, the levels of fatty acid-oxidizing genes significantly decreased at the later stage of NAFLD (p < 0.05, each). In parallel with these findings, the levels of SREBP1c (p < 0.05), FASN, and steatosis similarly decreased. On the other hand, hepatic iron score (HIS) tended to correlate with stage (p = 0.095). The levels of TFR1, TFR2, and HAMP were significantly higher in NAFLD patients than in normal livers, and they significantly correlated with those of Trx (p < 0.0001, each). The level of TFR1 significantly correlated with stage (p = 0.05), whereas the level of TFR2 significantly correlated with that of PPARα (p < 0.0001). The level of HAMP significantly correlated with HIS (0.01) as well as serum levels of ferritin (p < 0.001). Conclusion: Our results indicated that 1) although oxidation of fatty acid exacerbates oxidative stress in NAFLD, these effects attenuate as the disease progresses, 2) TFR1 and TFR2 may continue hepatic accumulation of iron, despite hepatic synthesis of HAMP in response to iron load, 3) upregulation of TFR2 may possibly be associated with PPARα.

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1116 NON-INVASIVE DIAGNOSTIC BIOMARKERS FOR NON-ALCOHOLIC STEATOHEPATITIS (NASH)

Ancha Baranova, Mohammed Jarrar, Clare Nugent, Arian Afendy, Caitlin Quigley, Maria Stepanova, Hazem Elariny, Zachary Goodman, Vikas Chandhoke, Zobair M. Younossi

From the spectrum of non-alcoholic fatty liver disease (NAFLD), only patients with NASH have been shown to progress to cirrhosis. To date, liver biopsy remains the gold standard for diagnosis of NASH. Liver biopsy is expensive and is associated with a small but definite risk. There is an urgent need for the development of non-invasive diagnostic biomarkers for NASH. Recent data suggest the role of apoptosis, hepatocyte necrosis as well as adipocytokines in the pathogenesis of NASH. AIM: To develop a non-invasive diagnostic biomarker panel which could reliably distinguish NASH from simple steatosis (SS). METHODS: 69 patients with a liver biopsy were included (22 patients with NASH, 15 with SS and 32 weight-matched controls with normal liver biopsies). Liver biopsies were interpreted by a single hepatopathologist. NASH was defined as steatosis, lobular inflammation, ballooning degeneration with or without Mallory bodies and/or fibrosis. Clinical data and serum samples were collected at the time of biopsy. Serum was assayed for adiponectin, resistin, insulin, glucose, TNF-alpha, IL-6, IL-8, total CK18 (M65, a measurement of overall cell death due to both apoptosis and necrosis) and caspase cleaved CK18 (M30, a specific measurement of apoptosis). RESULTS: In the entire cohort, M30 apoptosis measurements correlated with levels of TNF-alpha (R = 0.4986, p < 0.0001) and IL-8 (R = 0.3052, p = 0.0108), but not with IL-6, visfatin, resistin, adiponectin, or HOMA. An indirect measurement of necrosis, M65-M30, did not correlate with any of the parameters measured. In NASH patients (N = 37), M30 apoptosis measurements correlated with the Homeostasis Model Assessment (HOMA) (R = 0.5106, p = 0.0013), TNF-alpha (R = 0.4816, p = 0.003), and IL-8 (R = 0.3052, p = 0.015). These findings further support the role of insulin resistance and pro-inflammatory cytokines in NASH. In the entire cohort, histologic NASH could be reliably predicted by a combination of M30 (apoptosis), M65-M30 (necrosis), serum adiponectin and serum resistin (Model p-value = 0.0001; AUC = 0.908, Sensitivity = 0.773, Specificity = 0.872). Use of M30 (apoptosis) as a sole predictor of NASH was a less reliable diagnostic biomarker (Model p-value = 0.000089; AUC = 0.710, Sensitivity = 0.636, Specificity = 0.872). CONCLUSIONS: A combination of ELISA-based measurements of apoptosis and necrosis markers as well as adipocytokines could be used to develop a simple diagnostic biomarker panel for NASH. If validated, this biomarker could become very useful in the clinical management of patients with NAFLD.

Disclosures: The following people have nothing to disclose: Ancha Baranova, Mohammed Jarrar, Clare Nugent, Arian Afendy, Caitlin Quigley, Maria Stepanova, Hazem Elariny, Zachary Goodman, Vikas Chandhoke, Zobair M. Younossi
Effect of Pentoxifylline on Clinical, Biochemical, and Metabolic Parameters, Hepatic Necroinflammation, Fibrosis and Stellate Cell Activation in Patients with Nonalcoholic Steatohepatitis

Deepak K. Singh1, Puja Sakhija1, Archana Rastogi1, Ajay Kumar2, Ranjana Gondal1, Shiv K. Sarin2

Pentoxifylline inhibits proinflammatory cytokines including TNF-α and may be antifibrogenic. TNF-α may cause hepatic necroinflammation, fibrosis and impaired insulin sensitivity in patients with nonalcoholic steatohepatitis (NASH). Inhibition of TNF-α by Pentoxifylline may be an important therapeutic strategy in NASH. AIM: To study the effect of Pentoxifylline on clinical, biochemical and metabolic parameters, hepatic necroinflammation, fibrosis and stellate cell activation in patients with NASH. METHODS: Nineteen patients included in the study were given Pentoxifylline 1200mg/day. All baseline clinical and lab data was recorded. A pre-therapy liver biopsy was done in all cases. Histologic features, necroinflammatory grade and fibrosis stage on liver biopsy were scored as per Brunt et al (1999). All the patients tolerated the drug well. After one year, a repeat liver biopsy was done. All the pre and post therapy clinical and lab data was compared. To accurately assess changes in hepatic fibrosis, immunostaining with anti-α-SMA was done in paired liver biopsies and hepatic stellate cell index (HSCI) was calculated. RESULTS: Mean age of patients was 35±8.8 yrs. After 12 mo. of therapy there was a significant reduction in BMI (25.3±1.9 vs 23.1±2.2, p=0.000), AST (70.3±25.5 IU/mL vs 27.9±6.3 IU/mL, p=0.000), ALT (108.6±50.3 IU/mL vs 35.8±14.5 IU/mL, p=0.000), serum insulin levels (13.5±7.4 IU/mL vs 7.9±2.9 IU/mL, p=0.000) and insulin resistance (HOMA-IR) (3.6±1.9 vs 1.9±0.9, p=0.000) levels. No significant change was observed in bilirubin (p=0.105), albumin (p=0.517), blood sugar (p=0.461), HDL cholesterol (p=0.224). Though TNF-α levels reduced the difference was not significant (p=0.075). On histologic examination of liver biopsies, a significant decrease was observed in steatosis (p=0.008), lobular inflammation (p=0.042) and necroinflammatory grade (p=0.014). No decrease was observed in ballooning degeneration (0.155), portal inflammation (0.527) and fibrosis stage (0.298). HSCI increased significantly in zone 1, 2 and 3 of hepatic lobule (1.6±0.8 vs 5.3±2.4, p=0.013). The increased HSCI was seen even in cases which had decreased fibrosis stage or similar stage in pre and post therapy biopsies. CONCLUSION: Pentoxiphylline therapy significantly improves clinical, biochemical and metabolic parameters in patients with NASH. It also leads to reduction in steatosis, necroinflammation and lobular inflammation. TNF-α level are also reduced after therapy, but not statistically significant. The limited effects of Pentoxiphylline on hepatic fibrosis and HSCI could be due to need for a higher dose of the drug requiring greater suppression of TNF levels.

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The following people have nothing to disclose: Deepak K. Singh, Puja Sakhija, Archana Rastogi, Ajay Kumar, Ranjana Gondal, Shiv K. Sarin

Adipose Tissue of Insulin Resistance in NASH Patients

Masahiko Shimada1,2, Hiromu Kawahara1, Masayoshi Yamada1, Masayuki Fukura1, Mutsumi Tsuchishima1, Shuhei Yoshida2, Yoshiaki Kitamura2, Morihiko Yoshino2, Shujiro Takase1

Background/Aims: Insulin has the function of controlling the breakdown of fatty acids in adipocytes and reducing the level of free fatty acid (FFA) in the blood. However, if insulin resistance develops, then the effectiveness of controlling the breakdown of fatty acids is reduced, and FFA will also reach high levels, even while insulin is at a high level. For non-alcoholic steatohepatitis (NASH) patients, insulin resistance is often detected in the liver, but it remains to be elucidated whether insulin resistance can be detected in adipose tissue. Methods: The subjects consisted of 30 simple steatosis (SS) persons who were matched by BMI, age, and sex (BMI 26.4, age 51.3, 14 females) and 30 NASH persons (BMI 26.7, age 52.2, 15 females). Among these patients, the fasting blood sugar level (FBS), fasting immunoreactive insulin (IRI), HOMA-IR, serum adiponectin level, FFA, Tcho, HDL-cho, TG, and ALT were measured, after which it was determined whether the level of FFA in the blood is higher among NASH patients than among SS patients. In addition, in order to detect the presence or absence of insulin resistant adipose tissue in NASH patients, we investigated whether the effectiveness of insulin in controlling the breakdown of fatty acids had decreased and whether the FFA in the blood were at a high level (>800 μEq/l), even while IRI was at a high level (>10.0 ng/ml), in comparison with SS. Results: The hematological findings showed the following findings, with SS values preceding those of NASH—FBS: 91.8, 104 mg/dl; IRI: 6.2, 13.6 μg/ml; HOMA-IR: 1.3, 4.1; serum adiponectin: 8.4, 5.0 μg/ml; FFA: 431, 698 μEq/l; Tcho: 221, 212 mg/dl; HDL-cho: 54, 47 mg/dl; TG: 93, 126 mg/dl; and ALT: 70, 78 IU/l. FFA (P=0.0024) was at a significantly higher level in NASH patients. In addition, a significant difference in FBS, IRI, HOMA-IR, and serum adiponectin was detected between SS and NASH. Regarding the presence or absence of insulin resistant adipose tissue in NASH patients, the number of cases of high-level IRI was 22 for NASH, and the number of cases of high-level FFA was 9 (30%) of these cases. Conversely, 6 cases with high-level insulin were detected in SS, but there was only 1 case of high-level FFA (3%), and insulin resistance was significantly detected (P=0.0013) in the adipose tissue of NASH patients. Conclusions: Although no difference in the BMI was detected, the FFA showed a higher level in NASH than in SS. In addition, the effectiveness of insulin was also found to decrease in the adipose tissue of 30% of the NASH patients, thus indicating that this may be one possible cause for the development of NASH.

Disclosures:
The following people have nothing to disclose: Masahiko Shimada, Hiromu Kawahara, Masayoshi Yamada, Masayuki Fukura, Mutsumi Tsuchishima, Shuhei Yoshida, Yoshiaki Kitamura, Morihiko Yoshino, Shujiro Takase
1119 HIGH SENSITIVITY C-REACTIVE PROTEIN IS AN INDEPENDENT CLINICAL FEATURE OF NONALCOHOLIC STEATOHEPATITIS (NASH) AND ALSO OF THE SEVERITY OF FIBROSIS IN NASH

Yuichi Nozaki, Masato Yoned, Takuma Higurashi, Hiroshi Iida, Hironori Mawatari, Hiroki Endo, Ayako Tomimoto, Kyoko Yone-mitsu, Tomoyuki Akiyama, Koji Fujita, Hirokazu Takahashi, Masahiko Inamori, Noritsahi Kobayashi, Yasunobu Abe, Kenseke Kubota, Hiroyuki Kikikoshi, Satoru Saito, Atsushi Nakajima; Division of Gastroenterology, Yokohama City University, Yokohama, Japan

[Background and aims] The changes in nonalcoholic fatty liver disease (NAFLD) range over a wide spectrum, extending from steatosis to steatohepatitis (NASH). However, it has remained difficult to differentiate between NASH and non-progressive NAFLD by clinical examination. We investigated the interrelationships between serum high-sensitivity CRP (hs-CRP) and the presence of obstructive sleep apnea (OSA) in patients with NAFLD.

[Methods] hs-CRP were measured in 101 patients with histologically verified NAFLD (29 with steatosis and 71 with NASH) and a total of 200 patients with OSA. The results of the multiple regression analysis revealed that as compared to that in cases with steato-sis, hs-CRP was significantly elevated (p = 0.0048) in cases of NASH. Furthermore, among the patients with NASH, hs-CRP was significantly elevated in cases with advanced fibrosis as compared with that in cases with mild fibrosis (p = 0.0384), even after adjustment for age, gender, existence of diabetes, BMI, visceral fat area, subcutaneous fat area, HOMA-IR, HDL-cholesterol, triglyceride, and LDL-cholesterol. In regard to the results of the RT-PCR study, the intrahepatic mRNA expression of CRP, but not IL-6, was increased in patients with NASH as compared with steatosis (p = 0.0228).

[Conclusions] This is the first report to demonstrate consistent and profound elevation of hs-CRP in cases of NASH as compared with simple non-progressive steatosis, but also a clinical feature of the severity of hepatic fibrosis in cases of NASH.

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1120 PRESENCE OF OBSTRUCTIVE SLEEP APNEA IS ASSOCIATED WITH FIBROSIS IN PATIENTS WITH NONALCOHOLIC FATTY LIVER DISEASE

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[Background] Nonalcoholic fatty liver disease (NAFLD) is a common cause of chronic liver disease which is associated with obesity and insulin resistance. Obstructive sleep apnea (OSA) is also independently associated with insulin resistance and is a common disorder in obese patients. AIM: To determine the polysomnographic predictors of fibrosis in patients with nonalcoholic steatohepatitis (NASH). METHODS: 101 patients who underwent bariatric surgery were selected from our database. Pre-operative demographic, anthropometric, clinical, laboratory, liver histology and full overnight polysomnographic data were available. Excess alcohol intake (defined as ≥20gm/day for males and ≥10gm/day for females) and other causes of chronic liver disease were excluded. NASH was defined as steatosis, hepatocyte ballooning degeneration and lobular inflammation with or without fibrosis or Mallory bodies. Apnea was defined as the complete cessation of airflow ≥10 seconds and hypopnea was defined as a ≥50% or greater reduction in airflow or respiratory effort ≥10 seconds accompanied by a ≥4% or greater desaturation. The average number of episodes of apnea and hypopnea per hour of sleep (apnea-hypopnea index, AHI) was calculated. AHI > 5 is considered abnormal according to the American Academy of Sleep Medicine (AASM) consensus. RESULTS: Out of 101 patients, 79 had NASH and 22 had Non-NASH. Out of 79 NASH patients, 66 had mild fibrosis, 7 with advanced fibrosis and 6 had no fibrosis. Presence of metabolic risk factors such as diabetes, hyperlipidemia and hypertension were similar in all three groups. Presence of sleep apnea was similar in fibrosis vs. non fibrosis group (84% vs. 75%, p=0.32). Lowest desaturation (nadir oxygen saturation) was significantly lower in patients with fibrosis vs. non-fibrosis (76% vs 85%, p=0.004). Mean nocturnal oxygen saturation in patients with fibrosis was 90.4 vs. 93.0 in non-fibrosis group (p=0.02). Although patients with fibrosis had a trend towards increasing AHI, it did not reach statistical significance (p=0.08).

[Conclusions] Presence of sleep apnea is associated with significant fibrosis in patients with NAFLD. Further studies are warranted to study the effect of optimizing sleep apnea management on liver outcome.

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The following people have nothing to disclose: Poonam Mishra, Clare Nugent, Chunhong Bai, Mariam Afendy, Theresa Egasani-Elises, Hazem Elariny, Zobair M. Younossi

1121 PROTEOMIC ANALYSIS OF SERUM BIOMARKERS IN PATIENTS WITH NONALCOHOLIC STEATOHEPATITIS USING SELDI-TOF/MS OR MALDI-TOF/MS

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[Background and Aims] Nonalcoholic fatty liver disease (NAFLD) is a common chronic liver disease. Among NAFLD patients, only those with nonalcoholic steatohepatitis (NASH) are at increased risk for progression to cirrhosis and hepatocellular carcinoma compared to patients with simple steatosis. However, there is no serum biomarker for NAFLD or NASH, and identifying high-risk patients is extremely difficult. This study illustrates how surface-enhanced laser desorption and ionization (SELDI)- or matrix-assisted laser desorption/ionization (MALDI)-time of flight mass spectrometry (TOF/MS) is a valuable method to profile and identify serum proteins in patients with different NAFLD subtypes in order to discover a novel serum marker for NASH.

[Methods] NAFLD patients were classified as simple steatosis or NASH by liver biopsy. A total of 71 serum samples, including serum from 25 NASH patients, 20 patients with simple steatosis, and 26 healthy controls were examined by SELDI-TOF/MS using the IMAC 30 chip. Serum
protein profiles were analyzed with the accompanying software to screen for potential NASH markers. For protein identification, an affinity-bead-purified serum protein was subjected to MALDI-TOF/MS analysis, followed by Mascot identification of the peptide sequences and a search of the National Center for Biotechnology Information protein database. RESULTS: Three serum protein peaks with mass-to-charge ratios (m/z) ranging from 3000 to 5000 were significantly upregulated in NAFLD samples compared to healthy control samples. One protein peak was also significantly downregulated in NASH patients compared to simple steatosis patients by SELDI-TOF/MS. However, these 4 serum proteins were not identified by high-performance liquid chromatography. To optimize MALDI-TOF/MS analysis based on the best markers distinguishing NASH and control spectra, cation-exchange beads were used for serum protein profiling. Three markers between 1000 and 3000 m/z were upregulated in NAFLD sera compared to healthy control sera. These three protein peaks were detected in 44 NAFLD samples and were not detected in any healthy control samples. These three peaks were identified as different isoforms of the same protein with some portions deleted. CONCLUSIONS: Proteomics approaches such as SELDI-TOF/MS and MALDI-TOF/MS could greatly facilitate the discovery of serum biomarkers in NAFLD patients. Identifying these proteins will help clarify NASH pathogenesis and identify potential markers for NASH.

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1122 PIOGLITAZONE ENHANCES HEPATIC, MUSCLE AND ADIPOSE TISSUE INSULIN SENSITIVITY AND AMELIORATES SYSTEMIC INFLAMMATION IN PATIENTS WITH IGT OR T2DM AND NASH

Kenneth Cusi1, Renata Belfort4, Steve Harrison2, Bogdan Balas1, Stephen Schenker1, Celia Darland1, Amalia Gastaldelli1, Kenneth Brown1, Jean Hardies1; 1The University of Texas Health Science Center at San Antonio, San Antonio, TX; 2Brooke Army Medical Center, San Antonio, TX

Background: We reported that pioglitazone (PIO) improves glucose metabolism and histology (steatosis/necro-inflammation) in pts (pts) with IGT or type 2 diabetes (T2DM) and non-alcoholic steatohepatitis (NASH) (Belfort, NEJM 2006). Because insulin resistance (IR) and systemic inflammation are involved in the pathogenesis of NASH, we further examined the effect of PIO on both variables. Methods. Pts (n=55) received a hypocaloric diet (-500 kcal/d) and were randomized (double-blind) to PIO (45mg/d) or placebo (Pbo) for 6 months. Pts had the following studies before and after treatment: 1) liver biopsy; 2) liver fat by magnetic resonance spectroscopy (LMRs); 3) plasma levels of inflammatory markers; 4) double-tracer OGTT to assess glucose tolerance, endogenous (liver) glucose production (EGP) and glucose clearance. We calculated indexes of hepatic (Hep-IS = 1/[EGP x fasting insulin]), muscle (Muscle-IS= Rd/insulin during OGTT) and adipose tissue (Adipo-IS= 1/[FFA x fasting insulin]) insulin sensitivity. We also studied 15 controls (CON) without T2DM or NASH (no biopsy). Results. NASH pts (vs. CON) had higher fasting/OGTT plasma glucose, A1C, insulin and FFA levels, LMRs, and lower adiponectin and glucose clearance (all p<0.001) – each defect being reversed by PIO [all p<0.001; Belfort, NEJM 2006]. In NASH pts, Hep-IS, Muscle-IS and Adipo-IS were all reduced (~75%, ~43% and ~50%, respectively, p<0.01) and associated with reduced fasting/OGTT glucose clearance (r= 0.37 to 0.44, p<0.01). Pbo did not change glycemic control/insulin sensitivity, but PIO markedly increased Hep-IS, Adipo-IS and Muscle-IS from 1.2- to 1.8-fold (p<0.001 vs. pre-T and Pbo). Adiponectin increased ~3-fold (p<0.001) and correlated strongly with improved Hep-IS (r=0.37), Adip-IS (r=0.54), OGTT glucose clearance (r=0.44), steatosis (r=0.49) and necroinflammation (r=0.51, all p<0.01). NASH pts had an increase in markers of systemic inflammation: hsCRP x ~3-fold and IL-6, TNF-α, VCAM and ICAM by 40-50% (all p<0.01 vs. CON). PIO significantly reduced hsCRP (6.1±1.0 to 3.1±0.8 mg/L, p<0.04), TNF-α (2.2±0.1 vs. 2.0±0.1 pg/ml, p<0.01), TGF-β (31±2 vs. 26±2 ng/ml, p<0.05), VCAM (323±32 vs. 480±32 ng/ml, p<0.01) and ICAM (315±17 vs. 268±10 ng/ml, p<0.01). Conclusion: pts with IGT/T2DM and NASH have severe hepatic/muscle/adipose tissue insulin resistance and systemic inflammation. Amelioration of insulin resistance by PIO reduces hepatic lipogenesis and fat accumulation by acting at multiple levels, including substrate supply (glucose, FFA), hormonal control (lowering plasma insulin and increasing adiponectin) and lipid-associated inflammation, which helps explain its clinical efficacy.

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The following people have nothing to disclose: Renata Belfort, Steve Harrison, Bogdan Balas, Stephen Schenker, Celia Darland, Amalia Gastaldelli, Kenneth Brown, Jean Hardies

1123 OXIDATIVE STRESS PROFILES IN PEDIATRIC NON ALCOHOLIC FATTY LIVER DISEASE

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Background: Non Alcoholic Fatty Liver Disease (NAFLD) in the pediatric population is an under recognized pathologic entity since most patients are clinically asymptomatic until a late stage of disease. There is limited knowledge about oxidative stress variability in the pediatric literature. Oxidative stress may be related to alterations in immune response that preferentially enhance hepatic inflammation and NAFLD progression. Identifiable biomarker patterns that accurately represent oxidative stress would be an important advance in NAFLD recognition and therapeutic intervention. Aim: To explore oxidative stress biomarker models and lymphocyte immunophenotype profiles in pediatric patients within the clinical spectrum of NAFLD. Patients and Methods: Sixteen obese patients (BMI>95th percentile, mean age 11.5 years, range 3-17 years, 5 females) were included in the study. Standard liver function tests, creatinine-adjusted urinary F2-isoprostane, lymphocyte immunophenotype subsets and a liver sonogram were obtained. Other causes of liver disease were excluded. Three groups were compared: steatohepatitis group; hepatic steatosis group; an obesity only group without liver abnormalities was used as a control. Results: The steatohepatitis group had approximately 2-fold higher average creatinine-adjusted urinary isoprostane excretion compared to the hepatic steatosis and obese groups. Higher serum ALT values correlated with increased average F2-isoprostane; however, a linear relationship was not seen with or without NAFLD. In addition, there was a decreased percentage of CD3+CD4+ T-cells compared with the obese group (p<0.05) and normal controls (p<0.01) and an increased percentage of...
CD19(+) B-cell lymphocyte subsets in the steatohepatitis group compared with normal controls (p=0.06) studied in parallel. Conclusion: Higher levels of oxidative stress as measured by urinary F2-isoprostane and altered percentages of T- and B-cell lymphocyte subsets are associated with steatohepatitis as reflected in both liver function test and NAFLD-specific sonographic abnormalities compared to obese controls. The use of these modalities in identifying pediatric patients with NAFLD warrants further investigation.

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1124 SHORT-TERM VARIABILITY IN COMMONLY USED LIVER TESTS: IMPLICATIONS FOR CLINICAL PRACTICE

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Objectives: Although liver tests are routinely used in clinical settings to screen and monitor hepatic diseases, the short-term within-person variability of serum aminotransferases (AST, ALT), alkaline phosphatase (AP), gamma glutamyl-transferase (GGT) and total bilirubin is largely unknown. The magnitude of within-person variability has important implications for the use and interpretation of these tests. Methods: We analyzed repeated measurements (17 days apart on average) on 1,864 adults from the National Health and Nutrition Examination Survey III (NHANESIII) Second Examination. We describe the intra-individual variability in liver tests, as measured by the within-person coefficient of variability (CVw). We also assessed the proportion of subjects with persistently elevated levels as defined by NHANES III as follows: two measurements of AST >31 U/L for women and >40 U/L for men; ALT >31 U/L for women and >37 U/L for men; GGT >55 U/L; AP >117 U/L and total bilirubin >1 mg/dl. Results: There was significantly higher variability (p <0.05) within-person variability in the measurements of total bilirubin (CVw = 23.4%, 95% CI: 22.4%-24.3%) and ALT (CVw = 20.4%, 95% CI: 19.5%-21.2%) compared to AST (CVw, 13.9%; 95% CI: 13.2%-14.5%), and GGT (CVw, 13.8%; 95% CI: 12.6%-14.8%). AP was by far the most reliable of the five measurements (CVw, 6.7%; 95% CI, 6.4%-6.9%). Of those with initially elevated AST level, the proportion of persons with persistently elevated levels was 64%. Similarly, for ALT this proportion was 69%, for GGT 88%, for AP 83% and for total bilirubin 62%. Few people were reclassified as elevated on the 2nd exam after a normal result on the first exam (<5% for all tests). The results did not differ by gender, body mass index, level of alcohol consumption, or by laboratory evidence of hepatitis B or C infection. Conclusion: The usefulness of single measurements of liver tests in clinical practice and to screen large numbers of otherwise healthy subjects and/or monitor hepatic diseases may be limited by the low reliability of these measurements. If re-tested, over 30% of individuals with elevated AST, ALT or bilirubin levels will be reclassified as normal. GGT and AP are more reliable with ~15% of subjects being reclassified to normal after a second test. Clinicians should be aware of the relatively high within-person variability in common liver tests.

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The following people have nothing to disclose: Mariana Lazo, Elizabeth Selvin, Jeanne M. Clark

1125 ADIPOPHILIN EXPRESSION AND OXIDIZED PHOSPHATIDYLCHOLINE LOCALIZATION IN BALLOONED HEPATOCYTES IN NONALCOHOLIC STEATOHEPATITIS

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Background and Aims: Adipophilin, a member of PAT family proteins, plays a role in lipid droplet formation in diverse cell types. We previously reported that adipophilin expression was predominant in small lipid droplets and ballooned hepatocytes (BHs) in nonalcoholic steatohepatitis (NASH). Frequent expression of adipophilin in BHs is associated with advanced and/or aggressive diseases (Fujii H, et al., Hepatology 2006;44:199A-200A). In addition, ballooning degeneration in NASH is often accompanied by oxidized phosphatidylcholine (oxPC) localization, which indicates oxidative damage to hepatocytes (Ikura Y, et al., Hepatology 2006;43:506-514). Both adipophilin expression and oxPC localization may participate in pathology of NASH via a common pathway, hepatocyte ballooning. Thus, one can hypothesize that these two phenomena are potentially related. To further address this, we performed the following immunohistochemical analysis. Methods: Liver biopsies from 14 patients with NASH (F/M=10/4; Age, 60±9yr; BMI, 27.6±3.8kg/m2; ALT, 83±39IU) were examined. Disease activity was assessed according to the system of Brunt. Sections were stained with immunoperoxidase using anti-adipophilin (Progen) and anti-oxPC (DLH3), and examined under standard microscopy. Positivity in BHs was semiquantitatively as negative, mild (1-5 positive ballooned cells/10mm2 liver section), or marked (>6 positive cells/10mm2). Double staining was also performed using an immunofluorescent technique. Results: The samples showed diverse grade (2.0±0.9) and stage (2.4±1.0) scores. The presence of adipophilin-positive BHs positively correlated to the grade score (Rs=0.86, p=0.002). OxPC-immunoreactivity was also detected however its presence in BHs was linked with high stage scores (Rs=0.53, p=0.06) rather than grade scores (Rs=0.39, p>0.10). No significant relationship between adipophilin- and oxPC-positivity in BHs was evident as immunofluorescent staining infrequently revealed overlapping expression of adipophilin and oxPC in BHs. Conclusions: Both adipophilin expression and oxPC localization in BHs were definitely associated with the histological severity of NASH. However, these two phenomena seemed to be independent of each other. It is unclear whether this represents different pathophysiological processes in BHs or possibly to antigen modification due to oxidative injury to lipid droplet associated proteins like adipophilin in relation to oxPC.

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1126 ASSOCIATION OF AST AND ALT WITH LIVER HISTOLOGY IN ADULTS WITH NAFLD


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**Background:** Noninvasively distinguishing nonalcoholic steatohepatitis (NASH) from simple steatosis in people with nonalcoholic fatty liver disease (NAFLD) remains challenging and the role of individual liver enzymes for distinguishing NASH is unclear. **Methods:** We studied 383 adults with biopsy-confirmed NAFLD without other chronic liver diseases who enrolled in the NASH Clinical Research Network and who had liver enzyme levels obtained within 6 months of the biopsy. Liver enzymes were tested on fresh serum locally and biopsies were read centrally by hepatopathologists and classified by consensus using the validated NASH CRN NAFLD Feature Scoring System. We used bivariate and multivariable logistic regression to determine which of AST, ALT or the AST/ALT ratio best discriminates definite NASH from other forms of NAFLD. We also compared areas under receiver operating characteristic curves (AUROC) for NASH in relation to the two enzymes and the ratio adjusting for age, gender, race, BMI, PT, total protein and biopsy length. **Results:** The median age was 48 years; 61% were female, and 83% white. The median AST was 46 U/L, ALT 65 U/L, and AST/ALT ratio 0.70. Overall 62% had definite NASH. In bivariate analyses, the median enzyme levels were all significantly higher in those with NASH compared to those without: AST 54 vs. 38 U/L (p<0.001), ALT 71 vs. 59 U/L (p=0.004), and AST/ALT ratio 0.73 vs. 0.64 (p<0.001). The AUROC was highest for AST [0.69] compared to ALT [0.59] and AST/ALT [0.61]. Subjects with NASH were more likely to be women, and had significantly higher levels of total protein, triglycerides, fasting insulin and HOMA-IR, and longer biopsies (all p<0.01). Surprisingly, age, race/ethnicity and BMI were not significantly associated with NASH. In multivariable analyses, AST remained a better predictor for NASH than either ALT or AST/ALT. The AUROCs for the multivariate models were higher for ALT [0.68, p=0.005] and ALT/AST [0.69, p=0.007], but not significantly so for AST [0.73, p=0.06] than for each enzyme alone. A multivariate model with both AST and ALT did not improve the discrimination for NASH (AUROC 0.74, p=0.20) beyond the multivariate model with AST. **Conclusions:** We found that higher AST, ALT and AST/ALT ratio were all significantly associated with NASH, although AST was more strongly associated and had a higher AUROC for discriminating NASH from other forms of NAFLD. Even in the multivariable model with both AST and ALT, the probability a patient without NASH has a higher discriminating score than a patient with NASH is 26%, indicating that additional non-invasive markers of NASH are needed.

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1127 METABOLIC SYNDROME AMONG CHILDREN WITH VARIABLE FEATURES OF NONALCOHOLIC FATTY LIVER DISEASE

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**Background:** Nonalcoholic steatohepatitis (NASH) is considered the hepatic manifestation of metabolic syndrome (MetS) in adults. The relation between components of MetS and features of nonalcoholic fatty liver disease (NAFLD) in children has not been characterized. **Aim:** To determine the prevalence and correlates of MetS among children enrolled in a large multicenter NASH trial. **Methods:** Children ages 6-17 years enrolled in the NASH Clinical Research Network (CRN) with laboratory studies and anthropometry within 6 months of liver biopsy were included. The following features of MetS were evaluated: obesity (BMI >97th percentile), hypertriglyceridemia (≥5th percentile), low HDL cholesterol (<5th percentile), hypertension (HTN: systolic and/or diastolic blood pressure ≥95th percentile), and impaired glucose tolerance (IGT; serum glucose 140-199 mg/dL at 2 hours during oral glucose tolerance test). MetS was defined as the presence of ≥3 of these features. Liver biopsies were read by the NASH CRN Pathology Committee and are scored according to criteria by Kleiner et al (Hepatology 2005). **Results:** 131 children, predominantly males (78.6%), median age 12.9 years were studied. 89% were obesity, 30% had hypertriglyceridemia, 21% had low HDL, 53% had HTN, and 17% had IGT. The individual features of MetS were not associated with age, gender, race/ethnicity, or BMI status with the exception of IGT, which was less common among those of Hispanic ethnicity (10% vs. 24%, P=0.03). Overall, 32.8% of children met criteria for MetS: 3.1% had no features of MetS, 29% had 1 feature, 35.1% had 2, 22.1% had 3, and 10.7% had 4 features. Children with MetS did not differ from those without MetS in age, gender, race/ethnicity or Tanner stage but were more likely to be obese (100% vs. 83.9%, P=0.005). The histological features of NAFLD were not associated with MetS (mean ± SD values summarized in table). **Conclusions:** Features of MetS are prevalent among children and adolescents with NAFLD, 10.7% of whom had all 4 evaluated features present. Individual histological features of NAFLD did not vary according to the presence of MetS. The long-term implications of MetS, a cluster of cardiovascular risk factors in adults, needs to be determined in this age group.

<table>
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<tr>
<td>Stratosic Grease (0-3)</td>
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<td>2.1±0.9</td>
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<tr>
<td>Lobular Inflammation (0-3)</td>
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<td>1.58±0.63</td>
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<tr>
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<td>0.44</td>
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<td>NAFLD activity score (0-8)</td>
<td>4.5±1.41</td>
<td>4.5±1.55</td>
<td>0.90</td>
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Disclosures: The following people have nothing to disclose: Heather M. Patton, Ayunr Unalp, Katherine Yates, Jeffrey B. Schwimmer, Joel E. Lavine
Background: Cigarette smoking, alcohol use, diabetes (DM) and older age have all been linked with histological severity in certain chronic liver conditions. Associations between older age and DM with advanced histological stage in nonalcoholic fatty liver disease (NAFLD) have been suggested however, possible links between cigarette smoking or alcohol history with advanced liver fibrosis have not been studied in NAFLD. Aim: To study the associations between cigarette smoking history, alcohol use, DM and age with advanced fibrosis in patients with NAFLD, controlling for other patient characteristics. Methods: All adult patients enrolled in the NASH Clinical Research Network (NASH CRN) studies between February 2005 and December 2006 who had centrally read liver biopsies were included. Advanced fibrosis was defined as stage 3 or 4 by the validated NASH CRN Histological Scoring System for NAFLD. Significant smoking history was defined as a history of smoking >=10 pack-years. Alcohol consumption (abstinent or not) was derived from the Lifetime Drinking History (LDH) questionnaire based on reported history of lifetime abstinence (referred) or not, without quantification of exposure. Bivariate and multivariate logistic regression analyses were performed. Results: 666 subjects were included; 417 were female. Mean age was 48.5. Advanced fibrosis was present in 29.4%. Smoking history >=10 pack-years was reported 23% subjects. DM was present in 27% of subjects. Lifetime abstinence was reported by 50.2% of subjects. Significant bivariate associations were demonstrated between advanced fibrosis and age, significant smoking history, alcohol usage (versus lifetime abstinence), and DM. Alcohol use, DM, and age were independently associated with advanced fibrosis in the multivariate analysis (see Table); however, the association between >=10 pack-yrs smoking history and advanced fibrosis was not significant. Conclusions: Although a link between smoking and liver fibrosis has been demonstrated in some chronic liver diseases, this link was not found in NAFLD. Advanced fibrosis in NAFLD was linked to alcohol use, DM, and age. The possible association between lifetime abstinence or not and advanced fibrosis in NAFLD is an interesting observation that will be investigated further specifically regarding more detailed lifetime drinking histories.

Logistic regression model for advanced fibrosis and smoking, alcohol, DM, and age

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<th>Variable</th>
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<td>Alcohol, not abstinent vs. abstinent</td>
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<td>0.41, 0.85</td>
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<td>Age, years</td>
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<td>1.03, 1.08</td>
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1130 STUDY OF A POLYMORPHISM IN THE GLUTAMATE-CYSTEINE LIGASE (GCLC) GENE IN NONALCHOLIC FATTY LIVER DISEASE

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Background & Aims: Recently, considerable attention has been given to the oxidative stress and the defective cellular antioxidant defenses as cause of nonalcoholic fatty liver disease (NAFLD) progression. Glutamate-cysteine ligase is a heterodimer composed of a heavy catalytic subunit (GCLC) which catalyses the first and limiting step in the formation of glutathione, an important endogenous antioxidant. The -129C/T polymorphism of the GCLC gene, in which the T allele is associated with lower promoter activity, was associated with myocardial infarction in the Japanese population. In the Brazilian population, this polymorphism conferred a high risk for nephropathy development (OR=5.95; CI=2.29-15.46; p=0.0002) in patients with type 1 diabetes mellitus, a condition also associated with increased reactive oxygen species production and decreased intracellular antioxidant defenses. The frequency of this polymorphism in the GCLC gene is being preliminary investigated in NAFLD patients. Methods: Genomic DNA was extracted from peripheral blood cells and the promoter region of the GCLC gene was amplified by PCR. The GCLC -129C/T polymorphism was determined by the PCR-based restriction fragment length polymorphism (RFLP). Forty-one patients with NAFLD submitted to hepatic biopsy were divided in two groups: patients with hepatic steatosis (n=9) or steatohepatitis (NASH) (n=32), the latter defined by histologic aspects by NASH Activity Score (NAS) classification devised by the Pathology Committee of the NASH Clinical Research Network. Results: The presence of at least one T allele in the polymorphism -129 of the GCLC gene was higher in patients with NASH (9 out of 32 patients, 28%) than in patients with steatosis (0 out of 9 patients, 0%). Conclusions: Although the small sample size limited our ability to detect any statistically significant difference, further studies in larger populations are guaranteed to evaluate if the polymorphism in the GCLC gene is associated with NAFLD evolution.

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1131 INFLUENCE OF IMPAIRED GLUCOSE TOLERANCE, GLUCOSE CONTROL STATUS, LIGHT TO MODERATE ALCOHOL CONSUMPTION, AND REDUCED CARBOHYDRATE DIETS ON SERUM ALT CHANGE DURING RAPID WEIGHT LOSS IN THE RESIDENTIAL WEIGHT LOSS PROGRAM

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Background: We reported that serum ALT changes following weight loss are influenced by demography, lifestyle, and comorbidities. Rapid weight loss, smoking, sleep apnea, and glucose intolerance were adversely, while hypertriglyceridemia was favorably, associated with serum ALT response following significant weight loss [AASLD 2006]. We hypothesize that extent of fatty acid (FA) delivery to liver FA oxidizing enzymes, activity of FA oxidation, and hepatic redox status influence liver injury during significant weight loss. Aims: To determine how insulin resistance/diabetes (which increase FA delivery to liver), hyperglycemia status (which decreases mitochondrial FA oxidation), reduced carbohydrate (CHO) diets (which decrease de novo hepatic FA synthesis), smoking or alcohol use (which impact hepatic FA oxidizing enzymes and antioxidant defenses) and/or aging (which decreases antioxidant reserves) influence ALT response to rapid weight loss (which increases delivery of exogenous FA to the liver). Methods: Participants in a 4-week residential weight loss program who did not have other liver diseases, or excessive EtOH consumption (<7 women; <14 men, drinks/week) were examined (n=362). The 181 subjects whose rate of weight loss was greater than the median value (1.53 kg/week) were further analyzed. Putative predictor variables and ALT were collected at the baseline. ALT and body weight were re-evaluated at the end of the program. The associations of ALT deterioration (≥10 IU/L increase) with age, BMI, rate of weight loss, baseline ALT, comorbidities including diabetes mellitus/impaired fasting glucose (DM/IFG), serum glucose, high HbA1c (≥7%), current smoking, alcohol use (servings/week), reduced CHO diet during the program (CHO <35% dietary kcal) were assessed by multivariable logistic regression. Results: Total weight loss was 7.7+3.0 kg (2.36±0.67 kg/week). Of this subset, 20% showed ALT deterioration. Faster weight loss (OR=4. for 1 unit increase, p<0.01) and DM/IFG (OR=4.6, p<0.02) were associated with increased odds, while older age (OR=0.8 for 5 units increase, p<0.02), reduced CHO diets (OR=0.1, p<0.04), and possibly up to moderate alcohol consumption (OR=0.8 for 1 unit increase, p=0.08) were associated with decreased odds of ALT deterioration. Conclusions: Findings suggest that among rapid weight losers, ALT deterioration associates with factors that increase hepatic exposure to exogenous FA (faster weight loss, DM/IFG), while factors that reduce hepatic FA synthesis (reduced CHO diets) or induce FA oxidation/antioxidant defenses (light to moderate alcohol consumption) may be protective.

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1132
NON-ALCOHOLIC FATTY LIVER DISEASE IS ASSOCIATED WITH TRANSSCRIPTIONAL DYSREGULATION OF THE CORTICOSTEROID METABOLISING ENZYME 11βHSD-1 LEADING TO EXCESS INTRAHEPATIC STEROID EXPOSURE
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Background Glucocorticoids play a key role in determining body fat distribution. Excess circulating glucocorticoids lead to intrahepatic fat accumulation. Obesity is associated with enhanced steroid production but this is offset by enhanced degradation such that circulating steroid concentrations are normal. However, tissue-specific pathways may lead to different degrees of cortisol exposure in different organs. Local steroid exposure in the liver is determined by the activity of the enzyme 11 beta hydroxysteroid dehydrogenase type 1 (11βHSD-1) which converts inactive cortisone to active cortisol. Studies in ‘healthy’ individuals show a reduction in the activity of 11βHSD-1 with increasing BMI thus protecting the liver from the effects of excess cortisol. We tested the hypothesis that in obese people with non-alcoholic fatty liver disease (NAFLD) this adaptive response is impaired leading to enhanced local production of cortisol and steroid-induced steatosis. Methods We measured insulin resistance (HOMA-IR), % body fat (by DXA) and 11βHSD-1 activity/expression in patients with NAFLD/NASH undergoing biopsy and correlated these with the histological features. Global 11βHSD-1 activity was determined by analysis of urinary excretion of cortisol metabolites, with results expressed as a ratio of tetrahydrocortisols (THF, allo THF) over tetrahydrocortisone (THE). Intrahepatic 11βHSD-1 mRNA levels were determined in liver tissue by real-time PCR. Control patients had no-steatotic liver disease (chiefly hepatitis B, HCV patients were excluded). Results 11βHSD-1 activity was significantly higher in the 15 individuals with NAFLD (0.73±0.04) vs controls (0.57±0.03), p<0.02. In the 10 control patients 11βHSD-1 activity decreased with increasing BMI but this was not seen in patients with NAFLD/NASH. These changes in 11βHSD-1 activity were mirrored by changes in mRNA expression of 11βHSD-1 in liver tissue, suggesting that the changes were due to dysregulation of enzyme RNA expression. Thus in obese individuals with fatty liver disease 11βHSD-1 does not down regulate appropriately and this may be due to transcriptional abrogation that may play a role in the development of hepatic steatosis.

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The following people have nothing to disclose: F. M. Coyle, N. F. Taylor, J. P. Monson, W. M. Drake, G. R. Foster

1133
NORMAL ALT IN NAFLD SHOULD NOT PRECLUDE LIVER BIOPSY
Anna Ludovica Fracanzani1, Luca Valenti1, Elisabetta Bugianesi2, Giulia Marchesini3, Marco Andreolletti4, Agostino Colli, Cristina Bertelli, Erika Fatta, Silvia Fargion

Patients with NAFLD have an excess mortality for hepatic and extrahaepatic diseases. Some patients with NAFLD have normal ALT levels and it is uncertain whether this reflects a milder disease and whether these patients should undergo liver biopsy. The aim of this study was to compare clinical presentation and histological findings of patients with NAFLD with and without increased ALT. We reviewed a clinical database of Italian patients with NAFLD followed in four liver units (455 cases), who underwent liver biopsy between January 2002-December 2005, referred for abnormalities in liver function tests, dyslipidaemia, hyperferritinemia or for liver steatosis detected by US for non liver related causes. Patients were divided in two groups according to persistently normal ALT values (60 cases) and increased ALT (395 cases). Persistent hyperferritinemia, increased fasting glucose, impaired glucose tolerance or diabetes were the main reasons to biopsy patients with normal ALT. Patients with normal ALT were significantly older (48±10, 43±11 yrs, p=0.001), had significantly lower BMI (23.3±4, 27.4±3.7 Kg/m2, p=0.0001), triglycerides (124±70, 150±87 mg/dl, p=0.02), fasting insulin (13.0±7.3, 19.1±14.8, mIU/l p=0.002), HOMA-IR (3.0±2.0, 4.7±4.7, p=0.006), ALT (29±7, 84±44 IU/l, p=0.0001), and GGT(66±62, 101±14 IU/l, p=0.03), but higher prevalence of impaired glucose tolerance (16%, 8%, p=0.03) and hypertension (33%, 21%, p=0.03). NASH was diagnosed in 62% and 74% (p=0.04) patients with normal or increased ALT. No significant difference in advanced fibrosis (≥2) was observed in patients with (21%) or without (15%) increased ALT. Patients with normal ALT had significantly milder inflammation. At univariate analysis in patients with increased ALT variables significantly associated with fibrosis ≥2 were age (p=0.001), BMI (p=0.01), fasting glucose (0.002), ALT (0.01), ferritin (p=0.001), HOMA-IR (p=0.04) and prevalence of diabetes (p=0.01), while in patients with normal ALT were age (p=0.05), diabetes (p=0.03) and HOMA-IR (p=0.03). At logistic regression analysis, performed only in patients with increased ALT for the small number of patients with normal ALT, serum ferritin (p=0.03), age (p=0.02), ALT (p=0.005), and diabetes (p=0.01) remained independently associated with fibrosis. In the overall series, patients with higher ferritin and ALT level and diabetes were at higher risk to have NASH. In conclusion, our data indicate that also patients with normal ALT should undergo liver biopsy particularly in the presence of diabetes, for an early diagnosis of fibrosis and prevention of progression of hepatic and extrahaepatic diseases.

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The following people have nothing to disclose: Anna Ludovica Fracanzani, Luca Valenti, Elisabetta Bugianesi, Giulia Marchesini, Marco Andreolletti, Agostino Colli, Cristina Bertelli, Erika Fatta, Silvia Fargion

1134
ULTRASTRUCTURAL EVALUATION OF HEPATOCELLS WITH TYPE 2 MALLORY-DENK BODIES: CLUES TO BALLOONING IN NONALCOHOLIC STEATOHEPATITIS (NASH)
Stephen H. Caldwell1, Jan A. Redick2, Christine A. Davis2, Elizabeth M. Brunt3, Vicencia M. Lima3, Curtis K. Argo1, Abdullah M. Al-Osaimi1, Brent A. Tehrani1, GI/Hepatology, University of Virginia, Charlottesville, VA; 2Advanced Microscopy Facility, University of Virginia, Charlottesville, VA; 3Pathology, Saint Louis University, Saint Louis, MO; 4GI/Hepatology, Saint Louis University, Saint Louis, MO; 5GI/Hepatology, University of Sao Paulo, Sao Paulo, Brazil

Mallory-Denk bodies (MDB), or Mallory’s hyaline, are a common but less frequently encountered form of cell injury in NASH. MDB are often associated with hepatocyte ballooning by light microscopy (Denk et al J Pathol 2006;208:653-661). Although ballooning is one of the most significant light micro-
scopic (LM) findings in NASH, its mechanisms and ultrastructural definition remain controversial (Caldwell et al Am J Gastro 2006;101:1677). We recently completed a prospective, systematic transmission electron microscopic (TEM) survey of 40 liver biopsy specimens from 20 NAFLD patients in which we examined 1,597 grid holes - 40 grid holes per biopsy except one biopsy in which 37 gridholes were examined (Caldwell et al, Hepatology in press). Aim: This systematic study afforded us the opportunity to examine cells containing MDB and consider their possible relationship with other intracellular alterations that may relate to hepatocyte ballooning in NASH. Methods: Grid holes previously identified as containing hepatocytes with well-formed type 2 MDB (irregular intracytoplasmic aggregates of randomly oriented filaments of varying diameter) were identified and reviewed for the following characteristics: hepatocyte diameter >30µ, microsteatosis, dilation of endoplasmic reticulum, and mitochondrial alterations, i.e., crystalline inclusions. Results: MDBs were identified in 6 biopsies. Hepatocytes >30µ were present in 3 of these in association with MDB. The cytoplasm surrounding the MDB contained small droplet fat in 5 of 6 including all of the cells that were also >30µ. Dilated endoplasmic reticulum, often prominent at the border of the MDB, was evident in 5 of 6 samples also including all of those with hepatocytes >30µ. Crystal containing mitochondria were evident in two of six of the samples; neither of which were associated with hepatocytes >30µ. Conclusions: Mallory-Denk bodies occur in both normal sized and enlarged hepatocytes in human NASH. They are often associated with small droplet fat and dilated endoplasmic reticulum, especially in hepatocytes >30µ in diameter. Because MDBs are most easily identified in ballooned cells by LM, and these cells have rarefied cytoplasm, these associations suggest that ballooning in NASH may in part derive from a combination of small droplet fat and dilated ER. We hypothesize that this relationship results from oxidative injury in the rim of the ER-derived small fat droplets (Ikura et al Hepatology 2006;43:506-14) which disrupts trafficking of these droplets and impedes endoplasmic reticulum function.

Disclosures:
The following people have nothing to disclose: Stephen H. Caldwell, Jan A. Redick, Christine A. Davis, Elizabeth M. Brunt, Vicentia M. Lima, Curtis K. Argo, Abdullah M. Al-Osaimi, Brent A. Tetri

1135 RELATIONSHIP OF BMI AND THE METABOLIC SYNDROME IN NON-DIABETIC PATIENTS WITH NAFLD

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Background: The metabolic syndrome (MetS) and nonalcoholic fatty liver disease (NAFLD) are both associated with obesity and increasingly common in the USA. Aim: The aim of this study was to examine the relationship between BMI and MetS in non-diabetic patients enrolled in NASH Clinical Research Network (CRN) studies. Methods: Clinical, demographic, histologic, laboratory and anthropometric data were collected on 356 adult (≥18 yrs) non-diabetic patients with NAFLD. Patients were classified by BMI as follows: ≤24.9-normal weight; 25.0-29.9-overweight; ≥ 30.0-obese. The MetS was determined using the WHO criteria to include three or more of the following features: elevated waist circumference, elevated triglycerides, reduced HDL cholesterol, hypertension and elevated fasting blood glucose. Results: The majority of patients were obese (71%). The prevalence of the MetS was 67%. Obesity was significantly more common among patients with MetS compared to those without MetS (80% vs 52% respectively; p<0.001). The most common feature among MetS patients was elevated waist circumference (93%) followed by hypertension (85%). By contrast, elevated fasting blood glucose was present in only 27% of MetS patients; however, fasting insulin (27.2 vs 21.6 µU/ml, p<0.001), C peptide (4.6 vs 3.3 mg/dl, p<0.001) and HOMA IR (6.5 vs 4.9, p<0.001) were all significantly higher among those with MetS compared to those without MetS. The proportion of MetS patients with mild steatosis (5-33%) was lower compared to those without MetS and greater with severe steatosis (≥66%) suggesting a trend for greater severity (p=0.04). Chronic portal inflammation was more common among those with MetS compared to those without MetS (85% vs 74%; p=0.01). There was no significant difference in the NAFLD activity score or other histologic parameters nor the prevalence of NASH between the two groups (61% MetS vs 53% non-MetS p=0.12). Conclusions: Obesity and MetS are common among non-diabetic patients with NAFLD. These patients have greater insulin resistance compared to non-diabetic NAFLD patients without MetS based on higher fasting blood glucose, HOMA-IR, and fasting insulin and C peptide values. Patients with MetS tended to have more severe hepatic steatosis and chronic portal inflammation compared to those without MetS. However, presence of MetS was not associated with higher NAFLD activity score or fibrosis stage. These data suggest that the clinical and histologic features of non-diabetic NAFLD patients with MetS may be different than in those without MetS. Routine evaluation for MetS should be considered in non-diabetic NAFLD patients and may help guide diagnosis and therapy.

Disclosures:
The following people have nothing to disclose: James e. Nelson, Katherine Yates, Aynur Unalp, Kris V. Kowdley

1136 FATIGUE IN NAFLD IS SEVERE AND ASSOCIATES WITH EXCESSIVE DAYTIME SOMNOLENCE BUT NOT WITH LIVER DISEASE SEVERITY OR INSULIN RESISTANCE

Julia L. Newton, David E. Jones, Lara Kane, Elisbeth Henderson, Jessie Pairman, Katharine Wilton, Alastair D. Burt, Christopher P. Day; Institute for Cellular Medicine, Newcastle University, Newcastle, United Kingdom

Anecdotal reports suggest that patients with non-alcoholic fatty liver disease (NAFLD) experience disproportionate levels of fatigue. To date, there has been no attempt to quantify fatigue in NAFLD, to determine the extent to which perceived fatigue reflects true impairment of physical function and to examine the potential causes of this important symptom. We addressed each of these questions in a series of linked studies performed in a fully characterised patient cohort (156 NAFLD patients in total) using optimal methodologies. In phase 1; we explored the degree of fatigue experienced by NAFLD patients (assessed using the fatigue impact scale (FIS) a fully validated fatigue impact assessment tool) in comparison to normal and liver disease controls and its relationship to actual impairment of physical function. Mean FIS was markedly higher in NAFLD patients than in fully age, sex and BMI matched controls (mean FIS 51±38 (possible range 0-160) v 8±12, p<0.0001). NAFLD patients showed significantly lower levels of physical activity than normal controls over 6 days of physical activity monitoring (Actigraphy) (7089±2909 mean steps per day v 8676±2894, p<0.02). Significant inverse correlation was seen between FIS and physical activity (r2=0.1, p=0.02). Fatigue experienced by an appropriately case-matched subgroup of NAFLD patients was similar to that in a group of PBC patients (n=36) [FIS 64±9 v 61±2, p=ns]. Limitation in physical activity was also similar in

Diseases: EXCESSIVE DAYTIME SOMNOLENCE BUT NOT WITH LIVER DISEASE SEVERITY OR INSULIN RESISTANCE

Julia L. Newton, David E. Jones, Lara Kane, Elisabeth Henderson, Jessie Pairman, Katharine Wilton, Alastair D. Burt, Christopher P. Day; Institute for Cellular Medicine, Newcastle University, Newcastle, United Kingdom

Anecdotal reports suggest that patients with non-alcoholic fatty liver disease (NAFLD) experience disproportionate levels of fatigue. To date, there has been no attempt to quantify fatigue in NAFLD, to determine the extent to which perceived fatigue reflects true impairment of physical function and to examine the potential causes of this important symptom. We addressed each of these questions in a series of linked studies performed in a fully characterised patient cohort (156 NAFLD patients in total) using optimal methodologies. In phase 1; we explored the degree of fatigue experienced by NAFLD patients (assessed using the fatigue impact scale (FIS) a fully validated fatigue impact assessment tool) in comparison to normal and liver disease controls and its relationship to actual impairment of physical function. Mean FIS was markedly higher in NAFLD patients than in fully age, sex and BMI matched controls (mean FIS 51±38 (possible range 0-160) v 8±12, p<0.0001). NAFLD patients showed significantly lower levels of physical activity than normal controls over 6 days of physical activity monitoring (Actigraphy) (7089±2909 mean steps per day v 8676±2894, p<0.02). Significant inverse correlation was seen between FIS and physical activity (r2=0.1, p=0.02). Fatigue experienced by an appropriately case-matched subgroup of NAFLD patients was similar to that in a group of PBC patients (n=36) [FIS 64±9 v 61±2, p=ns]. Limitation in physical activity was also similar in
the matched NAFLD patients to that seen in PBC patients (mean steps 7501±1570 vs 7565±2905, p=ns). In phase 2; we examined the biological associations of fatigue in NAFLD. No association was seen between FIS and albumin, bilirubin, alkaline phosphatase, ALT or AST (all r² < 0.005), histological fat score (r² = 0.02), necroinflammatory score (r² = 0.01), fibrosis score (r² = 0.001) or HOMA (r² < 0.005). Significant association was, however, seen between fatigue severity and degree of daytime somnolence assessed using the Epworth Sleepiness Scale (r² = 0.2, p < 0.0001). In conclusion, we have demonstrated that fatigue is a significant problem in NAFLD, is similar in degree to that seen in PBC patients and reflects a true impairment in functional function. Fatigue in NAFLD appears to be unrelated to either severity of underlying liver disease (in terms of either inflammation or fibrosis) or insulin resistance but is associated with significant daytime somnolence. Further study will be required to determine whether the association with sleep disturbance reflects the presence of obstructive apnoea and the degree to which treatments aimed at reducing daytime somnolence are also effective at reducing fatigue in NAFLD.

Disclosures:  
The following people have nothing to disclose: Julia L. Newton, David E. Jones, Lara Kane, Elsbeth Henderson, Jessie Pairman, Katharine Wilton, Alastair D. Burt, Christopher P. Day

1137 INFLUENCE OF ADIPONECTIN GENE POLYMORPHISMS IN PATIENTS WITH NONALCOHOLIC FATTY LIVER DISEASE  
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(Aim) Adiponectin is one of the adipo-cytokines associated with insulin resistance, type-2 diabetes, liver fibrosis and the pathogenesis of nonalcoholic fatty liver disease (NAFLD). Single nucleotide polymorphism (SNP) of adiponectin gene has been reported to be associated with insulin resistance and the prevalence of type-2 diabetes. Therefore, we investigated the SNPs of adiponectin, comparing them with the respective clinical and pathological findings. In addition, we also investigated the associations between the SNPs of adiponectin gene and insulin resistance, and plasma adiponectin levels. (Patients and Methods) 106 patients were histologically diagnosed as having NAFLD. The diagnosis of NAFLD was based on three criteria; 1) histologically macrovesicular steatosis affecting at least 10% of hepatocytes, 2) intake of less than 20 g of ethanol per day, 3) appropriate exclusion of other diseases such as alcoholic liver disease, viral hepatitis, autoimmune hepatitis, and metabolic liver diseases. Adiponectin SNP sites were investigated at +45 of Exon 2, +276 of Intron 2, +112 of Exon 3 and +164 of Exon 3, sites indicated as being associated with diabetes or metabolic diseases. Adiponectin SNP sites were investigated at +45 of Exon 2, +276 of Intron 2, +112 of Exon 3 and +164 of Exon 3, sites indicated as being associated with diabetes or metabolic diseases. (Results) 1. Regarding +276 SNP, the frequency of G/G (57.3%) in NAFLD patients tended to be higher than that of control (50.9%), but the difference was not significant. In females only, the G/G frequency (59.6%) was significantly higher than in female control (37%). In addition, the G/G frequency of the severe-fibrosis group was 63.2%, indicating a higher frequency with fibrosis severity. 2. As for +45 SNP, the frequency of T/T (45.6%) in NAFLD patients tended to be lower than that of control, but the difference was not significant. In G/G homozygotes, the frequency of severe fibrosis was significantly higher than that of G/T and T/T genotypes (p=0.003). 3. Regarding +112 of Exon 3 and +164 of Exon 3 SNPs, there were no differences between NAFLD and control. 4. In NAFLD patients with adiponectin +45 G/G, the frequency of HOMA-2 was significantly increased compared with NAFLD patients without adiponectin +45 G/G. 5. In NAFLD patients with adiponectin +276 G/G, plasma adiponectin levels tended to be lower than in those without G/G. In NAFLD patients with adiponectin +45 T/T, plasma adiponectin levels tended to be higher than in those without T/T. (Conclusion) The frequencies of adiponectin SNP sites were significantly different between NAFLD and control in only females. In addition, associations between adiponectin SNP and progression of liver fibrosis and insulin resistance were suggested. In male patients with NAFLD, life style and some other genes might be involved.

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1138 HISTOLOGICAL CHARACTERISTICS OF PATIENTS WITH ‘CRYPTOGENIC’ CIRRHOSIS AND PRIOR BIOPSY SHOWING NASH  
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Prior serial biopsy studies have shown that the course of nonalcoholic steatohepatitis (NASH) may include a late stage in which steatosis decreases to the point that NASH is absent or evident only by residual markers of fatty liver disease. However, patients with prior biopsy-proven NASH are seldom re-biopsied if they present with complicated cirrhosis and patients presenting with advanced cirrhosis often lack prior pathological data to prove the existence of antecedent NASH. Aim: To expand the existing data regarding NASH and ‘cryptogenic’ cirrhosis, we sought to further characterize late stage histology in patients with prior biopsies showing unequivocal non-cirrhotic NASH. Methods: Patients were included who had at least two serial biopsies with the initial biopsy demonstrating non-cirrhotic NASH and subsequent biopsies showing cirrhosis without definitive findings of NASH. Controls were chosen from patients with serial biopsies showing NASH without progression to cirrhosis. Biopsy specimens were evaluated in a blinded fashion by a hepatopathologist using the NASCHR-CRN system. Results: 11 biopsy specimens from the 4 patients meeting criteria were included. All patients had a history of ongoing obesity and 3 of 4 were diabetic although only one had a history of thiazolidinedione (TZD) therapy. 3 were female and the age range at the latest biopsy was 41 – 63 yrs. The time between the first and last biopsy was 4 – 14 yrs. Although steatosis declined to trace (<5%) in 3 of 4, residual histological features characteristic of NASH including ballooning (4 of 4), lobular inflammation (4 of 4), Mallory-Denk Bodies (3 of 4) and glycogenated nuclei (2 of 4) were evident in the final specimens. One patient underwent liver transplantation (LT) and we documented progression from ‘NASH with cirrhosis’ on the explant to recurrent NASH with fibrosis 6 years post LT, to incomplete cirrhosis (Ishak stage 5) with only trace steatosis 14 years post transplant. Conclusions: Our results confirm that NASH can progress to a more bland histopathologic entity consistent with prior descriptions of ‘cryptogenic’ cirrhosis. The results also support histological characteristics of late stage NASH that have been used in classification schemes of cryptogenic cirrhosis (Contos, Liver Transpl 2001;7:363 and Ayata, Hum Pathol 2002;33:1098) including minimal steatosis, cellular ballooning, Mallory-Denk bodies, residual lobular inflammation and glycogenated nuclei. We further document post-transplant
recurrence of NASH with progression from histological NASH (year 6 post-LT) to cirrhosis with minimal steatosis (year 14 post-LT).

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The following people have nothing to disclose: Vanessa D. Lee, David Kleiner, Abdullah M. Al-Osaimi, Curtis K. Argo, Patrick G. Nortrup, Carl L. Berg, Timothy L. Pruett, Timothy M. Schmitt, Stephen H. Caldwell

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NAFLD AS A RISK FACTOR FOR THE DEVELOPMENT OF DIABETES AND THE METABOLIC SYNDROME: AN ELEVEN YEAR FOLLOW-UP STUDY
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Objectives: Hepatic steatosis increases hepatic insulin resistance and may therefore predispose to the development of diabetes and the metabolic syndrome. We sought to determine whether nonalcoholic fatty liver disease (NAFLD) with elevated aminotransaminase (ALT) levels was a risk factor for diabetes or the metabolic syndrome (MS) over an eleven year period. Methods: Adult residents of Busselton, Western Australia were assessed in 1994/5 as part of the Busselton Health Survey. NAFLD was diagnosed on the basis of a raised ALT (>40 IU/l) after the exclusion of alcohol, drug toxicity, viral, metabolic and autoimmune liver disease by clinical and serological assessment. Both NAFLD and non-NAFLD subjects were re-visited in 2005 for liver complications, diabetes (fasting glucose >7.0 mmol/l or history) and the metabolic syndrome (MS) as determined by ATPIII criteria. Results: 358 subjects, 68% male, (109 NAFLD, 249 non-NAFLD), mean age (SD) 59.9 (11.6) years, attended follow-up 11.1 years after initial assessment. In 1994/5, those with NAFLD were more likely to have diabetes (fasting glucose >7.0 mmol/l or history) and the metabolic syndrome (MS) (P<0.05 for all comparisons). Non-NAFLD subjects who developed elevated ALT levels on follow-up compared to those whose ALT remained normal, tended to gained more weight (BMI 2.6 (3.0) ± 1.5 (1.9) kg/m2, p=0.059) and had more progressive insulin resistance [HOMA 1.0 (1.4) vs. 0.2 (1.0), p=0.01] between assessments. After excluding subjects with diabetes at baseline, those with NAFLD were more likely to develop diabetes on follow-up (20/106 [18.9%] vs. 15/246 [6.1%], p=0.001). Furthermore, after excluding subjects with MS at baseline, those with NAFLD were more likely to develop MS at follow-up (27/81 [33.3%] vs. 51/226 [22.6%], p=0.056). Using logistic regression analysis, a diagnosis of NAFLD in 1994/5 was also a significant predictor of developing diabetes (r=0.2, p<0.001) and the metabolic syndrome (r=0.58, p=0.058) in 2005. However, a baseline diagnosis of NAFLD became non-significant after adjusting for baseline waist circumference, hypertension and insulin resistance as determined by HOMA. No subjects developed liver complications over the 11 year period. Conclusion: Subjects with NAFLD and elevated ALT levels are at increased risk of developing diabetes and the metabolic syndrome. However this may be due to the presence of associated metabolic risk factors such as central obesity, insulin resistance and hyperglycemia.

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ETHNIC AND GENDER DIFFERENCES IN LIVER HISTOLOGY IN OBESITY SURGERY PATIENTS
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There are limited data comparing liver histology by ethnicity in patients at risk for NASH, and Hispanics have not been represented in the available series of adult patients. The aim of the current study was to compare liver histology as a function of race and gender in a large, ethnically diverse population of obesity surgery patients. Methods: Case records were reviewed retrospectively from a surgeon who routinely performed liver biopsies during gastric bypass operations over a two year period. All biopsies were scored by a liver pathologist, masked to clinical data, using the NASH Activity Score (Hepatology 2005;41:1313-21). Clinical features were compared by chi square tests for categorical variables and ANOVA or t-tests for continuous variables. Results: 123 of 141 consecutive patients had available clinical data, liver histology, and no evidence of a cause of liver disease other than NAFLD. The ethnic distribution was 46% Non-Hispanic White, 44% African American and 11% Hispanic, by patient self report. There were no significant differences in age, or prevalence of diabetes, hypertension, or hyperlipidemia among the ethnic groups. The mean BMI (kg/m2) was greater in African Americans (55 ± 8), and Non-Hispanic Whites (55 ± 12), than in Hispanics (47 ± 16) [p=0.03]. Overall, 85% of liver biopsies had >5% steatosis and 33% had evidence of NASH (NAS ≥ 5). Steatosis was present in 93% of Non-Hispanic Whites, and 92% of Hispanics, compared to 74% of African American patients (p=0.02). Histologic evidence of NASH was greatest in Hispanics (62%), followed by Non-Hispanic Whites (36%), and African Americans (22%) [p=0.02]. There were no significant differences in lobular inflammation, hepatocellular ballooning, or advanced fibrosis (≥ F2) among ethnic groups, although the number of Hispanic patients was relatively small. Further analysis focused on gender differences. Seventy-six percent of patients were women. The prevalence of steatosis was higher in men (97%) than in women (81%) [p=0.03], although the prevalence of NAS in women was significantly higher in men (34%) and women (32%). Notably, advanced fibrosis (≥ F2) was observed in 48% of men compared to only 22% of women (p=0.007). There were no significant differences in lobular inflammation or hepatocellular ballooning by gender. Conclusions: Examination of liver histology in a large, ethnically diverse group of obesity surgery patients showed that: 1) Hispanics had the highest prevalence of NASH, despite the lowest mean BMI, 2) African Americans had the lowest rates of steatosis and NASH, and 3) men had a higher prevalence of steatosis and advanced fibrosis scores (≥ F2).

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The following people have nothing to disclose: Eric R. Kallwitz, Grace Guzman, Veronica Tencate, Murdula Kumar, Jamie L. Berkes, Joseph Vitello, Neelima Nadella, Roshan Patel, Thomas J. Layden, Scott Cotler.
INTRODUCTION: Retinol-binding protein 4 (RBP4) has recently been proposed as a new "adipokine" that regulates insulin action in muscles and liver, contributing to insulin resistance. Several studies suggested that RBP4 may play a key role in the pathogenesis of insulin resistance and type 2 diabetes. Non alcoholic fatty liver disease (NAFLD) is related to insulin resistance and obesity. We examined for the first time RBP4 levels in serum and liver in patients with NAFLD.

METHODS: 30 Caucasian patients with biopsy proven NAFLD were included in the study. They had persistently elevated aminotransferases, absence of any other liver disease and alcohol consumption <20gr/d. None had diabetes. 30 healthy controls, matched for age and sex, had been evaluated with a standard oral glucose tolerance test (OGTT). They had normal liver enzymes and abdominal ultrasound and no history of chronic liver disease. None had type 2 diabetes. BMI, ALT, fasting glucose and insulin line, OGTT and homeostasis model assessment of insulin resistance (HOMA-IR) were evaluated. RBP4 was measured by ELISA (AdipoGen). RBP4 expression in liver was examined by immunohistochemistry using a polyclonal antibody to human RBP4 and was semiquantitatively evaluated. Histology specimens were compared with ten normal liver biopsies.

RESULTS: NAFLD patients had significantly higher BMI, HOMA-IR and fasting insulin than controls. Serum RBP4 was significantly lower in NAFLD than in controls (26.14 vs 38.05 µg/mL, p<0.001). There was no significant difference of serum RBP4 between controls with normal and impaired OGTT (36.85 vs 39.25 µg/mL). No difference in serum RBP4 levels was found between patients with simple steatosis and patients with NASH (25.14 vs 25.2 µg/mL). Anti-RBP4 produced granular cytoplasmic hepatocellular immunostaining, which in normal liver was weak and evident in zone 3. In NAFLD cases, RBP4 expression was strongly intense in zone 3 and milder in zones 2 and 1 or perisinusoidal areas. RBP4 score was higher in NAFLD compared to normal liver and was correlated with the grade of steatosis (p<0.01). In NASH, RBP4 score was positively correlated with grade of activity and stage of fibrosis. There was no correlation of RBP4 score with age, serum ALT, BMI or OGTT.

CONCLUSIONS: In patients with NAFLD: a) serum RBP4 levels were significantly lower as compared to controls despite the presence of higher BMI, HOMA-IR and fasting insulin, b) serum RBP4 levels did not correlate with NAFLD disease activity. In contrast patients with NAFLD showed a more intense liver expression of RBP4 compared to controls that was related to grade of steatosis, grade of activity and stage of fibrosis.

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NON-ALCOHOLIC FATTY LIVER DISEASE IN CHINESE DOES LEAD TO PROGRESSIVE DISEASE

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Background: Non-alcoholic fatty liver disease (NAFLD) is an important cause of chronic hepatitis and liver cirrhosis in the West. However, the natural history of NAFLD in Chinese is uncertain. Unlike chronic hepatitis C virus (HCV) infection, the outcome of patients with chronic hepatitis B virus (HBV) infection in the presence of NAFLD is unknown. Aims: To investigate the outcome of Chinese patients with biopsy proven NAFLD compared with chronic HBV infected patients with or without co-existing NAFLD. Methods: Consecutive NAFLD and chronic HBV patients with baseline liver biopsy performed from 1998 to 2002 were included. Patients with liver cirrhosis on liver biopsy or hepatocellular carcinoma on ultrasound at the time of liver biopsy were excluded. Clinical and laboratory parameters related to their liver function and metabolic syndromes were recorded and analyzed. Liver biopsies were scored for degree of steatosis, necroinflammation and fibrosis ( Ishak fibrosis score). Patients were follow-up for a median of 73.1 (range 24.7-109.2) months after liver biopsy. The occurrence of liver complications was recorded. Results: Two-hundred and fifty-eight consecutive patients were studied (92 with chronic HBV, 68 with chronic HBV infection and NAFLD and 98 with NAFLD). Patients with NAFLD had a higher baseline serum bilirubin [median (range) 21 (4-50) vs. 13 (2-56) vs. 11 (4-51) umol/L respectively, p=0.01] and lower baseline platelet level [median (range) 155 (135-364) vs. 185 (139-353) vs. 192 (160-359) k/mm3 respectively, p=0.02] when compared with the chronic HBV and chronic HBV with NAFLD groups. The NAFLD group was also older when compared to the other 2 groups [median (range) 53 (25-86) vs. 38 (17-66) vs. 42 (21-69) years respectively, p=0.02]. There was no significant difference in the baseline liver necroinflammation and fibrosis score among the 3 groups. At the end of follow-up, 20 of the 258 patients (7.8%) had liver complications; 10 patients with uncompensated liver cirrhosis and 10 patients with hepatocellular carcinoma. On Cox proportional hazards analysis, NAFLD group were as likely to develop liver complications as those with chronic HBV infection and chronic HBV infection with NAFLD [8 of the 98 patients (8.2%) vs. 4 of the 92 patients (4.3%) vs. 8 of the 68 patients (11.8%) respectively, p=0.10]. Conclusion: NAFLD is a progressive liver disease in Chinese and more attention should be paid to the diagnosis of NAFLD in Chinese. However, unlike chronic HCV infection the presence of co-existing steatosis does not increase progression of liver disease in chronic HBV patients.

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NON-ALCOHOLIC FATTY LIVER DISEASE IN NON-OBESE AMERICAN CHEMICAL WORKERS

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Background: Occupational exposure to monomeric vinyl chloride (VC) is a well documented risk factor for the development of the rare liver tumor, angiosarcoma, but it has not been associated with NAFLD. Here, we document a high prevalence of advanced NAFLD in non-obese American VC workers. Following the identification of a cluster of hepatic angiosarcoma in VC workers at a single plant in Kentucky in 1974, a liver cancer screening program was begun. Between 1974 and 1976, baseline liver biopsies were obtained from 82 workers with exceptionally high VC exposures. Although NAFLD was not recognized until 1980, the original pathologist documented the presence of “fatty metamorphosis” and “lipoid granulomas”. We reviewed the original pathology reports and clinical data to calculate the prevalence of NAFLD in this group. The original slides from 26 of the 82 workers were available to be over-read by a single expert liver pathologist who, when relevant, graded and staged them by the Brunt criteria. Fatty liver disease was present in 46 workers (56%). They were all white males with an extremely high mean cumulative vinyl chloride exposure of 13,000 PPM-Yr (+5950, s.d.). Their mean age was 45 (+8) and mean body mass index was 26.6 kg/m2 (+4.05). While 10 of these employees reported at least some alcohol intake, only 3 consumed 3 or more drinks daily. Liver chemistries including mean AST (30.6±25.9 IU/L) and ALT (30.9±45.6 U/L) were usually normal. Although the mean serum hyaluronic acid (34±44 µg/L) was normal in most workers with fatty liver, it was elevated in 4 workers who either had or went on to develop angiosarcoma (269±333 µg/L). In the 26 over-read biopsies, the prevalence of hepatic steatosis was 81% and the prevalence of steatohepatitis (NASH) was 77%. While all biopsies originally read as “fatty metamorphosis” had NASH, others without this finding actually had mild NASH. The liver biopsies demonstrated typical morphologic features of NASH including macrovesicular steatosis, Mallory’s hyaline, ballooning degeneration, lipogranulomas, and inflammatory infiltrate. Sinusoidal dilation, an atypical finding for NAFLD, was noted in 80% of cases. 55% of NASH biopsies demonstrated fibrosis and 1 employee had cirrhosis. Three employees with NASH had repeat liver biopsies in 1-6 years. Two of the 3 showed worsening inflammation and fibrosis despite removal from the workplace. This group of American VC workers had a high prevalence of advanced NAFLD unexplained by obesity. This study highlights a potential role for industrial toxins in the development of NAFLD.

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A NEW APPROACH IN THE TREATMENT OF PATIENTS WITH NASH—RESULTS OF A PILOT STUDY

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BACKGROUND: Trimetazidine (Tmz) is a metabolic anti ischemic drug largely used in cardiology to treat angina. Ear-
lier studies using animal models have shown that Tmz produces a shift from mitochondrial fatty acid beta-oxidation to glucose oxidation, associated with down regulation of PPARalpha. NASH, usually associated with obesity, diabetes, hyperlipidemia, hyperinsulinemia and insulin resistance could benefit from the improvement in oxidative stress and ischemic conditions by Tmz. AIM: Trial of Tmz in the treatment of patients with NASH, on the hypothesis of improving liver histology and insulin resistance. METHODS: 24 histologically confirmed cases of NASH received Tmz 70 mg/day (gr.1), for 6 months, compared to 20 patients with NASH (gr.2) receiving placebo (vitamin E) in a randomised fashion. Liver morphometry, oral glucose tolerance test, plasma insulin, insulin sensitivity, aminotransferases, glucose, triglyceride, cholesterol, were followed up before and after 6 months. RESULTS: Elevated fasting serum glucose found in 10/24 patients from gr.1 and 8/20 from gr.2 and impaired glucose tolerance in 12/24 patients from gr.1 and 10/20 from gr.2, normalized in 83% of Tmz treated patients compared to none in placebo. Aminotransferases diminished by 75% in Tmz group vs. 22%. Hyperinsulinemia recovered in all patients from gr.1 vs.10% in gr.2, also insulin /glucose ratio (0.5 vs. 0.92) and hypertriglyceridemia (in 50% patients from gr.1). Liver morphometry index [grading and staging by rebiopsy after 6 months] improved in 75% of patients receiving Tmz vs. .10% in placebo, with no side effects. CONCLUSION: Trimetazidine seems to be a promising agent to ameliorate ischemic conditions in NASH, improving ATP level and oxidative stress. The treatment provided substantial metabolic and clinical benefits in patients with NASH, with respect to liver inflammation and fibrosis, diabetes, insulin resistance and hypertriglyceridemia.

Disclosures: The following people have nothing to disclose: Adriana Popescu  

1146 A NASH PREDICTIVE INDEX (NPI) FOR USE IN PATIENTS WITH NONALCOHOLIC FATTY LIVER DISEASE
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Background: A third of the U.S. population may have nonalcoholic fatty liver disease (NAFLD). Nonalcoholic steatohepatitis (NASH) is associated with progression. Identification of patients with NASH before the onset of advanced fibrosis would help recognize patients who would benefit most from aggressive interventions. Liver biopsy, the only accepted method for NASH identification, is invasive, costly, risky, and limited by sampling variability. Aim: To build a predictive model that would allow identification of NASH among patients with suspected NAFLD. Methods: Data were prospectively collected on consecutive patients with suspected NAFLD seen at an academic medical center in Cleveland between 2004-2006. An independent validation cohort seen at a separate institution between 2005-2006 was identified. Demographic, clinical, metabolic, and laboratory parameters were recorded. The homeostatic model assessment 2 (HOMA-2) was used to calculate HOMA-IR. Those on insulin therapy were excluded. Log transforms of variables with skewed distributions were used. Statistical analyses were done. A scoring system was created. Results: 177 patients were included. In the original cohort (n=122), mean age was 49; 80/122 (66%) were female; 34/122 (28%) were diabetic; and 84/122 (69%) had NASH. Subjects with NASH were older, had higher BMI, HOMA-IR, AST and ALT. By multivariate analysis, age, BMI, HOMA-IR, female sex, and log (ASTxALT) were independent predictors of NASH. A NASH Predictive Index (NPI) was created based on the model (Table). The area under the ROC curve for the NPI was 0.87 (95% CI, 0.81, 0.93). In the validation set (n=55), the AUC was 0.85 (95% CI, 0.75, 0.95). The ROC curves were not statistically different (p=0.68). A NPI value >=6 identified 56% of NASH cases in the original cohort with 100% positive predictive value (PPV), and 65.7% of NASH cases in the validation cohort with 95% PPV. Conclusions: 1. The NPI allowed identification of a significant proportion of patients with NASH, non-invasively and with a high positive predictive value; 2. The NPI, after further validation, may aid in the identification of a significant proportion of NASH cases, patients that will benefit most from aggressive interventions and future therapies, while avoiding liver biopsy.

Logistic regression model for prediction of NASH*

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<td>HOMA-IR</td>
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</tbody>
</table>

*Whole model test p<0.0001

Disclosures: The following people have nothing to disclose: Claudia O. Zein, John M. Edmison, Mark Schluchter, Ariel E. Feldstein, Nizar N. Zein, Arthur J. McCullough  

1147 PHOSPHOPROTEOMIC BIOMARKERS PREDICTING WEIGHT LOSS AFTER BARIATRIC SURGERY IN PATIENTS WITH NON-ALCOHOLIC FATTY LIVER DISEASE (NAFLD)
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Central adiposity and metabolic syndrome are associated with NAFLD. Weight loss through bariatric surgery has been associated with resolution of obesity-related complications, including insulin resistance and NAFLD. AIM: To develop a prognostic biomarker to predict successful weight loss after bariatric surgery. METHODS: 111 patients undergoing bariatric surgery were included [age 44+/=11 yrs, 29% male, 99% NAFLD, ALT 33+/=23 IU/L and AST 26+/=19 IU/L]. Clinical and demographic data was obtained at the time of surgery. A liver biopsy was performed and interpreted by a single hepatopathologist. A biopsy of omental fat/white adipose tissue (WAT) was obtained as well as serum which were both snap-frozen. Follow up data was also collected 10.25 +/-9.79 months after surgery. Successful weight loss was defined as losing >50% excess body weight (EBW) or achieving a normal waist circumference (=40 inches in men and <35 inches in women). From WAT, protein lysate was extracted and then used for Reverse Phase Protein Microarray analysis, which quantitatively measured the relative phosphorylation of 80 specific signaling molecules. Parametric and non-parametric analyses...
were performed to compare the cell signaling networks of those patients achieving successful weight loss to those without successful weight loss post-bariatric surgery. RESULTS: Comparing patients who achieved successful weight loss by normalizing their waist circumference to those who did not, the phosphorylation of 17 proteins [pAKT(S473), pERK (T202/Y204), pMEK1/2 (S217/221), pp38 (T180/Y182), pmTOR [S2448], pBAD [S155], penoS (1177), pFAF [S194], pPyk2 (Y402), pFHKR/FKHRL1 (T24/32), pGSK3α/β (S21/9), pp90RSK (S380), pSTAT3 (S277), pCREB (S133), pBAD (S112), pERα (S118) and pAMPKα1 (S485)] were significantly different (P<0.05). If successful weight loss was defined as achieving >50% EBW loss, the phosphorylation levels of 14 proteins [pAKT (S308), pmTOR (S2448), pRasGRF (916), pp70S6 (T389), ClCasase3 (D175), pBAD (S155), pc-Abl (T735), pPyk2 (Y402), pp90RSK (S380), pPKCa/BII (T638/641), pSMAD (S645/647), pSrc (Y527), pBAD (S112) and Cox2] were significantly (>0.05) different in those who achieved success. Nearly all of these proteins are within apoptosis control pathway or insulin/AKT/growth factor signaling pathways.

CONCLUSIONS: Signal pathway profiling using Reverse Phase Protein Microarrays of WAT appears to predict successful weight loss in obese patients with NAFLD after bariatric surgery.

Disclosures:
The following people have nothing to disclose: Valerie S. Calvert, Clare Nugent, Rochelle Collantes, Hazem Elariny, Aryan Alendy, Ancha Baranova, Yun Fang, Jianghong Deng, Zachary Goodman, Lance A. Liotta, Emanuel F. Petricoin, Zobair M. Younossi

1149 DIRECT COMPARISON OF TWO NON INVASIVE BIO-MARKERS FOR THE DIAGNOSIS OF ADVANCED FIBROSIS IN PATIENTS WITH NON ALCOHOLIC FATTY LIVER DISEASE (NAFLD) : NAFLD SCORE AND FIBROTEST
Monna Munteanu2, Frederic Charlotte1, Vlad Ratziu1, Sophie Jacqueminet1, Djamila Messous1, Philippe Podevin2, Laurence Serfaty3, Eric Bruckert1, Andre Grimald1, Thierry Paynard1,1; APHP GHPS, Paris, France; 2Biopredictive, Paris, France; 3APHP Cochin, Paris, France; 4APHP StAntoine, Paris, France

Background and aims: Patients with NAFLD and advanced liver fibrosis are at the highest risk for progressing to end stage liver disease. The aim was to directly compare two biomarkers that have been separately validated for the noninvasive diagnosis of advanced fibrosis in NALFD, the FibroTest (FT) and the NAFLD score (NS). Methods: Two populations of two prospective studies, initially, were included in the present study when FT and FN were available at the time of liver biopsies. One “diagnostic” cohort initially included 267 patients with baseline biopsy and one randomized trial with rosiglitazone or placebo with baseline and end of 1yr treatment paired biopsies included 63 patients (total biopsies n=126). The primary end point was a difference in area under the ROC curves (AUROC) for the diagnosis of advanced fibrosis defined as septal or bridging fibrosis or cirrhosis (Kleiner et al scoring system).

Results: A total of 246 cases with biopsy, FT and NS were included; these were not significantly different than the nonincluded cases for the main characteristics. The trial patients had more severe liver injury than the cohort patients: the prevalence of advanced fibrosis was 31% and NASH 100% versus 12% and 32% in the cohort. FT and NS both had very significant diagnostic value (P<0.001 versus 0.50 AUROC) for the diagnosis of advanced fibrosis (figure), but the FT AUROC was significantly higher than the NS AUROC both in the diagnostic cohort 0.89 (95%CI 0.81-0.94) vs 0.76 (95%CI 0.59-0.86 ;P=0.035. n=120) and in the therapeutic trial: 0.80 ;95%CI 0.71-0.88 vs 0.70 ;95%CI 0.60-0.78 ;P=0.04 n=126). There was no significant heterogeneity for the FT and NS AUROCs between the two studies results. Conclusion: FibroTest had better diagnostic value than the NAFLD score for the diagnosis of advanced fibrosis among patients with NAFLD in two retrospective analyses; however these differences must be confirmed prospectively.
Background and aims: Physical activity (PA) is commonly recommended for patients with NAFLD. However, evidence that PA is effective in NAFLD prevention and treatment is scarce. Aims: To examine the association between self-reported PA and NAFLD. Methods: A cross-sectional study of a sub-sample (n=375) of the Israeli National Health and Nutrition Survey. Exclusion criteria were any known etiology for secondary NAFLD. Participants underwent an abdominal ultrasound, biochemical tests and anthropometric evaluations (abdominal obesity defined as waist circumference >88 cm in women and >102 cm in men). During a face-to-face interview a detailed PA (leisure and occupational) questionnaire was administered including the name of the PA, its frequency and duration and how long did the subject engaged in the PA [multiplication of all categories was defined as “score”]. Results: After exclusion 349 subjects (52.7% male, mean age 50.7±10.4, 30.9% primary NAFLD) were included in the analysis. No association was found with occupational PA. The NAFLD group as compared to subjects with normal liver performed less than the half of the amount of all kind of sports (32.1 minutes/week vs. 67.3, P=0.001), almost half the amount of aerobic exercise (23.2 minutes/week vs. 42.3, P= 0.03) and only one third the amount of anaerobic exercise (8.9 minutes/week vs. 25.0, P=0.001). Adjusting for age and gender, performance of all kind of sports [OR=0.66, 0.44-0.96 95% CI per 413.2 increment in score units [1 SD]] and anaerobic exercise [OR=0.61, 0.38-0.85 95% CI per 162.5 increment in score units [1 SD]] were inversely associated with NAFLD. The association with anaerobic exercise remained significant with further adjustment for BMI [OR=0.61, 0.44-0.85 95% CI]. Only anaerobic exercise was associated with a lower rate of abdominal obesity irrespective of BMI, gender and age (P=0.03). Among NAFLD patients, performance of PA was associated with lower fasting serum insulin levels (25.7 vs. 32.0, P=0.03) and waist circumference (99.8 vs. 104.7, P=0.04) though BMI was similar. Moreover, performance of PA was associated with a lower rate of abdominal obesity irrespective of BMI, gender and age (OR=0.25, 0.07-0.93 95% CI). Conclusions: Higher leisure-time PA may protect against NAFLD. The protective role of anaerobic exercise can stem from its independent association with a lower rate of abdominal obesity. NAFLD patients that perform PA benefit lower insulin levels irrespective of BMI, probably mediated by a lower rate of abdominal obesity.

Disclosures:
The following people have nothing to disclose: Frederic Charlotte, Vlad Ratziu, Sophie Jacqueminet, Djamilia Messous, Philippe Podevin, Laurence Serfaty, Eric Bruckert, Andre Grimaldi.

1150
ROLE OF PHYSICAL ACTIVITY IN NON-ALCOHOLIC FATTY LIVER DISEASE (NAFLD): A POPULATION BASED STUDY
Shira Zelber-Sagi1,2, Dorit Nitzan-Kaluski2,3, Rebecca Goldsmith2, Muriel Webb1, Laurie M. Blendis1, Zamir Halpern1,3, Ran Oren1,3; 1Department of Gastroenterology, Tel Aviv Sourasky Medical Center, Tel Aviv, Israel; 2The Food and Nutrition Administration, Ministry of Health, Tel Aviv, Israel; 3The Sackler Faculty of Medicine, Tel-Aviv University, Tel Aviv, Israel

Background and aims: Physical activity (PA) is commonly recommended for patients with NAFLD. However, evidence that PA is effective in NAFLD prevention and treatment is scarce. Aims: To examine the association between self-reported PA and NAFLD. Methods: A cross-sectional study of a sub-sample (n=375) of the Israeli National Health and Nutrition Survey. Exclusion criteria were any known etiology for secondary NAFLD. Participants underwent an abdominal ultrasound, biochemical tests and anthropometric evaluations (abdominal obesity defined as waist circumference >88 cm in women and >102 cm in men). During a face-to-face interview a detailed PA (leisure and occupational) questionnaire was administered including the name of the PA, its frequency and duration and how long did the subject engaged in the PA [multiplication of all categories was defined as “score”]. Results: After exclusion 349 subjects (52.7% male, mean age 50.7±10.4, 30.9% primary NAFLD) were included in the analysis. No association was found with occupational PA. The NAFLD group as compared to subjects with normal liver performed less than the half of the amount of all kind of sports (32.1 minutes/week vs. 67.3, P=0.001), almost half the amount of aerobic exercise (23.2 minutes/week vs. 42.3, P= 0.03) and only one third the amount of anaerobic exercise (8.9 minutes/week vs. 25.0, P=0.001). Adjusting for age and gender, performance of all kind of sports [OR=0.66, 0.44-0.96 95% CI per 413.2 increment in score units [1 SD]] and anaerobic exercise [OR=0.61, 0.38-0.85 95% CI per 162.5 increment in score units [1 SD]] were inversely associated with NAFLD. The association with anaerobic exercise remained significant with further adjustment for BMI [OR=0.61, 0.44-0.85 95% CI]. Only anaerobic exercise was associated with a lower rate of abdominal obesity irrespective of BMI, gender and age (P=0.03). Among NAFLD patients, performance of PA was associated with lower fasting serum insulin levels (25.7 vs. 32.0, P=0.03) and waist circumference (99.8 vs. 104.7, P=0.04) though BMI was similar. Moreover, performance of PA was associated with a lower rate of abdominal obesity irrespective of BMI, gender and age (OR=0.25, 0.07-0.93 95% CI). Conclusions: Higher leisure-time PA may protect against NAFLD. The protective role of anaerobic exercise can stem from its independent association with a lower rate of abdominal obesity. NAFLD patients that perform PA benefit lower insulin levels irrespective of BMI, probably mediated by a lower rate of abdominal obesity.

Disclosures:
The following people have nothing to disclose: Frederic Charlotte, Vlad Ratziu, Sophie Jacqueminet, Djamilia Messous, Philippe Podevin, Laurence Serfaty, Eric Bruckert, Andre Grimaldi.

1151
SEVERITY OF NON-ALCOHOLIC FATTY LIVER DISEASE IS RELATED TO MTHFR 677C>T HOMOZYGOITY, LOWER GLUTATHIONE AND INSULIN RESISTANCE
John M. Edmison, Srinivasan Dasarathy, Satish C. Kalhan, Arthur J. McCullough; Department of Gastroenterology and Hepatology, Cleveland Clinic, Cleveland, OH

Background & Aims: Non-alcoholic fatty liver disease (NAFLD) is a spectrum of disease ranging from simple steatosis to non-alcoholic steatohepatitis (NASH) and is the most common chronic liver disease in the United States. Hepatic lipid accumulation and peroxidation with generation of reactive oxygen species and unopposed oxidative stress have been implicated in the pathogenesis of NASH. We hypothesized that insulin resistance (IR) and altered hepatic lipid metabolism cause perturbations in methionine-homocysteine metabolism, which are related to severity of liver injury and is compounded by methylenetetrahydrofolate reductase (MTHFR) 677C>T homozygosity. MTHFR is involved in the formation of N5-MTHF, the source of methyl groups for the conversion of homocysteine to methionine. The relation between magnitude of IR, MTHFR 677C>T homozygosity and alterations in the methionine-homocysteine transsulfuration pathway were examined in subjects with biopsy-proven NAFLD. Methods: 48 subjects with histologically diagnosed NAFLD (15 steatosis, 33 NASH) and 25 controls were studied after a 12 hour fast. IR was determined by HOMA; plasma glutathione, methionine, homocysteine and cysteine levels were measured with high-performance liquid chromatography; and MTHFR 677C>T polymorphism was determined by polymerase chain reaction. Results: Plasma glutathione levels were lower (P<0.017) and homocysteine and cysteine levels higher (P<0.017) in subjects with NASH. IR (HOMA) was higher (P<0.0001) in subjects with steatosis and NASH compared with controls. Severity of IR (HOMA > 2.5) was associated with a higher degree of steatosis, hepatocellular ballooning, hepatic fibrosis, and NASI activity score. The frequency of TT homozygosity for the MTHFR polymorphism was higher in subjects with steatosis (13.3 %) and NASI (18.2 %) compared with controls (4 %). Conclusions: Our data show that NASH is associated with perturbations in glutathione and methionine metabolism. Reduced levels of glutathione (the major antioxidant in the liver) may result from the effect of IR on γ-glutamyl-cysteine synthetase, the enzyme utilized in the conversion of cysteine to glutathione. The higher clustering of MTHFR 677C>T homozygosity in NAFLD may have compounded the hepatic injury by impairing the methylation of homocysteine to methionine. The present study provides a metabolic rationale for the progression of NAFLD. Additionally, severe IR (HOMA > 2.5), low plasma glutathione and presence of MTHFR 677C>T homozygosity are associated with NASH. These markers, in combination, may help in the non-invasive diagnosis of NASH as well as provide a rationale for potential new therapies.

Disclosures:
Background: Current upper limit of normal (ULN) for alanine aminotransferase (ALT) level was set at 40 IU/L regardless of age, sex or risk factors for fatty liver diseases (FLD). A recent Italian study suggested that the ULN for ALT should be adjusted to 30 IU/L for men and 19 IU/L for women by eliminating the influences of risk factors for FLD. However, there has been no data on Asian population, especially subjects with normal liver histology. We, therefore, investigated the normal range of ALT level for healthy Korean subjects. Method: We reviewed the medical records of 23,233 subjects who were examined at Health Promotion Center, Asan Medical Center, from January 2005 to April 2006. Questionnaires about past medical illness, medication, alcohol, and smoking were taken from all the study subjects. Subjects were excluded from the analysis if they had any of the followings: current medication, alcohol consumption>40 g/week), DM, hyperlipidemia or positive results on HBsAg or anti-HCV. Subjects were further stratified according to USG findings for FLD, sex, and body mass index (BMI). The UL was defined as 95th percentile. To validate the UL obtained from 'presumed' healthy subjects, we compared with that of 538 living liver donors. They were negative for, HBsAg and anti-HCV, and showed normal histology on liver biopsy.

Results: 1) The ULs for ALT in subjects with normal USG finding and BMI were 36 IU/L in men and 26 IU/L in women 2) Among those with normal USG finding and high BMI (>25), the ULs were 42 IU/L in men and 31 IU/L in women. 3) Presence of fatty change on USG significantly increased the ULs for ALT even in subjects with normal BMI (56 and 43 IU/L). 4) The ULs of ALT were highest in subjects with high BMI and fatty change on USG. They were 88 IU/L in men and 56 IU/L in women. 5) BMI and Triglyceride significantly affected the ULs of ALT, even in subjects with normal USG finding and BMI. 6) The ULs for ALT in normal living liver donors were 35 IU/L for men and 25 IU/L for women, which were comparable to those of the group with normal USG finding and BMI. Conclusion: Overweight and fatty change on USG significantly increased ALT levels, suggesting that subjects at high risk for FLD cannot represent normal population. However, ALT levels increased with BMI and TG, even in subjects at low risk for FLD. The ULN for ALT in Asian subjects are 35 IU/L for men and 25 IU/L for women, which were higher than those in Italian study. This discrepancy was probably due to the fact that our participants had higher BMI than those in Italian study. Therefore, the ULN for ALT obtained from young Western population cannot be used as a reference for Asian subjects.

Disclosures: The following people have nothing to disclose: Jae Keun Lee, Han Chu Lee, Kyoung Hoon Lee, Kang Mo Kim, Young Suk Lim, Young-Hwa Chung, Yung Sang Lee, Dong Jin Suh.
Background: Nonalcoholic fatty liver disease (NAFLD) includes a spectrum of hepatic steatosis, steatohepatitis, fibrosis, and cirrhosis. NAFLD is considered the hepatic representation of the metabolic syndrome and, to date, no effective drug therapy exists. Thus there is a need to expand our current understanding of its pathophysiology and explore novel therapeutic pathways. The renin-angiotensin system (RAS) is crucial in blood pressure regulation and plays a vital role in the pathogenesis of hypertension. Accumulating evidence suggests that RAS exerts deleterious effects through the production of reactive oxygen species (ROS). We hypothesized that increased RAS activity causes NAFLD due to increased hepatic oxidative stress. Methods: We employed a transgenic TG(mRen2)27(Ren2) hypertensive rat model, harboring the mouse Ren2 gene with elevated local RAS activities and Ang II levels. Hepatic indices for steatosis, inflammation, fibrosis, and oxidative stress were assessed and compared with normotensive Sprague-Dawley (SD) controls at ages of 8 and 12 weeks. The causative relationship between Angiotensin II, oxidative stress, and NAFLD were further evaluated by treating groups of 5 week old Ren2 rats (n=6-7 per group) with the angiotensin receptor blocker valsartan (30 mg/kg/day) or the superoxide dismutase/catalase mimetic tempol (1 mM) followed by sacrifice at 12 weeks of age. Hepatic indices for steatosis, inflammation, fibrosis and oxidative stress were assessed and compared with normotensive SD controls. Results: 8-week Ren2 rats developed significant hepatic steatosis, oxidative stress, and NAFLD were further evaluated by treating groups of 5 week old Ren2 rats (n=6-7 per group) with the angiotensin receptor blocker valsartan (30 mg/kg/day) or the superoxide dismutase/catalase mimetic tempol (1 mM) followed by sacrifice at 12 weeks of age. Hepatic indices for steatosis, inflammation, fibrosis and oxidative stress were assessed and compared with normotensive SD controls. Results: 8-week Ren2 rats developed significant hepatic steatosis, oxidative stress, and NAFLD were further evaluated by treating groups of 5 week old Ren2 rats (n=6-7 per group) with the angiotensin receptor blocker valsartan (30 mg/kg/day) or the superoxide dismutase/catalase mimetic tempol (1 mM) followed by sacrifice at 12 weeks of age. Hepatic indices for steatosis, inflammation, fibrosis and oxidative stress were assessed and compared with normotensive SD controls. Results: 8-week Ren2 rats developed significant hepatic steatosis, oxidative stress, and NAFLD were further evaluated by treating groups of 5 week old Ren2 rats (n=6-7 per group) with the angiotensin receptor blocker valsartan (30 mg/kg/day) or the superoxide dismutase/catalase mimetic tempol (1 mM) followed by sacrifice at 12 weeks of age. Hepatic indices for steatosis, inflammation, fibrosis and oxidative stress were assessed and compared with normotensive SD controls. Results: 8-week Ren2 rats developed significant hepatic steatosis, oxidative stress, and NAFLD were further evaluated by treating groups of 5 week old Ren2 rats (n=6-7 per group) with the angiotensin receptor blocker valsartan (30 mg/kg/day) or the superoxide dismutase/catalase mimetic tempol (1 mM) followed by sacrifice at 12 weeks of age.
1156
THE ZEBRAFISH FOIE GRAS MUTANT IS A MODEL OF FATTY LIVER DISEASE WITH ACTIVATED UNFOLDED PROTEIN RESPONSE
Ayca Cinaroglu, Smita Gopinath, Katherine Krahn, Kirsten C.Sadler; Division of Liver Disease/Dept. of Medicine and Dept. of Molecular Cell and Developmental Biology, Mt. Sinai School of Medicine, New York, NY

Hepatic fat accumulation (steatosis) is a frequent consequence of obesity and insulin resistance, and is considered the hepatic manifestation of metabolic syndrome. Steatosis predisposes hepatocytes to further damage, apoptosis and inflammation, which may progress to steatohepatitis and end stage liver disease. Recent studies indicate that activation of the unfolded protein response (UPR) and endoplasmic reticulum (ER) stress as important cellular processes leading to steatosis and hepatic insulin resistance. However, the interplay between hepatic damage, lipid accumulation and UPR activation is not understood. This study describes the zebrafish foie gras mutant embryos as a system for delineating the mechanism linking UPR activation and steatosis. The foie gras gene is highly conserved in animals, has only been studied in zebrafish and its function is uncharacterized. Mutation in foie gras results in hepatomegaly, steatosis, decreased hepatic function and hepatic apoptosis by day 5 of development, with 100% penetrance. Marked increase in the expression in a panel of genes, including bip (22-fold), chop (53-fold) and spliced xbp1 is found in the liver of MT embryos, signifying the specificity of this process. Importantly, gene expression analysis shows that activation of UPR occurs prior to the development of steatosis and hepatic damage, suggesting this to be a cause of steatosis. Moreover, Wild type embryos treated with tunicamycin to induce UPR activation phenotypically the morphological and molecular features of foie gras mutants. This indicates that activation of the UPR is sufficient for steatosis. We hypothesize that mutation in the foie gras gene leads to the activation of UPR and consequently results in steatosis and propose the foie gras mutant as a model to study the relationship between UPR and fatty liver disease.

Disclosures:
The following people have nothing to disclose: Ayca Cinaroglu, Smita Gopinath, Katherine Krahn, Kirsten C. Sadler

1157
C-REACTIVE PROTEIN INDUCES INSULIN RESISTANCE IN BOTH HUMAN HEPATOCYTES AND ADIPOCYTES
Adeline Bertola1, Rodolphe Anty1,2, Stephanie Bonnafous2, Yannick Le Marchand-Brustel2, Albert Tran1,2, Philippe Gual2; 1Pôle digestif, Centre Hospitalier universitaire, Nice, France; 2INSERM U568, Nice, France

Introduction: C Reactive Protein (CRP) is a nonspecific marker of inflammation which is elevated in obesity (~ 10 mg/l). We and others have shown that the liver but also the adipose tissue can produce CRP which might contribute to the elevated plasma CRP levels found in obesity. Since CRP could promote specific biological responses, we evaluated the role of local CRP production in human hepatocytes and adipocytes in the insulin signaling and its potential contribution in liver complications. Patients and Methods: This study was done in HepG2 cells and human adipocytes. After isolation of the pre-adipocyte cells in subcutaneous adipose tissue from abdominalplasty, the cells were differentiated into adipocytes. Insulin-stimulated glucose uptake, and CRP-induced lipolysis were evaluated in vitro. IRS1, IRS2 and SOCS3 gene expression was determined by Real-Time PCR. The expression levels of IRS1 were also evaluated by western blot analysis. Results: The long-lasting treatment of HepG2 with a high CRP concentration (10 mg/l for 24h) enhanced the gene expression of SOCS3, protein known to alter insulin signaling by binding with the IRS proteins and Insulin Receptor. Furthermore, the gene expression of both IRS1 and IRS2 was strongly decreased in response to CRP. In human adipocytes, a long-lasting treatment with a high CRP concentration (10 mg/l for 48h) strongly decreased insulin-stimulated PKB activation and glucose uptake, while a low concentration of CRP was without effect (1 mg/l). Moreover, the treatment of adipocytes with a high CRP concentration strongly decreases the expression of Insulin Receptor Substrat-1 at the protein level. The decrease in the amount of the major substrate of Insulin Receptor strongly reduced its action. Finally, the lipolysis was also stimulated by CRP stimulation leading to a strong increase in free fatty acids release from the adipocytes. Conclusion: These data provide the first evidence that in vitro CRP promotes insulin resistance in human hepatocytes and adipocytes. Since insulin-resistance is responsible for the development of steatosis and steatohepatitis, the enhancement of insulin resistance by CRP could contribute to the development of liver complications. CRP acts directly in the liver by inhibiting insulin signaling in hepatocytes and indirectly via the adipose tissue. In adipocytes, CRP stimulates the lipolysis and consequently provides fatty acids for the liver which could lead to the accumulation of triglycerides and hepatic steatosis.

Disclosures:
The following people have nothing to disclose: Adeline Bertola, Rodolphe Anty, Stephanie Bonnafous, Yannick Le Marchand-Brustel, Albert Tran, Philippe Gual

1158
MB07811, A HEPDIRECT PRODRUG OF A LIVER-TARGETED THYROID HORMONE RECEPTOR (TR) AGONIST, AND OTHER SYNTHETIC NON-LIVER-TARGETED TR AGONISTS, BUT NOT T3, REDUCE HEPATIC STEATOSIS
Edward E. Cable, Xiaohong Yang, Patricia D. Finn, Jian Li, Jeffery W. Stiebins, Michael P. Haughey, Jinzhao Hou, Bruce R. Ito, Paul D. van Poelje, David L Linemeyer, Mark D. Erion; Metabasis Therapeutics, La Jolla, CA

MB07811, an orally active HepDirect prodrug of MB07344 (a livers-targeted TR-β agonist), is currently undergoing clinical trials for the treatment of hyperlipidemia. Thyroid hormones (T3, and T4), known to reduce plasma cholesterol levels, also mobilize fatty acids (FA) from the periphery and induce hepatic lipogenic gene expression potentially causing hepatic steatosis. Therefore, the purpose of these studies was to assess the effects of MB07811 on whole body and liver lipid metabolism compared to those elicited by T3, and other TR agonists. Methods: Isolated primary rat adipocytes were used to test for lipolytic effects of TR agonists. Sprague Dawley (SD) rats, Zucker-diabetic fatty rats, ob/ob mice and diet-induced obese (DIO) mice were treated with test agents for up to 11 weeks. Endpoints measured were epididymal fat pad (EFP) weights; liver lipids, histology, mRNA levels; and plasma acyl-carnitine levels. Results: Initial characterization of TR agonists in SD rats demonstrated that T3 decreased EFP weights but not liver triacylglycerol (TG) levels. In contrast, the four synthetic TR agonists tested decreased liver TG levels but did not change EFP weights. Further characterization of MB07811 in animal models of hepatic steatosis indicated that the decrease in liver TG levels occurred across all the animal models tested. T3 consistently decreased peripheral fat with little or no effect on liver TG levels, whereas in the ob/ob mouse, a model with elevated transaminases, clearance of hepatic TG levels correlated with decreases in plasma AST and ALT levels. In DIO mice, hepatic levels of both TGs and diacylglycerols, a key mediator of hepatic insulin resistance, were decreased. These data provide a potential therapeutic strategy for treatment of hepatic steatosis.
resistance, were reduced following MB07811 treatment. Mechanistic differences in the hepatic effects of MB07811 and T3 could not be explained on the basis of liver TR agonism as both MB07811 and T3 induced TR target genes and enzymes, including carnitine palmitoyl transferase-1, a key enzyme controlling mitochondrial FA oxidation. Changes in acyl-carnitine levels were consistent with an increase in FA oxidation following MB07811 and T3 treatment in vivo. However, only T3 induced lipolysis in isolated adipocytes. Conclusion: Synthetic TR agonists, in addition to their potential beneficial cholesterol lowering properties, represent a novel treatment for reducing hepatic steatosis. The mechanism by which synthetic TR agonists, but not T3, reduce hepatic TG levels is likely related to the inability of the synthetic TR agonists to induce adipocyte lipolysis. Without the influx of FA from the periphery, the TR-driven increase in hepatic FA oxidation reduces hepatic lipid content.

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1159 HOMOCYSTEINE SUPPLEMENTATION ATTENUATES, RATHER THAN INCREASES, THE UNFOLDED PROTEIN RESPONSE IN A MURINE NUTRITIONAL MODEL OF STEATOHEPATITIS
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Severe hyperhomocysteinemia is often associated with hepatic steatosis, suggesting that homocysteine (Hcy) is involved in the pathogenesis of fatty liver diseases. In vitro studies and rodent models of severe hyperhomocysteinemia demonstrate activation of the unfolded protein response (UPR) [1]. Despite these correlations, a causal relationship between Hcy and the UPR and hepatic steatosis remains unestablished. Thus, we investigated the effects of Hcy supplementation on hepatic steatosis and activation of the UPR in normal mice and mice fed a methionine-choline deficient (MCD) diet. Methods: FVB/NJ mice (10 wks) were fed either: 1) control (CON); 2) HCY [1.8g/L in H2O]; 3) MCD; or 4) MCD+Hcy diet for 2 weeks. Serum Hcy, ALT, and hepatic lipid content were analyzed. Expression of hepatic genes (CHOP, BiP, XBP-1s) and proteins (p-eIF2α) involved in the UPR were measured by real-time PCR and Western blotting. Results: Mice fed Hcy developed a 2-fold elevation in serum Hcy level (29±6 vs 14±2 μM in CON, p<0.001) yet did not develop hepatic steatosis, serum ALT elevation, nor activation of the UPR. In contrast, mice fed the MCD diet developed severe hyperhomocysteinemia (103±17 μM, p<0.001) and hepatic steatosis (0.7±0.2 vs 0.22±0.06 mg trig/protein in CON, p<0.001). Unexpectedly, the MCD+Hcy group had less ALT elevation (129±30 vs 202±57 IU/L, p=0.01), and gained, rather than lost weight (+4±4% vs -15±1% bw, p<0.001) compared to the MCD group. The MCD diet increased hepatic cholesterol content (48±8 vs 26±3 μg chol/mg protein in CON, p<0.001) and increased hepatic HMG-CoAR expression (1.6-fold vs CON, p=0.02), which did not occur in the MCD+Hcy group. The MCD diet induced 1.3-, 4.8-, 2.4-, and >10-fold increases in expression of CHOP, BiP, XBP-1s and p-eIF2α, compared to CON, respectively (p<0.05). All of these effects were attenuated in the MCD+Hcy-fed mice, (p<0.05) except for BiP expression, which remained upregulated in this group. Hcy alone had no effect on any of the UPR activation pathways compared to CON. Conclusions: Homocysteine supplementation increases serum Hcy 2-fold, but does not cause hepatic steatosis, serum ALT elevation, nor activation of the UPR. The MCD diet causes 10-fold elevations in serum Hcy, significant hepatic steatosis, and UPR activation. Unexpectedly, Hcy supplementation attenuates the deleterious effects of the MCD diet including weight loss, serum ALT elevations, hepatic cholesterol accumulation, and activation of the UPR. This suggests that although hyperhomocysteinemia is often associated with UPR activation and hepatic steatosis, these effects may be a secondary response rather than a direct effect.

Disclosures:
The following people have nothing to disclose: Anne S. Henkel, Richard M. Green

1160 GENETIC EVIDENCE THAT LIPID TRAFFICKING PLAYS A KEY ROLE IN FIBROSIS IN NON ALCOHOLIC FATTY LIVER DISEASE
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Studies suggest that susceptibility to non-alcoholic fatty liver disease (NAFLD) is determined in part, by genetic factors. Previous small studies have reported an association between the Val175Met single nucleotide polymorphism (SNP) in the phosphatidylethanolamine N-methyltransferase (PEMT) gene and non-alcoholic steatohepatitis (NASH). The -493G/T SNP in the promoter of the microsomal triglyceride transfer protein (MTP) gene has also been reported to be associated with steatosis and severity of fibrosis in a small cohort of NAFLD patients. PEMT and MTP are involved in hepatic lipid trafficking and now increasingly implicated in the pathogenesis of progressive NAFLD. We therefore sought to replicate these associations in a large cohort of NAFLD patients. In addition, we also looked for associations between NAFLD severity and functional SNP’s in the genes encoding apolipoprotein E (APOE) (E2,E3,E4 alleles) and cholesteryl ester transfer protein (CETP) I405V which are also involved in lipid trafficking. Methods: A cohort of patients with biopsy proven NAFLD (n=395) from the North of England were included. SNPs in the genes for PEMT (n=287), CETP (n=310), MTP (n=296) and APOE (n=395) were determined using PCR-RFLP analysis. Multivariate analysis (MVA) was used to examine the effects of genotype independent of known risk factors for progressive NAFLD (Type 2 diabetes, age and body mass index). NAFLD severity was graded and staged using modified NASH CRN criteria Results: There were no associations observed for the PEMT Val175Met polymorphism and either steatosis or fibrosis severity or the presence of hepatocyte injury (ballooning). The -493G/T MTP SNP was associated with fibrosis severity (% with stage 3/4 fibrosis; GG 29.8% vs T/* 19%; p=0.025, on multivariate analysis (MVA) p=0.068; Odds Ratio [OR] 1.8 [0.96-3.3]). The I405V CETP SNP was also associated with fibrosis (% stage 3/4; I/I 32.4% vs V*/ V* 18.2%; p=0.003, on MVA p=0.013, OR 2.2[1.2-4.1] as was the APOE E2 allele (% stage 3/4; E2/* 35.9% vs non-E2 22.6%; p=0.022, on MVA p=0.04, OR 2.17 [1.0-4.5]). Neither the MTP, CETP nor APOE SNPs were associated with either steatosis or ballooning. Conclusion: Functional
SNPs in genes encoding proteins involved in hepatic lipid trafficking, MTP, CETP and APOE, but not PEMT, are associated with advanced fibrosis in NAFLD independently of BMI, age and the presence of diabetes. None were associated with cell injury or steatosis severity. These data further support recent studies dissociating steatosis from the pathogenesis of progressive NAFLD and confirm a key role for hepatic lipid transport in the pathogenesis of advanced disease.

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1161 ALTERATIONS IN LYMPHOCYTE LIPID RAFT COMPOSITION AND STRUCTURE VIA BETA GLYCOLIPIDS IS ASSOCIATED WITH AMELIORATION OF NON ALCOHOLIC STEATOHEPATITIS: A NOVEL THERAPEUTIC TARGET FOR NASH
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Lipid rafts are microdomains of dynamic assemblies of cholesterol and sphingolipids which organize the plasma membrane into functional units. Raft microdomains represent sites of accumulation for multiple glycosphingolipids (GPI)-anchored membrane proteins, and for proteins targeted to rafts by binding integral raft proteins. Lipid rafts are considered to be critical for proper compartmentalization of insulin signaling. Leptin-deficient ob/ob mice are an accepted model for NASH. Aim: To determine the effect of distinct beta-glycolipids on lipid rafts and the metabolic syndrome in a murine model of NASH. Methods: Leptin deficient ob/ob mice received daily IP injections of 2.5mg/kg beta-glycosylceramide (GC), beta-lactosylceramide (LC), a 1:1 combination of GC and LC (IGL) or PBS, for 6 weeks. Detergent-insoluble fractionation by Triton X-100, was used for studying hepatocytes membranes. Soluble and insoluble fractions extracted from liver were assessed by western blot analysis for ESA-2 (Flotillin-2). The Effect of glycolipids on the metabolic syndrome and on liver damage was assessed by glucose tolerance test (GTT), hepatic fat content (oil-red-O staining), liver enzymes and TUNEL assay. Results: Administration of beta-glycolipids induced alteration of the hepatic detergent-insoluble/soluble fractionation and led to increased expression of ESA-2 in both the soluble and insoluble fractions. Administration of GC and IGL significantly improved the glucose tolerance of Ob/Ob mice (459 and 454 vs. 163 and 190 mg/dl at 30 minutes; 116 and 131 vs. 69 and 71 mg/dl at 180 minutes for PBS and LC vs. GC and IGL respectively; P < 0.01 for GC and IGL vs. PBS). Insulin serum levels were significantly lower in GC group, (128±34 vs. 228 pg/ml for GC vs. PBS, respectively, P = 0.05). IGL administration produced a trend in reduction of total serum cholesterol and triglycerides (5.7 ± 6.18 mmol/L cholesterol for IGL vs. PBS, respectively). Liver weight was lower in all beta-glycolipid treated groups (4.13, 4.38 and 4.38 vs. 4.43 gr, for GC, LC and IGL vs. PBS). The oil-red-O staining of liver biopsies showed a microvesicular fat pattern in beta-glycolipid treated animals and a macrovesicular fat pattern in PBS treated ones. Conclusions: Alteration of cell membranes may provide a new mechanism for understanding the pathophysiology of NASH. Administration of naturally occurring beta glycolipids alters hepatocyte membranes and affects the intracellular signaling machinery, providing a novel therapeutic approach to NASH.

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1162 HIGH FAT DIET CHANGES EXPRESSION OF IRON RELATED MOLECULES IN MICE LIVER AND DUODENUM
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Mild or moderate excess iron is frequently accumulated in liver tissue with non-alcoholic steatohepatitis (NASH) and contributes to liver injury through production of free radical. However the mechanism of iron overload in NASH is still unknown. In this study, we evaluated the iron status in the mouse fatty liver model with high fat diet. Furthermore we investigate expression of iron related molecules in liver and duodenum tissue in this mouse fatty liver model. [Methods] C57Bl/6 mice were fed with control or high fat diet. Mice in each diet were sacrificed after 2, 4, 8, or 16 weeks. Serum alanine aminotransferase (ALT), iron (S-Fe), unsaturated iron binding capacity (UIBC), ferritin were measured and histopathologic evaluation (hematoxylin-eosin stain) were performed. The expression of mouse transferrin receptor 1 (TR1) and hepcidin-1 (Hepc) in the liver and divalent metal transporter 1 (DMT1) in the duodenum were evaluated in these mice model by quantitative real-time RT-PCR method. The protein expression of Hepc and lipid peroxidation product 4-hydroxy-2-nonenal (HNE) as a consequence of oxidative stress were evaluated by immunohistochemical analyses. [Results] Serum ALT and ferritin with high fat diet were higher than those of control after 16 weeks (serum ALT with control or high fat diet; 34 ± 14, 180 ± 65 IU/L, S-Fe; 127 ± 10, 150 ± 34 µg/dl, UIBC; 210 ± 16, 239 ± 39 µg/dl, ferritin; 315 ± 59, 369 ± 37 ng/ml, respectively). The expression of TR1 mRNA in the mice liver with high fat diet was significantly higher than the control diet after 2, 4, 8 and 16 weeks (p<0.05). Hepc mRNA expression in the mice liver with high fat diet was no difference from control diet after 2 and 4 weeks. However Hepc mRNA expression with high fat diet was significantly lower than control after 8 and 16 weeks (p = 0.01). Furthermore the expression of Hepc in the liver with high fat diet was decreased than control. Corresponding to it, DMT-1 mRNA expression with high fat diet was significantly higher than control after 8 and 16 weeks (p = 0.02). The expression of HNE in the liver with high fat diet was induced than control after 16 weeks. [Conclusions] The expression of TR1 mRNA in the mice liver with high fat diet was induced. In addition, the expression of Hepc was decreased and the expression of duodenal DMT1 was increased with high fat diet after 8 and 16 weeks. Our data suggests that fatty liver with high fat diet might up-regulate TR1 expression and down-regulate Hepc expression, following the increased iron uptake into hepatocytes and iron absorption from small intestine.

Disclosures:
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1163 THE CAR AGONIST, TCPOBOP, ATTENUATES STEATOHEPATITIS IN THE MCD-FED MOUSE
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Nonalcoholic steatohepatitis (NASH) is a progressive disease in a subset of patients with limited therapeutic options. NASH
is characterized by toxicity from the endobiotic, free fatty acids, and apoptosis. Recently, the constitutive androstane receptor (CAR) has been reported to effect both hepatic detoxification of endo- and xenobiotic pathways and cytoprotection against apoptosis. Thus the AIM of this study was to ascertain whether CAR activation by TCPOBOP modulates steatohepatitis in the methionine choline deficient (MCD)-fed animal. METHODS: C57/BL6 wild type mice (n = 5) were fed either the MCD or CHOW diet for 2 weeks and were treated with vehicle (corn oil), or the CAR agonist, TCPOBOP ((3mg/kg) daily for the first 3 days at the start of week one of the MCD diet), or the CAR inhibitor, androstanol ((100mg/kg) daily for 3 days at the start of weeks one and two of the MCD diet). Gene expression was determined by RT-PCR. Hepatic steatosis was assessed by H&E and digital image analysis. Liver cell apoptosis and inflammation were assessed by TUNEL assay, immunohistochemistry for active caspase 3/7 and CD68 (Kupffer cell marker). RESULTS: mRNA expression of CYP2B10 and CYP3a11, known CAR target genes were increased 45-50-fold in TCPOBOP-treated mice fed the MCD diet (p<0.001), this phenomenon was abated by treatment with the CAR inhibitor, androstanol. Thus CAR can be activated in this animal model of NASH. TCPOBOP treated MCD-fed mice had 25% less hepatic macrosvesicular fat when compared to vehicle-treated MCD-fed mice, p<0.001. In contrast, MCD-fed mice treated with androstanol had 15% more hepatic steatosis as compared to vehicle, p<0.01. Similarly, serum triglyceride levels in MCD-fed mice treated with TCPOBOP were reduced compared to vehicle-treated and androstanol-treated mice, p<0.03. This reduction in hepatic steatosis was accompanied by an increase in enzymes involved in fatty acid microsomal β-oxidation and peroxisomal β-oxidation, namely CYP4A10 (p<0.01), LPBE (p<0.001), and 3-ketoacyl-CoA thiolase (p<0.04) in TCPOBOP treated compared to vehicle-treated MCD-fed mice. Finally, the reduction in steatosis was also accompanied by a reduction in liver cell apoptosis and inflammation. The percent/field area of TUNEL (p<0.01) and caspase 3/7 positive cells (p<0.03) were significantly higher in vehicle-treated MCD-fed mice than TCPOBOP. Immunoreactivity for CD68 was significantly reduced in TCPOBOP treated MCD-fed mice compared with vehicle and androstanol-treated animals, p<0.002. In CONCLUSION: CAR activation can stimulate a unique induction of genes involved in fatty acid oxidation, ameliorating hepatic steatosis, apoptosis and inflammation.

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1164 ROLE OF VISCERAL FAT REMOVAL IN THE PREVENTION OF HEPATIC INSULIN RESISTANCE AND TRIGLYCERIDE ACCUMULATION IN RESPONSE TO HIGH FAT DIET IN VIVO

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Background: Visceral fat (VF) is associated with the metabolic syndrome and insulin resistance. Therefore, we reasoned that VF accretion might be important in the pathogenesis of non alcoholic fatty liver disease. Aim: The aim of the present study was to determine if surgical removal of VF could prevent hepatic triglyceride (TG) accumulation and insulin resistance induced by short term high fat diet (HFD). Methods: Four groups of 5 young rats were studied: Two groups that preemptively underwent surgical VF removal (VF-) and were fed with either 30% HFD for three days or regular chow (RC). These were compared with 2 groups of age-matched sham operated rats (SO), either with HFD or RC. All visible perinephic and epididymal fat was removed in the VF- groups (~ 2.5 g). Insulin sensitivity was measured by hyperinsulinemic euglycemic clamp (3uU/kg/min) 2 weeks following VF-. Results: Body weight and basal hepatic glucose production (HGP) of the four groups were comparable (13.13.5 mg/kg/min). In SO, HFD significantly decreased insulin-induced suppression of HGP (i.e. hepatic insulin sensitivity) compared to RC (10.7 vs. 6.7 mg/kg/min p<0.01). Preemptive VF- restored HGP under HFD to the level of SO with RC (HGP=7.2mg/kg/min). In accordance with the clamp results, short term HFD significantly increased hepatic TG in SO, while preemptive VF- maintained hepatic TG under HFD (fig 1). During clamping in SO, HFD abolished insulin suppression of plasma free fatty acid (FFA) levels (0.73 meq/l), compared to 0.42 meq/l in RC (p<0.01). However, VF- in HFD group did not improve the ability of insulin to suppress FFA plasma levels (FFA=0.75 meq/l). Conclusion: This study underlines the significance role of VF in the pathogenesis of hepatic insulin resistance and TG accumulation. Moreover, the mechanism by which VF affects the liver does not necessarily involve modulation in FFA delivery.

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1165 RESOLUTION OF HEPATIC STEATOSIS DESPITE PERSISTENT OBESITY IN MICE FED STANDARD RODENT CHOW AFTER INDUCTION OF TYPE 2 NAFLD USING THE AMERICAN DIET INDUCED OBESITY SYNDROME MODEL

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A recently described model of rapidly progressive severe type 2 NAFLD in mice with obesity and impaired glucose tolerance was developed by combining the major components of diet and lifestyle observed in patients with NAFLD (high fat content, high trans-fat content, sedentary lifestyle and relevant amounts of high fructose corn syrup in the drinking water) [Gastroenterology 2007;132:A-818]. This intervention was termed the American Diet Induced Obesity Syndrome (ADIOS) model. The aim of the present study was to determine the impact of resuming standard rodent chow on obesity, NAFLD and other metabolic abnormalities in ADIOS mice. Methods: 6 week old male C57/BL6 mice were kept sedentary and fed ad libitum a high fat diet (43% of kcal from fat, 30% of fat as trans-fat) and water
containing high fructose corn syrup equivalent (6 g/kg/d) or standard chow and water for 24 weeks. After 16 weeks of treatment, a group of ADIOS mice (n=10) was crossed over to receive standard rodent chow (13% of kcal from fat) for another 8 weeks and compared to ADIOS mice (n=10) and control mice (n=10). Results: Mice crossed over to control chow at 16 weeks remained obese at 24 weeks (see table). Plasma leptin and resistin levels remained elevated in crossover mice, consistent with adipokine production from adipose tissue. Energy consumption was similar among the treatment groups. Despite these persistent similarities between ADIOS mice and crossover mice, NAFLD markedly improved with reduced liver fat and improved plasma ALT and AST. Plasma cholesterol also improved but plasma triglycerides became elevated in crossover mice. Summary: Feeding mice that have NAFLD a standard diet improved liver histology and liver injury despite persistent obesity and hyperleptinemia. Plasma triglycerides increased during the resolution phase, consistent with increased hepatic export. Conclusions: The type of food consumed had a greater impact on the resolution of NAFLD than the presence of obesity in the ADIOS mouse model.

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<td>50 (6)</td>
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Different numbers of asterisks indicate significant differences (P<0.01 by ANOVA)

Disclosures:
The following people have nothing to disclose: Brent A. Neuschwander-Teti, Metin Basaranoglu, Lisa M. Yerian, Laura H. Tetri, Elizabeth M. Brunt

1166 THE DEVELOPMENT OF NEW DRUG SCREENING SYSTEM USING STEATOHEPATITIS MEDAKA FISH MODEL INDUCED BY HIGH-FAT DIET
Toshihiko Matsumoto, Shuji Terai, Shinya Kuvashiro, Kaichi Fujisawa, Naoki Yamamoto, Yoshihiko Hamamoto, Isao Sakaida

Objective: The Medaka fish (Oryzias latipes) is small aquarium fish, have the advantage of having a short generation time and low costs of purchasing, housing and feeding to perform a large-scale screening of drugs. Here we tried to produce and characterize steatohepatitis caused by feeding of a high-fat diet in Medaka fish and analyzed the effect of Eicosapentaenoic acid (EPA) on steatohepatitis. Material and method: Male 8-weeks old see-through Medaka T5 and orange-red color Medaka Cab were administrated the high-fat diet (HFD) for 12 weeks. The process of alteration of the liver and increasing of abdominal adiposity was monitored by repeated observations from outside of the body in the same living T5. At 4, 8, 12weeks, plasma FBS and ALT level were determined. Then composition of hepatic free fatty acid was measured, and paraffin embedded sections of liver were stained with HE and with Gitter and Sirius red. Next we did DNA-chip analysis. Total RNA was obtained from liver each group (Group1:control, Group2:HFD4w, Group3:HFD8w, n=3) and the RNA was analyzed with DNA-Chip system (NANDEMO ARRAY, Gene Frontier). Next Cab were administrated HFD or HFD+10%EPA for 2weeks. The histological change, plasma ALT level and fatty acid composition of hepatic triglycerides were assessed to evaluate the efficacy of EPA. Result: HFD induced medaka fish into obesity, increased abdominal adiposity, fasting hyperglycemia (control:57±21, HFD8w:196±42, mg/dl, p<0.05), and showed severe hepatic steatosis. HFD model showed steatohepatitis by inflammatory infiltrate, sinusoidal fibrosis accompanying with elevation of plasma ALT levels (control:123±42, HFD8w:488±64 IU/L, p<0.05). HFD feeding provoked a 2.4-fold increase of oleic acid and one-tenth decrease of n-3 polyunsaturated fatty acid (PUFA) including Docosahexaenoic acid (DHA) and EPA in the liver. We compared the gene expression profiles among control, HFD4w, HFD8w group using DNA-chip. We found that 2470 genes, including fatty acid binding protein (FABP), phosphoenolpyruvate carboxykinase (PEPCK), α2 type1 collagen, tissue inhibitor of metalloproteinase 2 (TIMP-2) were detected as differentially expressed genes among the three groups. In this model, EPA improved plasma ALT level, hepatic steatosis and inflammatory infiltrate. Then the ratio of the n-3 PUFA to total free fatty acids in the liver was increased. Conclusion: We succeed to develop a new non-alcoholic steatohepatitis model and drug screening system using Medaka fish. EPA will be one of the candidate drugs for hepatic steatosis and steatohepatitis.

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1167 HYPOXIA WORSENS NON-ALCOHOLIC STEATOHEPATITIS
Piquet Anne-Christine, Monika Ledermann, Arthur Zimmermann, Deborah Stroka, Jean-Francois Dufour

Background/Aim. Patients with obstructive sleep apnoea syndrome are predisposed to non-alcoholic steatohepatitis (NASH) and insulin resistance. It is unclear whether cellular hypoxia plays a direct pathogenetic role along with the metabolic disorders that are frequently associated with obesity. Conditional knockout mice lacking hepatocellular expression of PTEN develop spontaneously a steatohepatitis (Horie et al., JCI 2004). To investigate the effect of hypoxia on NASH we studied the influence of hypoxia on the development of steatohepatitis in hepatocyte-specific PTEN-deficient mice. Methods. 8 weeks old female hepatocyte-specific PTEN-deficient mice were exposed to a 10% O2 (hypoxic) atmosphere or to 21% O2 (control) atmosphere. Food intake was matched in both groups. After 7 days, mice were euthanized and livers analysed by histology (H&E and Oil red O). NASH lesions were graded according to Kleiner (Hepatology, 2005). Hematocrit, liver morphology (H&E and Oil red O). NASH lesions were graded according to Kleiner (Hepatology, 2005). Hematocrit, liver test, glucose, triglycerides and insulin tolerance were determined in blood. Genes expression of SREBP-1, FOXO1, PPARy, CYP2E1 and hepcidin in the liver were determined by TaqMan. Means±standard deviations, Mann-Whitney test. Results. As
expected the hematocrit level increased in hypoxic mice 61±3% [n=15] compared to controls 49±2% [n=15] (p<0.01). The ratio liver weight/body weight was also increased in the hypoxic animals (4.7±0.3% vs 5.4±0.2% p<0.01). Steatosis was significantly more pronounced in hypoxic mice (2.4±0.8 vs 1.9±0.6, p<0.05) as well as the NAS score (5.2±1.7 vs 2.8±1.4, p<0.01) and the total score (7.7±3.2 vs 3.5±2.5, p<0.01). Oil red O-staining revealed that lipid droplets were more abundant and significantly larger in hypoxic livers. AST (105±25 IU/L vs 70±17, p<0.01) was higher in hypoxic ani- mals as well as triglycerides (1.8±0.4 mmol/l vs 1.1±0.4, p<0.01) and glucose (305±13 mg/dl vs 204±29, p<0.01). Hypoxic mice were more resistant towards insulin than control animals. Expression levels of genes encoding for SREBP1, CYP2E1 and PPARγ were increased by respectively 2.1±0.2, 2.3±0.6 and 4.2±1.9 fold (p<0.05) in liver of hypoxic mice, while hepcidin gene expression was decreased by 5.0±1 fold (p<0.05) and expression of FOXO1 was not affected. Conclusion. This is the first experimental demonstration that hypoxia directly worsens non-alcoholic steatohepatitis. The expression of several genes involved in metabolism was affected by hypoxia in this model. These data suggest that one mechanism is a hypoxia-induced insulin resistance.

Disclosures: The following people have nothing to disclose: Zhouji Chen, Elizabeth P. Newberry, Jin Y. Norris, Jianyang Luo, Susan M. Kennedy, Nicholas O. Davidson

1168 DISTINCT REQUIREMENT OF APOB-100 FOR INCREASED SECRETION OF VLDL-TRIGLYCERIDE IN RESPONSE TO HEPATIC OVEREXPRESSION OF MTP IN OB/OB MICE

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Microsomal triglyceride transfer protein (MTP) is a key genetic restriction point in hepatic assembly and secretion of very low density lipoproteins (VLDL). MTP polymorphisms have been linked to variations in plasma lipoprotein levels and non-alco- holic steatohepatitis in patients with type II diabetes and/or obesity. There are distinct requirements for MTP-mediated lipida- tion of apoB isoforms (i.e., apoB48 and apoB100) with apoB100 being exquisitely sensitive to defective MTP expres- sion. We determined the effects of MTP overexpression on hepatic triglyceride (TG) metabolism and VLDL production in the leptin-deficient (ob/ob) mouse and have additionally deter- mined the importance of apoB isoforms in mediating these effects. We crosssed apobec-1/- mice with ob/ob mice to gen- erate an apoB100 only ob/ob mouse (A-ob/ob). These mice, like humans, synthesize and secrete exclusively apoB100-con- taining VLDL from the liver. The obesity phenotype of ob/ob mice of both apobec-1 genotypes was similar, but A-ob/ob mice had increased serum cholesterol levels and higher hepatic TG. A recombinant adenovirus was constructed to express a mouse MTP cDNA (AdmMTP) in vivo and an empty adenovirus expressing GFP alone was used as control. Infusion of AdmMTP elevated hepatic MTP contents 3 fold. MTP overexpression signif- icantly increased hepatic VLDL-TG secretion in A-ob/ob mice but there was no change in ob/ob mice in the wild type apobec-1 background. Hepatic MTP overexpression did not significantly affect hepatic TG contents in any ob/ob genotype. Metabolic labeling studies on primary hepatocytes isolated from A-ob/ob mice demonstrated that MTP overexpression resulted in a 2-fold increase in rates of triglyceride secretion without affecting synthesis and secretion of apoB-100, indicat- ing that MTP overexpression promotes secretion of enlarged VLDL-particles by the liver of A-ob/ob mice. By contrast, over- expression of MTP did not affect hepatic VLDL secretion in the lean apobec-1/- and C57/BL-6 mice. These findings demon- strate that hepatic apoB100 and apoB48 in the lean mice are sufficiently lipidated by endogenous MTP and that there is no gain of function phenotype with MTP supplementation. Taken together, these findings demonstrate that MTP overexpression increases VLDL-TG secretion in ob/ob mice only in the apoB-100 background. Our findings emphasize the importance of apoB genotype in understanding the regulation of hepatic VDL- TG secretion by MTP.

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1169 5-LIPoxyGENASE INHIBITION ABROGATES HEPATIC STEATOSIS BY REDUCING LIVER FATTY ACID-BINDING PROTEIN EXPRESSION AND INCREASING HEPATOCYTE MICROsomAL TRIGlycerIDE TRANSFER PROTEIN ACTIVITY

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The 5-lipoxygenase (5-LO) pathway is an emerging target in obesity, insulin resistance and atherosclerosis. In this study, we explored the possible contribution of this key enzyme of the arachidonate cascade to the progression of hepatic steatosis. The administration of a potent and selective 5-LO inhibitor to leptin deficient mice (ob/ob mice), an experimental model of non-alcoholic fatty liver and steatohepatitis, significantly abro- gated hepatic steatosis as revealed by reduced oil red-O stain- ing and decreased hepatic triglyceride (TAG) content. In these animals, 5-LO inhibition down-regulated liver fatty acid-binding protein (L-FABP) expression while increasing microsomal TAG transfer protein (MTP) activity. No changes in the expression of lipogenic genes and transcription factors such as fatty acid synthase (FAS) and sterol response element-binding protein-1c (SREBP-1c) were observed. The anti-steatotic actions exerted by pharmaco- logical 5-LO blockade were further confirmed by genetic inhibition of this pathway. As compared to wild-type, mice deficient for the 5-LO gene showed reduced susceptibility to develop hepatic steatosis when submitted to a lipoperoxida- tive-induced model of liver injury. Interestingly, analysis of hepatic gene expression by microarrays revealed that a num- ber of genes involved in lipid metabolism including CCAAT/enhancer binding protein beta (C/EBP), car- boxylesterase 3, stearoyl-CoA desaturase 2 (Scd2), ATP-citrate lyase (Acly) and elongation of very long-chain fatty acids 3 and 6 (Elov3 and Elov6), were differentially expressed in 5-LO deficient mice. Consistent with in vivo findings, studies per- formed in cultured murine hepatocytes demonstrated that 5-LO- derived products (i.e. leukotriene [LT] B4 and LTD4) triggered TAG accumulation in the cytosolic compartment, while decreasing extracellular TAG levels. These effects were apparently not mediated by binding to cell surface receptors since neither CP-105,696, a selective LTB1 receptor antagonist, nor MK571, a
selective Cys-LT1 receptor antagonist, were able to prevent LTB4 and LTD4-induced intracellular TAG accumulation. Moreover, both LTB4 and LTD4 significantly reduced MTP activity in hepatocytes without affecting the expression of lipogenic factors (i.e. FAS and SREBP-1c). Taken together, these findings strongly support the concept that the 5-LO pathway participates in the pathogenesis of hepatic steatosis, probably by regulating fatty acid uptake and TAG export in hepatocytes.

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1170 DAILY EXERCISE INCREASES HEPATIC FATTY ACID OXIDATION AND PREVENTS STEATOSIS IN OTSUKA LONG-EVANS TOKUSHIMA FATTY (OLETF) RATS

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Background: The most common prescribed therapy for individuals with non-alcoholic fatty liver disease (NAFLD) is exercise training and weight loss; however, the effect of daily exercise training upon development of NAFLD in an obese model has not been prospectively examined. This study sought to determine whether exercise training prevents hepatic fatty acid accumulation and the development of NAFLD in Otsuka Long-Evans Tokushima Fatty (OLETF) rats and to elucidate the molecular mechanisms underlying the effect of exercise on hepatic steatosis.

Methods: Four-week old male OLETF rats were randomly assigned to either a sedentary control group (SED, n=8) or were given access to voluntary running wheels for 6 weeks (EX, n=8). Running wheels were locked two days before sacrifice in the EX animals, and both groups were sacrificed at 26 weeks of age. Liver morphology, hepatic triglycerides, fatty acid oxidation, and expression of key proteins involved in fatty acid metabolism were assessed in SED and EX animals. Results: Voluntary wheel running significantly attenuated weight gain and significantly reduced blood glucose, insulin, free fatty acids, and triglycerides in EX animals compared with SED controls (p<0.001). In addition, EX animals exhibited significantly reduced hepatic triglyceride levels compared with SED controls [1.62 ± 0.42 nmol/g (mean ± SE) vs. 2.94 ± 0.61, respectively, P<0.01]. A further analysis of hepatic triglycerides by Oil-Red O revealed that EX animals displayed fewer lipid droplets and reduced lipid droplet size compared to SED animals. Wheel running increased by 3-fold the percent of palmito-lipid droplets and reduced lipid droplet size compared to SED animals.

Conclusion: These results unequivocally demonstrate that daily physical activity attenuates hepatic steatosis and NAFLD in an obese rodent model through enhancement of hepatic fatty acid oxidation and reduction in key protein intermediates of fatty acid synthesis.

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1171 PHYLANTHUS URINARIA AMELIORATES THE SEVERITY OF NUTRITIONAL STEATOHEPATITIS

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Aims: Hepatic oxidative stress plays a critical role in the pathogenesis of non-alcoholic steatohepatitis (NASH). Phyllanthus Urinaria (Phyllanthus), a herbal medicine, has been reported to have potential anti-oxidant and anti-inflammation property. We tested the effects of Phyllanthus and the mechanisms of its action on metabolic forms of steatohepatitis both in vitro and in vivo.

Methods: AML-12 hepatocytes were exposed to the methionine and choline deficient (MCD) culture medium and the control medium in the presence or absence of Phyllanthus for 24 hours. Hepatocyte triglyceride, release of alanine aminotransferase (ALT) and lipoperoxides levels were determined. Mice were fed with control or MCD diet for 10 days with or without Phyllanthus (500, 1000 and 2000 ppm). The lowest dosage of Phyllanthus was the equivalent to the recommended dosage used in human. Hepatic steatosis, necroinflammation, triglycerides and lipid peroxide levels were determined. Hepatic expression of inflammatory factors and lipid regulatory mediators were assayed. Results: Phyllanthus significantly reduced steatosis and ALT levels in AML-12 hepatocytes in a dose-dependent manner in vitro. In addition, Phyllanthus prevented MCD-induced hepatic fat accumulation and necroinflammation in mice. This effect was associated with a repressed levels of hepatic lipid peroxides (12.3±7.5 μM/g liver vs 29.7±12.7, P<0.01) as determined by thiobarbituric acid reactive substances assay, reduced expression of cytochrome P450 2E1 (2.5 ± 0.6 vs 4.6 ± 0.14, P<0.05), pro-inflammatory tumor necrosis factor-alpha (1.1 ± 0.8 vs 5.7 ± 4.4, P<0.01), interleukin-6 (1.6 ± 1.1 vs 12.4 ± 7.8, P<0.01) and increased expression of Cyp4a10 (4.1±1.0 vs 1.2 ± 0.6, P<0.05). Hepatic acyl coenzyme A oxidase that regulated hepatic beta-oxidation of fatty acid and other lipid regulators including proliferator-activated receptor gamma (PPARγ), PPARα, CYP2E1, CYP4A14, long-chain acyl-CoA dehydrogenase, stearoyl coenzyme A desaturase-1, liver X receptors-alpha (LXRα), LXRβ, fatty acid synthase, were not affected by Phyllanthus. Conclusions: Phyllanthus clearly attenuated the liver injury of steatohepatitis in cultured hepatocytes and in MCD diet-fed mice in vivo. The mechanisms that Phyllanthus ameliorated steatohepatitis could be attributed to its antioxidant properties via suppression of CYP2E1, anti-inflammatory effects by down-regulating critical inflammatory mediators TNFα and IL-6, and induction of fatty acid oxidation through up-regulation of CYP4A10.

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The following people have nothing to disclose: Bo Shen, Jun Yu, Shiyian Wang, Eagle SH Chu, Vincent WS Wong, Xin Zhou, Ge Lin, Joseph JT Sung, Henry LY Chan
1172
OXIDATIVE STRESS-RELATED DAMAGE IS IMPlicated IN LPS-INDUCED ACTIVATION OF HEPATIC STELLATE CELLS
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In the progression of fatty liver to steatohepatitis a key role is played by mitochondrial β-oxidation impairment and disruption of the respiratory chain leading to reactive oxygen species (ROS) production. We previously reported that altered gut mucusal barrier function is associated to increased portal endotoxemia leading to fibrogenic activation of hepatic stellate cells (HSCs). Since oxidative damage is implicated in lipopolysaccharide (LPS) induced-liver injury, we tested the hypothesis that bacterial endotoxins may cause mitochondrial dysfunction and increase ROS formation in cultured HSCs. Low passage murine HSCs were exposed to LPS 10µg/ml with or without 30 min pre-treatment with antioxidant agents: glutathione (GSH, 200µm) and vitamin E (vitE, 500µM). Mitochondrial DNA (mtDNA) damage was assessed by quantitative real-time PCR whereas ROS production and mitochondrial membrane potential (MMP) were determined by flow cytometry analysis using respectively dichlorodihydrofluorescein diacetate fluorophore and tetramethylrhodamine methylester. Redistribution of mitochondrial proteins were studied by Western blot analysis performed on sub-mitochondrial fractions. LPS exposure induced a rapid (within 8 hrs) mtDNA deletion involving the coding regions for NADH dehydrogenase ND1 (0.6±0.15 fold decrease over control, p<0.05) and ND2 (0.4±0.2 fold decrease over control, p<0.05). Deletion of cytochrome c oxidase gene became evident only following 24 hrs of LPS incubation (0.38±0.13 fold decrease over control, p<0.05). LPS-exposed HSCs showed a disrupted MMP within 30 min and an increased ROS production within 75 min. However, HSCs pretreatment with vitE or GSH significantly reduced ROS accumulation and prevented mtDNA deletion. Moreover, LPS exposure induced in HSCs an altered expression of mitochondrial proteins, since Bax was cleaved from the mitochondrial membrane but the translocation to mitochondrial matrix was prevented by GSH treatment. LPS stimulation did not induce a significant mtDNA damage in HSCs isolated from TLR4/-/- mice. Our results indicate that LPS in murine HSCs induces alterations of the mitochondrial machinery such as decreased expression of the complexes I and IV genes, disrupted MMP and redistribution of the mitochondrial protein. We suggest that in the fatty liver LPS-induced ROS production may contribute together with the impaired mitochondrial β-oxidation to establish the oxidative stress responsible for the clinical progression of liver damage.
Disclosures:
The following people have nothing to disclose: Paola Brun, Melania Scarpa, Alessia Grillo, Federica Ditadi, Giorgio Palò, Diego Martines, Ignazio Castagliuolo

1173
PIOGLITAZONE RESTORES REGENERATION FAILURE FOLLOWING PARTIAL HEPATECTOMY IN OBSE and DIABETIC KK-Ay MICE
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Pathogenesis of metabolic syndrome-related NASH in part involves abnormal tissue-repairing responses in the liver. Although poor hepatic regeneration has been demonstrated in a variety of experimental models of hepatic steatosis/steatohepatitis, no effective therapeutic approach has been established yet. In this study, we investigated whether pioglitazone, a thiazolidinedione derivative (TZD), improves hepatic regenerative responses in obese, diabetic KK-Ay mice. Methods: Male KK-Ay and C57Bl/6 mice 12 weeks after birth underwent 2/3 partial hepatectomy (PH). Some mice were given repeated intragastric injection of pioglitazone (25 mg/kg) for 5 days prior to operation. Hepatocyte proliferation was evaluated by BrdU uptake, and hepatic expression of cyclin D1 and phosphorylation of STAT-3 were detected by Western blotting. TNF-α and socs-3 mRNA levels in the liver were determined by real-time RT-PCR. Serum IL-6 and adiponectin levels were measured by ELISA. Results: Half number of KK-Ay mice which underwent PH died within 48 hr, whereas this mortality was completely prevented in mice given pioglitazone for 5 consecutive days prior to PH. In KK-Ay mice, BrdU incorporation to hepatocyte nuclei 48 hr after PH was as low as 2%; however, pioglitazone pretreatment increased BrdU-positive cells to 8% (p<0.05), the levels being almost 1/3 of Bl/6 given PH. Cyclin D1 was barely detectable in hepatotomized livers in KK-Ay mice within 48 hr. In contrast, weak, but overt expression of cyclin D1 was observed 24 hr after PH in KK-Ay pretreated with pioglitazone. Hepatic expression of TNF-α mRNA was tremendously increased 1 hr after PH in KK-Ay, the levels reaching 9-fold over Bl/6 given PH. Pretreatment with pioglitazone blunted this increase almost 3/4. Serum IL-6 levels were also elevated extensively 24 hr after PH in KK-Ay mice, while the levels were largely decreased in KK-Ay mice given pioglitazone. Further, aberrant increases in phosphorylation of STAT-3 and socs-3 mRNA in the liver in KK-Ay mice were also tended to be normalized by pioglitazone. Interestingly, hypothalamic neocortex observed in KK-Ay mice was corrected by pioglitazone pretreatment to the levels almost similar to Bl/6 controls. Conclusions: These findings indicated that pioglitazone reverted, in part, poor regenerative responses in the liver in KK-Ay mice. The mechanism underlying the effect of pioglitazone on regeneration failure most likely involves normalization of expression pattern of adipokines and subsequent cytokine responses during the early stage of PH. It is therefore postulated that TZDs prevent progression of NASH in part through restoration of tissue repairing responses in the liver.
Disclosures:
The following people have nothing to disclose: Tomonori Aoyama, Kenichi Ikejima, Kazzuyoshi Kon, Kyoko Okumura, Kumiko Arai, Sumio Watanabe

1174
MECHANISM OF SERUM ALT INCREASE IN EXPERIMENTAL NASH
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Purpose: Serum ALT is a biomarker of hepatocyte injury in hepatitis of various etiologies including NASH. Increased activity is thought to be related to increased release from hepatocytes. We sought to understand the mechanisms involved in models of experimental NASH hypothesizing that serum values reflect increased hepatocyte expression rather than hepatocyte injury. Methods: Experimental NASH was produced in female A/J mice by feeding a methionine and choline deficient (MCD) diet versus an identical replete (MCR) diet and by treating mouse AMI-12 hepatocytes in culture with MCD versus MCR medium ± the P13-kinase inhibitor LY294002 (LY). ALT activity was measured enzymatically, expressions of the ALT isoforms ALT1 and ALT2 by Western blot, and abundance of ALT1 and ALT2
mRNA by real-time PCR. Significance of results for MCD v. MCR was determined by t-test: all reported results were significant at p<0.05. Results: Serum ALT activity in mice fed the MCD diet for 12 weeks was 383 ±5.6 U/L v. 92 ± 2.1 for MCR. The expressions of ALT1 and ALT2 protein in serum were proportionately increased; 2-fold and 1.5-fold, respectively. The expressions of ALT1 and ALT2 protein in liver tissue were increased to a degree greater than in serum; 7-fold for ALT1 and 10-fold for ALT2. Hepatic protein expression correlated with mRNA expression: 2-fold increased for ALT1 and 4-fold for ALT2. The medium from 18-hr MCD treated AML-12 cells showed increased ALT activity (81.5 ± 7.8 U/L v. 57.5 ± 0.7), and the cellular ALT activity was proportionately increased (80.1 ± 1.6 U/L/mg protein v. 44.1 ± 1.6). Cellular ALT protein and mRNA expressions were correspondingly increased after treating with MCD medium. The increased medium ALT, cellular ALT activity and cellular ALT1 and ALT2 protein and mRNA expressions were completely eliminated by treating with LY. In all in vivo and in vitro conditions, hepatocyte necrosis and apoptosis were measured to be insignificantly increased. Conclusions: Increased serum and medium ALT activity in these mouse models of experimental NASH correlates closely with the increased hepatocyte expression of ALT protein and not with the degree of hepatocyte death. The increased ALT protein expression in liver/hepatocytes correlates with mRNA expression, suggesting a translational mechanism for increased expression. Increased in vitro ALT release and cellular protein and mRNA expression during MCD treatment is entirely dependent upon activating the PI3-kinase cell survival pathway. These findings suggest increased serum ALT in experimental NASH results from increased hepatic expression with a constant “leak”. The pathway of leak is to be explored.

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The following people have nothing to disclose: Rui Liu, Xiaomin Pan, Peter F. Whitington

1175 THE MITOCHONDRIAL SUPEROXIDE DISMUTASE 2 (SOD2) TARGETING SEQUENCE POLYMORPHISM IS ASSOCIATED WITH FIBROTIC NAFLD: CONSISTENT EVIDENCE FROM CASE-CONTROL AND INTRA-FAMILIAL ALLELIC ASSOCIATION STUDIES

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Family studies and studies in different ethnic groups suggest that susceptibility to progressive non-alcoholic fatty liver disease (NAFLD) - non-alcoholic steatohepatitis (NASH) and fibrosis - has a genetic component but the genetic variants underlying this susceptibility remain largely unknown. Studies in both animals and humans suggest that oxidative stress/lipid peroxidation are important in the pathogenesis of fibrosis in NAFLD with mitochondria an important source of reactive oxygen species (ROS). A small study in 63 patients has reported that homozygosity for the T allele, which encodes a valine in the mitochondrial targeting sequence of superoxide dismutase 2 (SOD2), associated with less efficient mitochondrial import, is more common in patients with NASH than in healthy controls. We have used two complementary approaches to examine this mechanistically plausible association. First, we performed a classical case-control allelic association study in a large series of patients with biopsy proven NAFLD to determine if the Ala/Val SOD2 single nucleotide polymorphism (SNP) is associated with disease severity. Second, we performed an intra-familial association study in “trios”, with an index child with fibrotic NAFLD and two surviving parents, to look for preferential transmission of either SOD2 allele to the affected child using transmission disequilibrium testing (TDT). This approach is not subject to the potential confounding effects inherent in case-control studies and is significantly more powerful at detecting true associations. Methods: In the case control study, 282 adult patients with biopsy-proven NAFLD were genotyped for the Ala/Val SOD2 SNP and associations were sought with the severity of fibrosis and the presence of hepatocyte ballooning using the NIH NASH CRN staging system for fibrosis. In the family study 71 trios with an index child with fibrotic NAFLD were genotyped for the SOD2 SNP and the TDT was applied. Results: In the case control study the presence of significant fibrosis (stage >1) increased with the number of Val (T) alleles; Ala/Ala 23%, Ala/Val 37%, Val/Val 45%, p=0.034. There was no association with hepatocyte ballooning. In the family study 55 families were “informative” in that one or both parents were heterozygous for the SNP. In these families the Val allele was transmitted on 47/76 (62%) possible occasions whereas the C allele was transmitted on only 29/76 (38%) occasions, p=0.038.

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1176 STUDY OF THE MITOCHONDRIAL COMPLEX I BY BLUE NATIVE ELECTROPHORESIS IN LEAN AND OB/OB MICE

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In previous studies we have shown that the activity of the mitochondrial respiratory chain (MRC) is decreased in patients with nonalcoholic steatohepatitis and in ob/ob mice. Moreover, treatment of ob/ob mice with rosiglitazone (RGZ) reduces the activity of complex I of the MRC. Mechanisms leading to this dysfunction are not known. The aims of this study were to determine the integrity state of mitochondrial complex I in normal and ob/ob mice and the effect of RGZ on this complex. Methods: In liver samples from normal and ob/ob mice as well as in liver from both type of animals treated with 1 mg/Kg/day RGZ for 12 weeks we measured: (1) activity of complex I by in-gel activity assay, (2) the level of fully assembled complex I in a BN-PAGE system, blotted, and incubated with antibody against subunit p39, (3) complex I assembly by second dimension SDS-PAGE, blotted, and incubate with antibodies against complex I subunit p39, p30 and p20. Results: (1) In-gel activity assay confirmed that activity of complex I was almost absent in the liver of the ob/ob mice and that RGZ treatment decreased markedly the activity of this complex in normal mice. (2) Complex I remained fully-assembled in RGZ-treated mice, but was completely unassembled in ob/ob mice. (3) Second dimension SDS-PAGE system demonstrated that levels of fully-assembled complex I was increased in RGZ-treated animals, while assembly of complex I was severely affected in ob/ob mice. Conclusion: This study demonstrates that the activity of complex I is markedly decreased in RGZ-treated and ob/ob mice, but, while in RGZ-treated animals it is likely due to a catalytic defect, in ob/ob mice it is due to a defect in the assembly process.

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TRB3 IS ASSOCIATED WITH HEPATIC LIPOGENESIS AND DYSLIPIDEMIA IN NAFLD
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Background & Aims: The AKT kinase mediates the insulin drive on lipogenesis in hepatocytes by inducing the transcription factor SREBP-1. The Drosophila Tribbles homolog TRB3 has been reported to inhibit AKT activity and lipogenesis in animal models of insulin resistance, and to promote ubiquitination and degradation of acetyl-coenzyme A carboxylase, rate limiting enzyme in lipid synthesis. Aim was to evaluate TRB3 expression and the correlation with steatosis and dyslipidemia in patients with NAFLD, and evaluate the effect of the functional TRB3 Glu84Arg polymorphism on the metabolic picture. Patients and Methods: Liver biopsies of 20 patients with NAFLD (10 NASH and 10 with simple steatosis according do Kleiner), and 15 without metabolic diseases were considered; gene expression measured by qRT-PCR and Western blotting; the TRB3 Glu84Arg polymorphism evaluated by restriction analysis. Results: TRB3 expression was lower in NASH compared to simple steatosis and controls (p<.05), and was negatively correlated with steatosis percentage, triglycerides/HDL ratio, and HOMA-IR index compared to those with simple steatosis and without metabolic diseases (p<.05). Decreased TRB3 expression was associated with increased lipogenesis and insulin resistance in NAFLD.

TRB3 IS ASSOCIATED WITH HEPATIC LIPOGENESIS AND DYSLIPIDEMIA IN NAFLD

Disclosures:
The following people have nothing to disclose: Luca Valentì, Raffaela Rametta, Paola Dongiovanni, Anna Ludovica Fracanzani, Giancarlo Roviaro, Silvia Fargion

HEPATIC ADIPOR2 SIGNALING REGULATES THE DEVELOPMENT OF NONALCOHOLIC STEATOHEPATITIS IN MICE
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Background and Aims: Decreased expression of AdipoR2 in the livers of patients with NASH has recently been reported, indicating the possibility that adiponectin signaling mediated by AdipoR2 plays an important role in the pathogenesis of NASH. How hepatic adiponectin resistance and sensitivity mediated by its receptor, AdipoR2, contributes to the progression of nonalcoholic steatohepatitis (NASH) is unclear. We examined the roles of hepatic AdipoR2 in NASH using an animal model. Methods: We fed C57BL/6 mice a methionine- and choline-deficient (MCD) diet for up to ten weeks and analyzed changes in liver pathology caused by a mouse AdipoR2 short hairpin RNA-expressing adenovirus, an AdipoR2-overexpressing adenovirus, or an adiponectin-overexpressing adenovirus. Results: Inhibition of hepatic AdipoR2 expression aggravated fatty changes, inflammation and fibrosis in steatohepatitis, increasing lipid peroxidation, and decreasing hepatic glutathione content. It decreased hepatic peroxisome proliferator-activated receptor (PPAR)-α activity and the expression of acyl-CoA oxidase (ACO) and catalase, and increased the expression of proinflammatory cytokines, chemokines, and adhesion molecules. Hepatic AdipoR2 overexpression significantly alleviated steatohepatitis, decreasing lipid peroxidation while increasing hepatic glutathione content, PPAR-α activity, and the expression of ACO and catalase. It reduced the expression of inflammatory cytokines, chemokines, and adhesion molecules. In primary cultures, the expression of AdipoR2 in activated hepatic stellate cells controlled the inhibition of proliferation and the promotion of apoptosis of these cells by adiponectin. Conclusions: Hepatic AdipoR2 signaling regulates steatohepatitis progression by changing PPAR-α activity and reactive oxygen species (ROS) accumulation, making liver AdipoR2 signaling a promising target in treating NASH.

Disclosures:
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PREVENTION OF HEPATIC FIBROSIS IN A MURINE MODEL OF METABOLIC SYNDROME WITH NONALCOHOLIC STEATOHEPATITIS (NASH)
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D-4F is an apolipoprotein A1 mimic, being investigated in clinical trials for amelioration of atherosclerosis. D-4F reduces lipid hydroperoxides in lipoproteins, increases paraoxonase

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activity in pre-beta HDL, converts HDL from pro- to anti-inflammatory, and enhances endothelial eNOS activity. We have studied D-4F in a mouse model of NASH with significant fibrosis. Methods: C57BL/6J male mice were given 7 months of a Western diet (WD) followed by 2.5 months of: group 1- WD, group 2-WD + D-4F (125 microgram/mouse/day), group 3-chow, group 4- chow + D-4F. Control groups: 6-month-old C57BL/6J mice (normal control), C57BL/6J mice fed WD for 6 months (baseline for fibrosis progression). Steatosis and inflammation were determined by semi-quantitative assessment. Fibrosis was determined by whole-section-scanning morphometry of Sirius red stained slides with correction for staining of large vessels. Results: Group 1 had very marked increases in body weight (40.0 ± 5.2 g), hypercholesterolemia (288.0 ± 5.4 mg/dl), hyperglycemia (270.2 ± 10.0mg/dl), hyperinsulinemia (3.3 ± 3.0 ng/ml), insulin resistance (HOMA-IR 62.8 ± 5.1), and hyperleptinemia (32.6 ± 1.9 ng/ml). Liver weight (2.7 ± 0.7g) was twice that of controls, AST (268 ± 29.6 U/L) and ALT (235.2 ± 23.5 U/L) were 5-12 x elevated, and there was evidence of oxidative stress in the liver with increased tissue TBARS (27.1 ± 4.0 nmol/g tissue) and decreased GSH (39.6 ± 4.8 nmol/mg protein). There was marked steatosis and inflammation, increased alpha-smooth muscle actin staining, and increased fibrosis with 4.6 ± 1.9% of surface area positive for Sirius red staining (compared to 0.1 ± 0.0% for 6 month control mice). In group 2, total cholesterol and leptin levels were decreased by half compared to group 1. There was no improvement in body weight, liver weight, hyperglycemia, hypertriglyceridemia, insulin resistance, or oxidative stress with D-4F. Group 2 showed no improvement in steatosis and a small decrease in inflammation. Nevertheless, D-4F reduced fibrosis from 4.6% in group 1 to 1.3 ± 0.3% in group 2 (p<0.025 group 1 vs 2). 6 month WD (fibrosis baseline): 0.42 ± 0.04% fibrosis. Group 3 and 4 showed comparable improvements in all biochemical measures, albeit no normalization, and comparable improvements in steatosis and inflammation. However fibrosis in group 4 (0.89 ± 0.21%) was significantly less than in group 3 (2.79± 0.66%), p<0.05. Conclusion: C57BL/6J mice given a WD for 9 months develop features of the metabolic syndrome with NASH and fibrosis. In this model, D-4F significantly prevented progression of fibrosis without improving steatohepatitis, oxidative stress, or insulin resistance.

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HEPATOCYTE SPECIFIC GP130 PROTECTS AGAINST THE DEVELOPMENT AND PROGRESSION OF STEATOHEPATITIS

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The metabolic syndrome and the related development of hepatic steatosis is a growing medical problem in western industrial countries. Inflammatory processes play an important role in the development of hepatic steatosis progressing to non-alcoholic steatohepatitis (NASH) and ultimately liver fibrosis. The role of pro-inflammatory IL-6-type cytokines in NASH remains unclear. Thus, we aimed to investigate the involvement of gp130, the shared receptor subunit of IL-6 cytokines in liver steatosis and subsequent NASH. For this aim we used hepatocyte-specific conditional gp130 knockout mice (hepaΔgp130). Furthermore we combined conditional knockout and knockin technology to achieve hepatocyte specific deletion of gp130-dependent RAS/Erk (hepaΔgp130RAS) or STAT (hepaΔgp130STAT) activation. Wildtype and knockout mice were fed a cholin-deficient, ethionin supplemented diet (CDE). As early as two weeks after the start of CDE diet, hepaΔgp130 mice exhibited signs of hepatic steatosis confirmed by Oil red and H&E staining, in contrast to their wildtype littermates that displayed no significant phenotype. After four weeks, the effect was even stronger with a higher level of hepatic steatosis in the hepaΔgp130 mice while wildtype mice remained unaffected. Additionally, we observed accumulation of collagen-fibres as initial signs of fibrosis in the hepaΔgp130 group. Metabolic analyses revealed an increased content of liver triglycerides and elevated fasting blood glucose levels in CDE fed hepaΔgp130 mice four weeks after treatment, further corroborating the histological phenotype. Significantly increased TNF-α and decreased adiponectin mRNA levels were found in liver samples of hepaΔgp130 mice compared to wildtype controls, demonstrating the inflammatory response. Moreover, gp130 deficient mice displayed a lethal phenotype after insulin stimulation, further showing the profound impairment of metabolic pathways. In order to identify intracellular signalling pathways, hepaΔgp130STAT and hepaΔgp130RAS mice also received the CDE diet. While no liver pathology was observed in hepaΔgp130RAS, hepaΔgp130STAT exhibited a phenotype similar to the gp130 deleted mice. This clearly demonstrates the central role for STAT3 in gp130 mediated liver protection in this novel model of NASH. We conclude that gp130 exerts important protective effects in the establishment and progression of steatohepatitis and fibrosis and might therefore represent a potential target for therapeutic intervention.

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NOVEL THERAPEUTIC APPROACH FOR NAFLD USING ANTIPLATELET AGENTS IN AN ANIMAL MODEL

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[BACKGROUND AND AIMS] We found that some NASH patients treated with antiplatelet agents showed a tendency towards decrease in the plasma levels of the transaminases and triglyceride. However, the precise mechanisms underlying the therapeutic efficacy of antiplatelet agents in NASH have not yet been elucidated. Therefore, we investigated the therapeutic effects of antiplatelet agents against NASH in an animal model. [METHODS] Experimental design 1: We used three classes of antiplatelet agents (aspirin, ticlopidine, cilostazol). Three hundred forty-four 6-week-old male Fisher rats were divided into five groups (choline-deficient, l-amino acid defined (CDAA) diet, CDAA diet + aspirin 5mg/kg/day, CDAA diet + ticlopidine 3mg/kg/day, CDAA diet + cilostazol 3mg/kg/day, normal diet) and sacrificed after 16 weeks. Experimental design 2: Three hundred forty-four 6-week-old male Fisher rats were divided into four groups (high-fat high-calorie (HF/HC) diet, HF/HC diet + cilostazol 3mg/kg/day, HF/HC diet + cilostazol 9mg/kg/day, normal diet) and sacrificed after 16 weeks. The serum levels of markers of inflammation, fibrosis, lipid metabo-
lism and glucose metabolism were measured along with high-sensitivity lipoprotein profiling. Liver fibrosis, inflammation and steatosis were quantified by Masson's Trichrome, HE and Oil red staining. Protein and RNA were isolated from various tissues, such as the liver, subcutaneous fat, mesenterial visceral fat and muscle. Protein kinase A, MAP kinase, hepatocyte growth factor (HGF), PDGF were also measured. [RESULTS] All of the antiplatelet agents produced improvement of the steatohepatitis. Especially, cilostazol markedly attenuated the hepatic steato-sis, inflammation and fibrosis in the CDAAM-fed rats as compared with other antiplatelet agents. The quantitative parameters of steatosis, inflammation and fibrosis were also ameliorated by treatment with cilostazol. We further investigated the mechanisms underlying the therapeutic effect of antiplatelet agents against NASH in the HF/HC model. The cilostazol-fed model showed low plasma leptin, low plasma triglyceride and high plasma HDL-cholesterol levels and also improved insulin resistance (p<0.001). Moreover, the cilosta-zol-fed models showed upregulation of protein kinase A and HGF, and downregulation of MAP kinase and PDGF (p<0.001). [CONCLUSION] Antiplatelet agents, as a novel therapeutic candidate for NASH, in particular, cilostazol, a selective cAMP phosphodiesterase inhibitor, produced marked improvement of the steatohepatitis through cAMP activation and PDGF downregulation. Our results open up a novel therapeu-tic lead for the treatment of NASH.

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ENDOCANNABINOID PRODUCTION BY MONOCYTES IS INCREASED IN NONALCOHOLIC STEATOHEPATITIS Onpan Cheung1, Carol C. Sargeant1, Vincenzo Di Marzo2, Puneet Puri1, Faridoddin Mirshahi1, Arun J. Sanyal1

The hepatic content of endogenous cannabinoids (EC) is increased in an animal model of diet-induced fatty liver. Monocytes are an important source of EC and also produce many cytokines that affect both metabolism and inflammation. Hepatic macrophages are derived from both circulating monocytes and resident macrophages. There are no data on EC production in human nonalcoholic steatohepatitis (NASH) or their effects on cytokine production by macrophages. SPECIFIC AIMS: (1) To compare EC production by circulating peripheral blood monocytes (PBM) from subjects with NASH to those with the metabolic syndrome but without nonalcoholic fatty liver disease (NAFLD) and lean normal controls. (2) To define the effects of methanandamide (mAEA), an EC, on PBM cytokine production. (3) To determine if PBM derived EC modulate the cytokine response of PBM to their natural activator lipopolysaccharide (LPS). METHODS: PBM from 15 subjects (NASH=5, metabolic syndrome=5, lean normal=5) were isolated on a Histopaque (LPS). METHODS: PBM from 15 subjects (NASH=5, metabolic syndrome=5, lean normal=5) were isolated on a Histopaque column. Anandamide (AEA), palmitoylethanolamide (PEA) and 2-arachidonylglycerol (2AG) production following LPS stimulation was measured by HPLC and results normalized to lipid content. Cytokines (interleukin (IL)-1β, 2, 4, 5, 6, 8, 10, 12p40, 12p70, 13, TNF-α, interferon-γ, IP-10, MIP-1β) produced by PBM were measured by EUSA following exposure to mAEA, LPS and LPS after CB1 and CB2 receptor antagonists (SR141716 and SR144528) pretreatment. RESULTS: Compared to normal controls and those with the metabolic syndrome, subjects with NAFLD produced significantly more AEA (mean values, pmol/mg lipid: 21 vs. 14 vs. 13, p<0.05) and PEA (48 vs. 31 vs. 20, p<0.05). 2AG was not detected. PBM treated with mAEA increased pro-inflammatory cytokines IL-8 (mean values, pg/ml: 1,335 vs. 848, p<0.03) and IL-1β (142 vs. 116, p<0.03) production while decreased that of TNF-α (136 vs. 223, p<0.001) from baseline. It also decreased anti-inflammatory cytokine IL-4 (33 vs. 102, p=ns) production. LPS increased IL-8 (1,380 vs. 762, p<0.03) and decreased IL-4 (18 vs. 116, P<0.002), IP-10 (33 vs. 68, p<0.0001) and IL-6 (1,094 vs. 1,185, p<0.005) production. Only LPS induced increase in IL-8 was abrogated by CB1 and CB2 receptor antagonists pretreatment. CONCLUSIONS: (1) EC production by PBM is significantly increased in NASH. (2) AEA causes a pro-inflammatory profile of cytokine production by PBM by increasing IL-1β and IL-8 while decreasing IL-4. (3) PBM production of IL-8 in response to LPS is mediated by EC acting via CB receptors in an autocrine manner. Disclosures: The following people have nothing to disclose: Onpan Cheung, Carol C. Sargeant, Vincenzo Di Marzo, Puneet Puri, Faridoddin Mirshahi, Arun J. Sanyal

THE TRADITIONAL JAPANESE KAMPO FORMULA KEISHIBUKURYOGAN AMELIORATES STEATOSIS, REDUCES OXIDATIVE STRESS AND INFLAMMATION, AND ULTIMATELY PREVENTS LIVER FIBROSIS IN A RABBIT MODEL OF NON ALCOHOLIC STEATOHEPATITIS Makoto Fujimoto1,2, Koichi Tsuneyama2,3, Masaburo Kainuma4, Nobuyasu Sekiya2, Yasuo Takano5, Katsutoshi Terasawa6,9, Carlo Selmi7,8, M. Eric Gerstwin7, Yukata Shimada1,2,3, Japanese Oriental Medicine, University of Toyama, Toyama, Japan; 4Diagnostic Pathology, University of Toyama, Toyama, Japan; 521st Century COE Program, University of Toyama, Toyama, Japan; 6Environmental Medicine and Infectious Diseases, Kyushu University, Fukuoka, Japan; 7Frontier Japanese Oriental Medicine, Chiba University, Chiba, Japan; 8Japanese Oriental Medicine, Chiba University, Chiba, Japan; 9Internal Medicine, University of California, Davis, Davis, CA; 8Clinical Sciences ‘Luigi Sacco’, University of Milan, Milan, Italy

The mechanisms responsible for the progression of non alco-holic fatty liver disease (NAFLD) to steatohepatitis (NASH) remain unknown although oxidative stress and inflammation appear to play a crucial role. Although traditional Chinese and Japanese medicine has been advocated for a variety of human diseases, the vast majority of such studies lack the appropriate quality controls, standardization, and evidence-based blind evaluation. We have been intrigued with specific Japanese herbal compounds (Kampo formulas) alleged to have significant antioxidant activity. We studied the effects of 12-week supple-ments with three Kampo formulas [keishibukuryogan (1% KBG, TJ-25), orenegokoto (1% OOT, TJ-15) and shoaikoto (1% SST, TJ-9)] or two proposed NASH treatments [i.e. vitamin E (VE) and pioglitazone (PG)] on a cholesterol-fed rabbit model of NAFLD/NASH. Control groups were fed with standard rabbit chow and SRC containing only 1% cholesterol (C). Our readouts included (i) markers of liver function, lipid and glucose metabolism; (ii) liver histology and tissue lipid contents; (iii) oxidative stress; and (iv) plasma mediators of inflamma-tion and fibrosis. Results demonstrate that (i) total cholesterol levels in group C were significantly higher compared to each of the other supplemented groups (P<0.01 for all comparisons) and the KBG group had the lowest total cholesterol levels with the expected exception of the group not receiving cholesterol nor treatments while no significant differences were observed
between treatment groups in fasting glucose, insulin levels, or transaminase activities. (ii) KGB treatment led to significantly lower levels of total cholesterol (P<0.01), free cholesterol (P<0.01), and triglycerides (P<0.05) in the liver tissues. KGB treatment was associated with significantly higher adiponectin levels. (iii) KGB treatment was associated with significantly lower levels of plasma lipid peroxide compared to group C; urinary amounts of 8-OhdG were significantly reduced in all 5 supplement groups. (iv) KGB treatment was characterized by significantly reduced proportions of α-SMA positive areas and hepatic stellate cells at immunohistochemistry. Plasma levels of hyaluronic acid and TGF-β1 were significantly lower in groups KGB, OGT, and VE compared to group C. In conclusion, Kampo formulas (KGB in particular) exert a significant protective effect in a dietary rabbit model of NAFLD/NASH through several mechanisms that ultimately prevent the progression to liver fibrosis. We should also emphasize the rigor of these data and the need not only for further observations but also for the evaluation of cellular and molecular mechanisms.

Disclosures:
The following people have nothing to disclose: Makoto Fujimoto, Kaichi Tsucheyama, Masaburo Kainuma, Nobuyasu Sekiya, Yasuo Takano, Katsusabu Terasawa, Carlo Selmi, M. Eric Gershwin, Yutaka Shimada

1184 PALMITATE-INDUCED LIPID ACCUMULATION IN HEPATOCYTES LEADS TO ACTIVATION AND PRO-FIBROTIC GENE EXPRESSION IN HEPATIC STELLATE CELLS – A NEW IN VITRO MODEL TO STUDY FIBROGENESIS IN NAFLD

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Nonalcoholic fatty liver disease (NAFLD) is a frequent and potentially progressive chronic liver disease. A subset of patients with NAFLD develop non-alcoholic steatohepatitis (NASH), hepatic fibrosis and end-stage liver disease. Current evidence indicates that hepatic stellate cells (HSC) play the central mediators of liver fibrosis. The molecular mechanism linking hepatocyte steatosis to activation of HSC, thereby promoting inflammation and fibrosis, are mainly unknown. Here, we demonstrate a novel in vitro model to study the effect of hepatic lipid accumulation on HSC. Methods and Results: After exposure of primary human hepatocytes (PHH) and human hepatoma cells (HUH7) to the saturated fatty acid palmitate (PA), significant intracellular lipid accumulation was documented morphologically and by colorimetric assays. Subsequently, conditioned-media (CM) from PA-treated cells were used for stimulation of human hepatic stellate cells (HSC), leading to a significant induction of Collagen I, TGF-β1, TIMP-1/2 and MCP-1 mRNA and protein expression, as assessed by quantitative PCR and ELISA. Furthermore, CM from PA-treated hepatocytes induced the proliferation and activation process of HSC. Moreover, reporter gene assays revealed a significantly enhanced NFκB activation in HSCs after stimulation with CM from PA-treated hepatocytes as compared to CM from control hepatocytes or HSC without CM exposure, respectively. Summary and Conclusion: These data indicate that lipid accumulation in hepatocytes causes the secretion of soluble mediators that induce activation of HSC as well as proliferation and expression of pro-fibrotic and pro-inflammatory genes in activated HSC. These findings demonstrate a potential mechanism how hepatic lipid accumulation contributes to the progression of inflammation and hepatic fibrosis in NAFLD. This study therefore provides an attractive in vitro model to study molecular mechanisms of fibrogenesis in NASH and to establish potential anti-inflammatory and anti-fibrotic therapeutic strategies.

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1185 DIETARY CONSTITUENTS IN MICE ARE REFLECTED IN THE HEPATIC LIPID PROFILE AS DETECTED BY CARBON-13 MAGIC ANGLE SPINNING MAGNETIC RESONANCE SPECTROSCOPY

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BACKGROUND AND AIMS: Techniques to enable assessment of hepatic lipids in intact tissue are required to assess the effect of dietary constituents on the development and progression of non-alcoholic fatty liver disease (NAFLD). Magic angle spinning magnetic resonance spectroscopy (MAS-MRS) is a quantitative, non-destructive technique for metabolic profiling of intact tissue. Studies have demonstrated that proton (1H) MAS-MRS can be used to detect and quantify lipid. In this proof-of-principle study, carbon-13 (13C) MAS-MRS was applied to the study of hepatic tissue from murine models of NAFLD. We aimed to validate 13C MAS-MRS by comparison with 1H MAS-MRS findings. We hypothesised that differences in the hepatic lipid profile, in response to feeding diets with differing quantities of total lipid, monounsaturated (MUFA) and polyunsaturated (PUFA) fatty acids, are detectable by 13C MAS-MRS. METHODS: 6 mice of the C3H/HeH strain (susceptible to steatohepatitis) were housed under standard conditions and were fed either standard diet (StD) (4.2% fat by mass; 33% monounsaturated, 46% polyunsaturated) or high fat (HFD) (24% fat; 45% monounsaturated, 18.5% polyunsaturated) diets from age 6 weeks to culling at 36 weeks. In vitro hepatic MAS-MRS was carried out using a 500MHz JEOL ECP NMR spectrometer, with spin speed of 3500Hz at 4°C. 13C, 1H and 2-dimensional (2D) heteronuclear multiple quantum correlation (HMQC) MR spectra were obtained. Spectral peaks were assigned using published data and cross-correlated using HMQC. Quantification was by integration of peaks divided by total signal. A MUFA to PUFA index (MUFA:PUFA) was derived from a ratio of lipid resonance integrals at 128 and 129.8ppm. Livers were graded histologically for steatosis, fibrosis and inflammation. RESULTS: High quality 13C MAS-MRS spectra were obtained from all samples. The total lipid was greater in HFD mice compared to StD (p<0.05) and lipid differences were consistent between 13C and 1H MR spectra (absolute lipid CH2 resonance 2.9x (13C) and 2.7x (1H) higher in HFD vs StD). The mean (±SEM) MUFA:PUFA index was 1.7 (±0.15) in the HFD group versus 0.35 (±0.09) in StD (p=0.001), closely reflecting dietary constituents. A higher MUFA:PUFA index was also associated with increased disease severity as assessed histologically. CONCLUSIONS: 13C MAS-MRS has been applied to the study of intrahepatic lipid in intact tissue in response to dietary intervention and demonstrates a quantitative lipid profile, which corresponds to both dietary constituents and disease severity. This technique may be applied to further murine models and diets, and the findings translated to longitudinal in vivo studies.
1186 ADENOVIRUS-MEDIATED TRANSFER AND INDUCTION OF HEPATIC HEME OXYGENASE-1 IN THE LIVER PROTECTS AGAINST STEATOHEPATITIS IN VITRO AND IN VIVO

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Background and Aims: Non-alcoholic fatty liver disease can range from a benign condition (steatosis) to inflammation, and fibrosis (steatohepatitis). Oxidative stress has been suggested to play a novel role in transition from steatosis to steatohepatitis. Heme oxygenase-1 (HO-1), an antioxidant defense enzyme, has been shown to protect against oxidant-induced tissue injury. The aim of the present study was to investigate the role of HO-1 induction by adenovirus-mediated gene transfer or by chemical inducer in nutritional steatohepatitis both in vitro and in vivo. Methods: AML-12 hepatocytes were cultured in lipogenic medium and transfected with or without a recombinant adenovirus expressing the HO-1 gene (Ad-HO-1) or with or without HO-1 inducer SnMP (0, 1, 3, 5, 10 and 15 µM) or HO-1 inhibitor SnMP (0, 1, 3, 5, 10 and 15 µM) for up to 24 hours. Male C57BL6 mice were fed MCD or the control diet, with or without HO-1 inducer Hemin (25 mg/kg, 3 times per week) or HO-1 inhibitor SnMP (30 µM/kg, once weekly) for up to 3 weeks. AML-12 cells or livers were assessed for inflammatory and hepatocellular injury, triglyceride content, lipid peroxidation and HO-1 levels. Results: AML-12 cells exposed to MCD medium developed significant steatosis, increased loss of ALT into the medium and increased oxidative injury. Infection of AML-12 cells with Ad-HO-1 resulted in a dose-dependent increase in the expression of HO-1 mRNA and protein concomitant with a dose-dependent suppression of steatosis (0.16±0.03 mmol/L at highest Ad-HO-1 concentration vs 0.25±0.01 MCD control), ALT (71.0±8.0 U/L vs 207.5±7.8) and lipid peroxidation levels (1.96±1.03 nmol/mg protein vs 4.76±0.83). A similar effect was observed by treated with Hemin. Co-treatment with SnMP completely abolished Ad-HO-1 or Hemin-mediated protection in hepatocytes. Treatment of mice with hemin significantly attenuated MCD-induced steatohepatitis. In contrast, the severity of liver histology was more pronounced in MCD feeding mice treated with SnMP compared with MCD only. Conclusions: Our results have demonstrated that HO-1 is critical in the progression of nutritional steatohepatitis. Induction of HO-1 through adenovirus-mediated transfer of the HO-1 gene or HO-1 inducer may provide a novel therapeutic approach in treating this disorder.

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1188 GREEN TEA POLYPHENOLS IMPROVE LIVER INJURY OF NONALCOHOLIC STEATOHEPATITIS (NASH) MODEL MICE EXPRESSING NUCLEAR STEROL REGULATORY ELEMENT-BINDING PROTEIN 1C (nSREBP-1c) IN ADIPOSE TISSUE

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Background/Aim: Part of patients with NASH progress to end-stage liver disease such as cirrhosis and hepatocellular carcinoma, and the therapy of NASH is very important. In the present study, we examined whether green tea polyphenols (GTPs) including catechins improve liver injury of NASH model mice expressing nuclear sterol regulatory element-binding protein 1c (nSREBP-1c) in adipose tissue. Materials and Methods: nSREBP-1c transgenic C57BL6 male mice aged 30 weeks, that showed the typical NASH in liver histology at this age, were prepared in this study. These mice were shared three groups (Group 1; mice given water containing high dose GTPs (0.1%), Group 2; mice given water containing low dose GTPs (0.01%), and Group 3; control mice given water without GTPs). After treatment for 12 weeks, these mice were bled, humanely killed and examined as follows. 1) Ratio of liver weight to body weight, 2) Biochemical assays containing serum AST and ALT. 3) Morphometry of liver specimens according to the grading and staging of NASH proposed by Brunt et al., 4) Immunohistochemistry using antibody of 8-hydro-2'-deoxygenansine (8OhdG) to estimate oxidative stress, 5) Western blotting using antibodies of insulin receptor (IR), insulin receptor substrate (IRS)-1 and phosphorylated IRS-1 (pIRS-1) for insulin signaling, and using antibodies of Akt, pAkt, pIKKβ, NFkβ and pNFkβ for TNFα signaling. Results: 1) Ratio of liver weight to body weight in the high dose GTPs-treated group (group 1) was significantly lower than those of the groups 2 and 3 (p<0.05, respectively). 2) Blood AST, ALT, glucose, total cholesterol, and triglyceride levels of the group 1 were significantly low compared with that of the GTPs-no treated group (group 3) (p<0.05, respectively). 3) The degrees of steatosis, inflammation, ballooning hepatocytes, Mallory bodies, lipogranuloma and fibrosis in the group 1 significantly improved compared with those in the group 3 (p<0.01, respectively). 4) 8OhdG immunolocalization in liver tissues of the group 1 obviously decreased compared with those of the groups 2 and 3. 5) In Western blotting, the expressions of IR and pIRS-1 in liver tissues of the group 1 increased compared with those of the groups 2 and 3. On the other hands, the expressions of pAkt, pIKKβ and pNFkβ decreased compared with those of the groups 2 and 3. Conclusions: These results indicate that GTPs have the effects of anti-inflammation, anti-insulin resistance and anti-oxidative stress, and improve the liver injury of the transgenic mice expressing nSREBP-1c in the adipose tissue. Moreover, GTPs containing catechins are suggested to be useful for the therapy of patients with NASH

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1189 TRANS-FATTY ACID POTENTIATES ACCUMULATION OF TRIGLYCERIDE AND SUSCEPTIBILITY TO OXIDATIVE STRESS IN HEPATOCYTES

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Intake of trans-fatty acid is well known as potent risk factor of coronary heart disease. It has been reported that trans-fatty acid can aggravate insulin resistance, however, it has not been addressed the effect of trans-fatty acid on steatohepatitis. Therefore, our aim in this study was to investigate the effect of trans-fatty acid on fatty accumulation and susceptibility to reactive oxygen species (ROS) in isolated mouse hepatocytes. Methods: Hepatocytes were isolated from C57BL/6 mice 12 weeks after birth by collagenase perfusion and differential centrifugations, and cells were incubated in Waymouth's medium supplemented with 10% FBS for 20 hours. Cells were preincubated for 20 hours with oleic acid (cis-fatty acid, 0.1-1 mM) or elaicid acid (trans-fatty acid, 0.1-1 mM), and accumulation of triglyceride in hepatocytes was identified by Oil red O staining. Cell killing and production of ROS in isolated hepatocytes were determined fluorometrically using propidium iodide and 5-(and-6)-chloromethyl-2',7'-dichlorodihydrofluorescein diacetate acetyl ester (DCF), respectively. Results: Hepatocytes treated with 1 mM oleic acid accumulated triglyceride deposit in cytosol occupying 8±1% of cell area, and treatment with elaicid acid increased accumulation of triglyceride deposit to 20±2%, significantly (p<0.001). Exposure to 20 µM tert-butyl hydroperoxide (t-BuOOH), which induces NAD(P)H oxidation and mitochondrial ROS generation, did not cause significant cell killing within 30 min, and when cells were pretreated with 1 mM oleic acid, t-BuOOH did not affect until 20 min and increased cell killing to 29±3% after 30 min (p<0.05). Surprisingly, treatment with 1 mM elaicid acid increased cell killing even after 15 min of treatment significantly (p<0.05 vs. control) and reached to 34±3% after 30 min. Exposure to 20 mM t-BuOOH increased DCF fluorescence to 354±25% of baseline. Increment of DCF fluorescence after t-BuOOH was enhanced by pretreatment with 1 mM oleic acid to 488±34% of baseline after 30 min, and 1 mM elaicid acid increased DCF fluorescence to 59±21% of baseline after 30 min, significantly (p<0.05, vs. oleic acid). Pretreatment with elaicid acid at concentration as low as 0.1 mM increased DCF fluorescence after t-BuOOH to 489±42% of baseline significantly (p<0.05, vs. control), whereas 0.1 mM oleic acid did not affect DCF fluorescence. Conclusions: Our data demonstrated that trans-fatty acid enhances fatty accumulation and increases susceptibility to t-BuOOH-induced mitochondrial ROS generation in isolated hepatocytes. Therefore, it is concluded that trans-fatty acid is a potent exacerbating factor for hepatocellular injury in steatohepatitis.

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1190 LOSS OF β-CATENIN EXACERBATES MEHONINE-CHOLINE-DEFICIENT DIET INDUCED STEATOHEPATITIS IN MICE

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Background and Purpose: The protein β-catenin plays an important role in liver development, growth, and homeostasis. The
purpose of this study was to evaluate the effect of the methionine-choline-deficient (MCD) diet, which induces steatohepatitis in wild-type mice, in liver-specific β-catenin knockout mice. Methods: Mice with liver-specific disruption in β-catenin and wild-type littermates were fed MCD or control (replete in methionine and choline) diets for 2 weeks. Animals were then sacrificed for analysis of liver histology, gene expression by real-time PCR, and serum biochemistries. Results: On the MCD diet, β-catenin knockout animals exhibited striking macrovesicular hepatic steatosis (80-100%), grade 3-4 inflammation, and stage 1-2 fibrosis, compared with mild to moderate steatosis and no fibrosis in control animals. On the control diet, knockout mice had mild microvesicular steatoh hepatitis and two-fold higher serum transaminases compared with normal histology and transaminase levels in wild-type animals. Knockout mice had higher levels of hepatic lipid peroxidation on both control and MCD diets. No significant change in total β-catenin protein levels was noted in wild-type animals on the MCD diet. In knockout mice, liver injury occurred despite low expression of peroxisomal Cytochrome P450 2E1, a downstream target of β-catenin. Knockout mice had significantly lower expression of Peroxisome proliferator-activated receptor-alpha and microsomal triglyceride transfer protein, suggesting that decreased beta-oxidation of fatty acids and defective very low density lipoprotein secretion may be responsible for the liver phenotype seen in the knockout mice. Expression of SREBP1 was decreased in the knockout mice on the MCD diet, which may represent a compensatory decrease in lipid biosynthesis. Conclusion: Liver-specific β-catenin knockout mice show striking increase in liver injury on the MCD diet, suggesting an important protective role of β-catenin in the pathogenesis of liver injury in this model of steatohepatitis.

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1191
Dietary zinc supplementation reverses alcoholic fatty liver in the presence of chronic alcoholic administration
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Zinc deficiency has been documented in alcoholic liver disease and may contribute to the pathogenesis of liver injury. Alcoholic fatty liver, which is characterized by lipid droplets in the hepatocyte, is one of the earliest pathologic changes in the liver. The present study was undertaken to determine whether or not dietary zinc supplementation modulates lipid metabolism through regulation of gene expression, thereby attenuating alcoholic fatty liver. Mice (129 SEV) were pair-fed Lieber-DeCarli liquid diets containing alcohol or isocaloric maltose dextrin for 3 months, followed by dietary zinc supplementation for 3 weeks with the concomitant presence of alcohol exposure. Chronic alcohol exposure caused hepatic steatosis as demonstrated by oil red O staining and quantitative assay of triglyceride and cholesterol concentrations. Zinc supplementation reduced alcohol-induced lipid accumulation in the liver. To understand the mechanism of zinc action, the gene expression involved in lipogenesis, fatty acid β-oxidation, and very-low-density lipoprotein (VLDL) secretion were determined by real time RT-PCR assay. Zinc supplementation significantly increased the mRNA levels of acyl-coenzyme A dehydrogenase, microsomal triglyceride transfer protein, and apolipoprotein B, although the fatty acid synthase was not affected by zinc. Zinc supplementation also enhanced the gene expression of transcription factors involved in regulation of lipid metabolism, including hepatocyte nuclear factor-4α, PPAR-α and C/EBP. The capacity of lipid export from the liver was estimated by in vivo assay of VLDL triglyceride secretion using the Triton WR1339 method. The secretion rate of hepatic VLDL triglycerides was decreased by alcoholic exposure, but increased by zinc supplementation. These results suggest that zinc reverses alcoholic fatty liver, at least in part, by enhancing both fatty acid β-oxidation and the lipid export capacity. (Supported in part by the National Institutes of Health grants and the Veterans Administration, Louisville)

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1192
Caveolin-1 upregulates endothelial capillary-like tubular formation and fenestral contraction in association with Rho GTPases in sinusoidal endothelial cells
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Background and aims: Endothelial tubular formation depends on cell migration, adhesion, and proliferation. Rho GTPases are major regulators of cell polarization and motility. Caveolin-1 (Cav-1) is essential for caveolae formation. We have proposed that hepatic sinusoidal endothelial fenestrae (SEF) correspond to the fused and interconnected caveolae. The present study was designed to elucidate the roles of the cytoskeleton, Rho GTPases, and Cav-1 processes in isolated rat sinusoidal endothelial cell (SEC) s in vitro tube formation. Materials and Methods: The SECs obtained from rat livers by collagenase infusion method were subjected to primary monolayer culture on Matrigel for 5-18 hours. The morphology of was observed by scanning electron microscopy (SEM), transmission electron microscopy (TEM). Formation of F-actin stress fibers was observed by confocal microscopy. RhoA, Rac1 and phosphorylated myosin light chain kinase were analyzed by Western blotting. Rho and Rac activation were measured by the binding assays. Results: The overlay of Matrigel initially induced changes in endothelial cell morphology characterized by the formation of dynamic cellular protrusions, enhancing cell migration and finally leading the assembly of discrete aggregates or cords of aligned cells resembling primitive capillary-like structures, concomitant with a decrease in number and diameter of the characteristic SEF. In this process, time course analysis of RhoA and Rac1 activation matches specific morphological aspects. Rho A activation increased at 5 hr, and then declined after 6 hr, but increased again at 18 hr. The second increase of RhoA activation corresponded to the stage of stress fibers formation running along the major cell axis. During the remodeling phase of network formation, the SECs showed an intense staining for phosphorylated MLC by Western blotting. Rac1 activation was detected at 5 hr, then progressively decreased and lower than the basal level after 18 hr. The levels of endogenous Cav-1 expression increased in a time-dependent manner when endothelial cells underwent proliferation, reaching the maximum level of 6 hr. Cav-1 expression occurred just prior to the formation of capillary-like tube. Conclusions: Spatial activation of Rac1 and RhoA is considered to be involved in the formation of capillary-like tubular network accompanying fenestral contraction in SEC, implying that endothelial migration and...
adhesion are essential for sinusoidal angiogenesis in the liver. Taken together, the above results indicate that Cav-1 may play an important role in the regulation of SEC proliferation as a prerequisite step in the process of angiogenesis.

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ROLE OF THE N-TERMINAL SEQUENCE OF BSEP (ABCB11) IN PROTEIN TRAFFICKING AND STABILITY

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Background: The bile salt export pump (Bsep, Spgp, ABCB11) is a polytopic membrane glycoprotein that exports bile salts out of the hepatocyte across the apical (canalicular) membrane into the bile canaliculus. The molecular mechanisms responsible for targeting Bsep to the apical membrane and its dynamic trafficking are poorly understood. It is suggested that Bsep has a unique trafficking pattern as it leaves the Golgi and goes to an intermediate compartment before it reaches the apical membrane. Comparison of the different Bsep and MDR1 homologues indicate that the N-terminus of Bsep is highly divergent.

Aim: To understand the role of the divergent N-terminus in Bsep trafficking and protein stability. Methods: We have generated a series of N-terminus deletion mutants of rat Bsep by primer design deletion including, Δ19, Δ38, Δ56, Δ61 and one C-terminal deletion of Δ5 amino acids, fused to yellow fluorescent protein (YFP) at the C-terminus. Constructs were transiently transfected into both a non-polarized human derived kidney cell line (HEK293) and the polarized canine derived kidney cell line (MDCK) in order to investigate cellular localization by confocal microscopy. In addition, cell lines that stably express the different mutant proteins were isolated for biochemical analysis. Western blots were used to measure the mutant protein levels, the effects of temperature shift on protein expression, and half-life. Results: In both polarized and non-polarized cells lines Bsep-YFP localizes to the apical and plasma membrane. Nevertheless expression levels are lower in the cells expressing deletions of Bsep compared to the wild type construct, indicating that the mutant protein might be unstable and partially degraded as they traffic to the apical membrane. Removal of up to 38 amino acids from the N-terminus has no effect on Bsep localization to the apical membrane. Both the wild type Bsep and the Δ38 have complex glycosylation indicated by PNGase F and EndoH treatment. Removal of 56 amino acids or more from the N-terminus causes Bsep to localize to intracellular compartments. Immunofluorescence of the Δ56 suggests that the protein localizes in the ER. Conclusion: The results of this study suggest that the N-terminus amino acid sequence of Bsep does not have an apical localization sequence. The deletion of the first 38 amino acids of Bsep still reaches the apical membrane. The larger deletion up 56 amino acids inhibits apical localization of the mutant protein in the ER, possibly due to changes in protein folding or stability. Thus it suggests that the N-terminus of Bsep is important for protein folding or stability.

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LIVER CELL VOLUME REGULATION AND ATP RELEASE REQUIRE INTACT VESICULAR TRAFFICKING PATHWAYS

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Physiological changes in liver cell volume serve as a signaling pathway that modulates a broad range of liver functions. Following solute uptake, hepatocyte volume increases, this in turn initiates an adaptive regulatory volume decrease (RVD). RVD depends on cellular ATP release and autocrine stimulation of P2 receptors. In neuronal cells, ATP release depends on exocytosis of ATP-enriched vesicles. The role of vesicular trafficking pathways in liver cell ATP release and RVD are unknown. The aim of the present study was to determine the role of vesicular trafficking in ATP release and RVD in a hepatocyte model. METHODS: Studies were performed in HTC rat hepatoma cells. Changes in cell volume were measured by Coulter Multisizer and ATP release by a luciferin-luciferase assay, reported as arbitrary light units (ALU). RESULTS: Exposure to hypotonic buffer (20%) resulted in relative increase in cell volume of 1.25 +/- 0.06 followed by complete RVD (100% recovery to basal levels) by 15 minutes. Incubation of cells with monensin, which disrupts secretory vesicle formation, inhibited RVD to only 43%, while exposure to nocodazole, which disrupts microtubules, resulted in RVD of only 41%. In parallel studies, hypotonic exposure (25%) increased relative ATP release by 3.96 +/- 0.43 ALU. Volume-stimulated ATP release was attenuated by both monensin (2.57 +/- 0.15 ALU, p<0.01) and nocodazole (1.79 +/- 0.08 ALU, p<0.01). Since neuronal synaptic vesicle exocytosis requires “priming” by PKC, the effects of a panel of PKC isoform-selective agents were assessed on volume-stimulated ATP release in HTC cells. Chelerythrine, which inhibits all PKC isoforms, decreased volume-stimulated ATP release by 56.1 +/- 6.4 % and calphostin C, which inhibits conventional and novel isoforms, decreased ATP release by 56.8 +/- 11.3% (p<0.01). Acute exposure to phorbol 12-myristate 13-acetate (PMA), which activates conventional and novel PKC isoforms, did not affect basal ATP release, but significantly augmented relative ATP release by 6.43 +/- 1.2 ALU after hypotonic exposure (25%) vs. 2.7 +/- 0.1 ALU without prior PMA (p<0.05). CONCLUSION: Together these studies demonstrate that i) liver cell volume regulation and ATP release require intact vesicular trafficking pathways, ii) PKC is necessary but not sufficient for ATP release, and iii) volume-stimulated ATP release is potentiated by PKC activation. These findings suggest that PKC may be functioning in part to dock or prime a pool of ATP-containing vesicles so that they enter a readily-releasable state responsive to other signals.

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THE SORTING OF ENDOCYTIC PROTEIN ALONG ACTIN AND MICROTUBULES IN THE HEPATOCYTE

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Introduction: Viruses, proteins, lipids and other molecules enter cells through endocytosis wherein they enter a complex of intracellular vesicles and tubules that undergo movement, fusion, and fusion along the cytoskeleton, as demonstrated here using primary rat hepatocytes. Presumably, these cytoskeletal-based processes lead to the sorting of endocytic material to different cellular compartments. However, previous studies using
Depolymerizing drugs have shown that vesicle processing leading to protein segregation can occur in the absence of an intact cytoskeleton. **Methods:** To determine if microtubule and actin-based movement of endosomes can lead to the segregation of protein, individual endocytic vesicles and their cargo were tracked during high resolution, multi-channel fluorescence microscopy assays using endocytic vesicles that were purified from rat liver as well as primary cultured rat hepatocytes. **Results:** An in vitro biochemical assay successfully reconstituted both actin and microtubule based movement associated with endocytic processing. Endocytic cargo underwent fission and protein segregation along actin and microtubules at characteristic rates. Fission resulted in the segregation of proteins that are targeted for different cellular compartments. The bile salt transporter (ntcp) and transferrin receptor (TR), segregated away from asialoorosomucoid (ASOR), an endocytic ligand targeted for degradation, while the epidermal growth factor receptor (EGFR), which is degraded, and the asialoglycoprotein receptor (ASGPR), which remains partially bound to ASOR, segregated less efficiently from ASOR. Fission rates for ntcp and transferrin receptor were dramatically elevated when in the presence of the ASOR ligand. Additionally the inhibition of PI3-Kinase reduced fission but not overall motility indicating that PI3-Kinase functions in the fission of intracellular vesicles. **Conclusion:** The segregation of protein was achieved by the generation of ‘pure’ vesicles, which were depleted of endocytic ligand. Pure vesicles resulted from cytoskeletal based trafficking both in living hepatocytes and in the reconstituted in vitro assay. The identification and modulation of the cytoskeletal based fission machinery has implications for diverse fields such as drug delivery and viral replication.

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**PROTEOMIC AND IMMUNOFLOURESCENCE ANALYSIS OF EARLY AND LATE ENDOCYTIC VESICLES ISOLATED FROM RAT HEPATOCYTES**

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**Introduction:** In hepatocytes, following binding of asialoorosomucoid (ASOR) to the asialoglycoprotein receptor (ASGPR), the complex internalizes into early endocytic vesicles that acidify, resulting in dissociation and eventual segregation of the receptor-ligand complex into discrete vesicles. The proteins that mediate these events have been only partially elucidated. To identify and characterize the molecular mediators of this endocytic processing we have developed a proteomic and immunofluorescence based analysis of purified early and late endocytic vesicles. **Methods:** Early and late endocytic vesicles were isolated from rat livers that were removed at 5 and 15 minutes respectively after portal venous injection of fluorescent ASOR. Following partial purification by gel chromatography and sucrose gradient centrifugation, fluorescent vesicles were highly purified by flow cytometry. Sorted endocytic vesicles were subjected to 1D SDS-PAGE and gel slices were digested with trypsin and peptides were analyzed by nanoLCMS/MS. Using Mascot search criteria with peptide score of ≥45 and with peptide hits ≥ 2 or more, over 200 proteins were identified. **Results:** Aside from the ASGPR, a number of integral membrane proteins were identified, including the bile salt export pump, and organic anion and glucose transporters. Several Rab and SNARE proteins were identified, as well as motor proteins including dynein heavy chain, myosin 1 heavy chain and non-muscle myosin heavy chain A. To validate identification of proteins in vesicles, we employed an in vitro microscopy assay to quantify co-localization between the identified proteins and vesicles containing fluorescent ASOR. 92% (n=1145) of 5 min ASOR-containing early vesicles and only 19% (n=453) of the 15 min ASOR-containing late vesicles co-localized with the ASGPR, consistent with late vesicles being largely post segregation. Rab 1a (early-52%, late-36%), Rab 8a (early-67%, late-54%), Rab 14 (early-76%, late-78%), and Rab 18 (early-58%, late-54%) were highly co-localized with both early and late vesicles. Rab27a (early-22%, late-58%) and SNAP-23 (early-35%, late-64%) were more enriched in late as compared to early vesicles. **Conclusions:** Proteins that may be important in vesicle trafficking and processing (e.g. Rab’s and SNAP’s) have been discovered by proteomic analysis of endocytic vesicles that have been highly enriched by flow cytometry. We hypothesize that these proteins, which were previously not known to be associated with these endocytic vesicles, form a scaffold that directs interaction of these vesicles with other regulatory and motor proteins. This will be examined in future studies.

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1198 EMODIN IMPAIRS THE REARRANGEMENT OF ADHESION MOLECULES INDUCED BY HCV

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Hepatocellular carcinoma (HCC) is a common malignancy in the world. Unresectable or metastatic HCC carries a poor prognosis, and local, nonsurgical therapies are applied. In our study of the role of hepatitis C virus (HCV) proteins in inducing progression of HCC, leading cells to spread in liver tissue and promoting metastasis, we investigated the role of HCV proteins in regulating the invasive properties of human HCC cell line, HepG2. HepG2 cells were used to establish stable polyclones expressing the entire genome or core protein of HCV. When we examined cells for their differences in invasion ability we found that HCV proteins influence HCC cell adhesion and cell migration. Since adhesive and migratory ability of cells depends on expression, localization and activity of a cluster of proteins named focal adhesion molecules, we performed a deeply analysis by using western blotting, fluorescence and immunoprecipitation techniques. Our results demonstrate that in HCV positive polyclones, FAK (focal adhesion kinase) as well as beta1-integrin and paxillin are over-expressed. In addition, HCV proteins alter localization of beta1-integrin and alpha-actinin and induce activation of FAK. The silencing of HCV core protein by a specific small interference RNA (siRNA) completely abrogates HCV-related effects. To assay a possible gene therapy approach we used a specific siRNA against FAK. FAK silencing only partially reverted the HCV-dependent focal adhesion dysregulation, thus we treated cells with emodin, an anthraquinone derivative, with anti-microbial, immunosuppressive and anti-proliferative effects. Interestingly, emodin was able to completely abrogate the acquired HCV-related invasiveness of cells, fact probably due to its capacity to influence both ECM molecules and a wide range of intracellular pathways, which include FAK but also other kinases such as PI3K/AKT, MAPK, and PKC.

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The following people have nothing to disclose: Anna Alisi, Alessandra Spaziani, Simona Anticoli, Clara Balsano

1199 EFFECTS OF HINT2, A PUTATIVE TUMOR SUPPRESSOR, ON MITOCHONDRIAL FUNCTIONS

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Background: We identified HINT2, a protein expressed predominantly in the liver and the first human HIT protein to be localized in mitochondria. HINT2 is an AMP-lysinase hydrolase. We reported that over-expression of HINT2 sensitizes cells to Fas-induced apoptosis and that expression of HINT2 was down-regulated in hepatocellular carcinoma. Aim: to investigate the effects of HINT2 expression on mitochondria functions. Methods: HepG2 cells stably transfected to over-express HINT2 (20-fold excess), cells stably transfected to knock down HINT2 expression (10-fold decrease) and cells transfected with each control vectors. Enzymatic activities of the respiratory chain complexes were measured. Oxygen consumption of isolated mitochondria was assessed with a Clark-type electrode. Data were normalized to the activity of the citrate synthase. Oxygen consumption of whole cells was measured by high-resolution respirometry using an Oroboros Oxygraph-2k. Intracellular ATP levels were determined with kit HS II (Roche Applied).

Results: In isolated mitochondria, the activities of the complexes I to IV as well as the oxygen consumption were not affected by modifications of HINT2 expression. Respirometry on whole cells expressing less HINT2 show consistently less state 3 respiration in the presence of ADP using pyruvate/malate, succinate or ascorbate/TMPD as substrates respectively. This was associated with a significantly lower intracellular ATP content. Conclusion: Diminished expression of HINT2 impairs cellular respiration and affects ATP content. These effects are detected at the level of the whole cells and not at the level of isolated organelles suggesting on effect on the cellular mitochondrial mass.

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1200 HEPATITIS C VIRUSINDUCES IFN-A IN PLASMACYTOID DENDRITIC CELLS AND AFFECTS DIFFERENTIALLY STIMULATION VIA TOLL-LIKE RECEPTORS 7 AND 9

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Plasmacytoid dendritic cells (PDC) are responsible for production of a vast amount of type I IFN during viral infection. Therefore, eradication of hepatitis C virus (HCV) in more than 50% of chronically infected patients treated with IFN-a-based therapy suggests a major role of PDC in the control of HCV infection. In previous studies, the functional activity of PDC obtained from patients with chronic HCV infection was tested after ex vivo exposure to synthetic stimulators of Toll-like receptor 7 (TLR7) or TLR9. Here, we assayed the direct effects of HCV on functionality of purified PDC. We found that exposure of PDC to molecular clone HCV JFH-1 or to HCV from sera of chronically infected patients resulted in production of IFN-a and TNF-a.
an optimal multiplicity of infection, the HCV-exposed PDC produced significantly less IFN-α and TNF-α than did PDC exposed to influenza virus or human herpesvirus type 1. HCV did not induce maturation of PDC and had no effect on PDC apoptosis. Our experiments with thymoactivated virus and with inhibitors of endocytosis suggest that HCV stimulates PDC predominantly via TLR by the endocytosis pathway. Importantly, HCV reduced PDC-associated production of IFN-α induced with TLR9 agonist CpG more profoundly than that stimulated with TLR7 agonist resiquimod. Differential effect of HCV on stimulation of PDC via TLR7 or TLR9 may be important in the development of new anti-HCV therapies based on TLR agonists.

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HEPATIC STEATOSIS AND GLOMERULAR DISEASE IN MICE WITH C-TERMINALLY TRUNCATED GP73 (GOLPH2)

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GP73 (Golph2) is a type II integral membrane Golgi-localized protein of unknown function that is expressed constitutively in epithelial cells of many human tissues. GP73 is overexpressed in hepatocytes of patients with liver disease, and appears in the serum of hepatocellular cancer patients (Marrero, J.A., et al., J Hepatol 43:1007; Block, T.M., et al., PNAS 102:779). We hypothesized that GP73 deficiency might affect epithelial tissue integrity. We tested this hypothesis using gene trap technology to generate mice with a truncation of the C-terminal 146 amino acids of GP73. The truncated protein is fused to a β-galactosidase (β-gal) marker. Expression analysis by RT-PCR and β-gal staining revealed that wild-type and truncated GP73 were highly expressed in epithelial cells of the murine gastrointestinal tract and uterus, but present at low levels in liver and kidney. Notably, carbon tetrachloride-induced liver injury was associated with increased mRNA levels of wild-type GP73. Homozygous GP73-truncated mice were born at the expected rate and were fertile; the life span of males was normal. In contrast, females exhibited a 50% reduced cumulative 1.5 year survival compared to wild-type controls. Increased mortality rates first became evident at age 3 weeks, and continued to rise to late adulthood. Death in homozygous females was due to severe renal disease, as evidenced by advanced glomerular sclerosis, glomerular enlargement, glomerular hyaline thrombi, and degenerative changes in the proximal tubules. Homozygous mice developed hepatic microvesicular steatosis, intranuclear inclusions, and striking circumferential nuclear membrane irregularities. These nuclear changes suggested a loss of hepatocyte nuclear membrane integrity, and possibly of membrane stability. The mechanisms responsible for kidney and liver abnormalities in GP73-truncated mice are under investigation. Our study suggests a role for GP73 in normal epithelial cell function in the liver and kidney. This model will be a valuable tool to help elucidate the role of GP73 in hepatic carcinogenesis and renal function.

Disclosures:
The following people have nothing to disclose: Lorinda M. Wright, Maria Picken, Sherri Yong, Don C. Rockey, Scheherazade Nisar, Claus J. Fimmel

1202
PRIMARY HUMAN HEPATOCYTES UNDERGO DNA SYNTHESIS IN RESPONSE TO ENGINEERED FORMS OF HEPATOCYTE GROWTH FACTOR (HGF/SF): A PRE-REQUISITE TO USING HGF ANALOGUES CLINICALLY

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Hepatocyte Growth Factor (HGF/sf) is a 6-domain complex protein synthesised by mesenchymal cells, acting on target epithelial cells via a tyrosine kinase receptor (c-met). HGF/sf inactive single-chain precursor is converted to active two-chain form by cleavage of the linker connecting kringle 4 and sp domains. In experimental animals, HGF/sf can enhance regeneration, prevent and reverse fibrosis in chronic liver disease and enhance size and function at liver surgery. These effects would offer major therapeutic advantages if applicable in man, but production of wild-type protein is costly and difficult. Engineered variants of HGF/sf may offer advantages in respect of stability, altered ligand affinity, and ease of production, but require to be tested in human cells to justify development as therapeutic agents. Aim: To test the activity of engineered proteins in vitro in human hepatocytes by demonstrating the mitogenic effects of HGF/sf variants in human hepatocytes in culture, and the extent of DNA synthesis induced compared with wild type HGF/sf. Methods: We used the methylotrophic yeast P pastoris system to express hgf/sf fragments (Holmes 2007) nk1 and 1k1, using induction in BMGY medium and purification on Heparin-Sepharose CL-4B followed by cation exchange (Mono S) chromatography. We use the mouse myeloma N50 line for expression of full length proteins. Purification of hp21 mutant employed a Ni-NTA step prior to ion exchange. We dissociated the binding sites for the met receptor and for heparan-sulphate proteoglycans (HSPG) both in HGF/sf and in nk1. In nk1, which needs an intact high-affinity site, we mutated a secondary binding site for HSPG located in kringle 1, to create 1k1. Primary human hepatocytes were stimulated with nM doses of wild-type HGF/sf, HGF/sf21, nk1 or 1k1. 3Hthymidine incorporation was analysed in cells harvested for DNA. Results are expressed as fold stimulation over basal conditions. Results: Primary human hepatocytes undergo significant DNA synthesis in response to both wild-type (15-fold) and mutant (22-fold) HGF/sfs. The polypeptides (Nk1 and 1k1) although less active than wild type HGF/sf show a 3-4-fold stimulation. Conclusions: Engineered HGF isoforms, therefore, have the potential for use in man. The full length mutant hp21 is likely more active than the natural proteins in vivo as HSPGs greatly limit diffusion of the natural proteins in the tissue, and therefore access to target cells; moreover HSPG binding promotes degradation of HGF/sf. Whilst less active on a nanomolar basis than the full length protein, the polypeptides may have greater in vivo potential due to greater diffusivity and altered degradation.

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1203 THE ROLES OF LYSOSOMAL PROTEINASES IN PRIMARY HEPATOCYTE DEATH INDUCED BY TNF-α AND ACTINOMYCIN D

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Tumor necrosis factor-α (TNF-α) is a cytokine signaling that is comprised in cell growth and apoptosis in the liver. It has been suggested that hepatocyte death in the liver induced by TNF-α is similar to the morbidity of liver dysfunction, while the mechanism of this hepatocyte death is caspase-dependent/apoptosis. Cathepsin B (CB), a lysosomal cysteine proteinase, has been shown to be involved in primary hepatocyte death induced by TNF-α and actinomycin D (TNF/ActD). Lysosomal proteinases including CB and cathepsin D (CD), an aspartic proteinase, have also been suggested to participate in the execution of the caspase-dependent or -independent cell death pathway, using various types of cells. In these cell death pathways, lysosomal proteinases are suggested to be released into the cytosol because of lysosomal destabilization, while released proteinases may further be involved in the caspase-dependent or -independent cell death pathway. However, the precise molecular mechanism of how lysosomal proteinases participate in caspase-dependent or -independent cell death remains controversial. We therefore examined how lysosomal proteinases participate in TNF/ActD-induced hepatocyte death (adult mouse liver, 25 ng/ml TNF-α and 0.2 µg/ml actinomycin D). The treated hepatocytes died within 12 hr, and lysosomal proteinases in TUNEL-positive hepatocytes were immunohistochemically localized in granules but not in the cytosol. Such hepatocyte death was prevented by the treatment with BAF or Z-VAD-fmk, a pan-caspase inhibitor, until 18 hr after the start of cultures. The survival rate of hepatocytes treated with BAF decreased to that of hepatocytes treated without BAF at 24 hr; dying hepatocytes frequently possessed TUNEL-positive nuclei without activated forms of caspase-3/-7 but with LC3-positive granules in the cytoplasm. Since CA074 is known to inhibit specifically CB at a lower dose (10 µM) and not only CB but also cathepsin L (CL) at a high dose (300 µM), we examined the survival rate of hepatocytes that were cultured in the presence of low and high doses of CA074, respectively; it was significantly higher at a higher dose than at a lower dose. Moreover, the survival rate of hepatocytes was further increased by the combined treatment of CA074 with a high dose and pepstatin A, and this survival rate was similar to that treated by BAF alone. Moreover, TNF-α-induced hepatocyte death was inhibited by the treatment with both BAF and CA074 + pepstatin A in an additive fashion. These results suggest that lysosomal proteinases such as CB, CL and CD is involved in the TNF/ActD-induced hepatocyte death pathway that is independent of the apoptotic pathway.

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The following people have nothing to disclose: Noriyuki Yamamura, Toshiro Nishida, Kiyokazu Nakajima, Masahiro Shibata, Masato Koike, Yoshiki Sawa, Yasuo Uchiyama

1204 NATURAL POLYMORPHISM OF NS3 PROTEASE DOMAIN STRAINS HCV-1 IN HCV AND HIV/HCV COINFECTED PATIENTS: VIROLOGICAL AND CLINICAL IMPLICATION FOR DRUG RESISTANT VIRUSES

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Background: HCV NS3 protease is a chymotrypsin-like serine protease responsible for cleavage of the non-structural proteins of HCV and plays a pivotal role in viral life cycle. Selection of drug-resistant mutants was evidenced by in vitro and clinical studies with HCV NS3-4A protease inhibitors (PIs). It appeared that the mutations A71T, T72I, P88L, R155Q, A156T, D168V were selected in each studies conferring resistance level to each PI. Aim: The aim of this study was to describe the natural polymorphism of NS3 sequence in different HCV 1 strains and to compare the diversity of the protease in HCV monoinfected patients and in HIV/HCV coinfected patients receiving an HIV Protease inhibitor. Methods: 24 mono-infected genotype1 patients (13 g1b and 11 g 1a) and 24 HCV/HIV coinfected patients receiving anti-HIV PI therapy were selected in this study. NS3 protease domain was amplified by RT-PCR. PCR products were purified and directly sequenced (54-197). Multiple alignment of nucleotide and deduced amino acid sequences was inferred by Clustal_X version 1.64b. Statistical analysis was performed using Fisher’s exact test to compare the proportion of mutation at position 71, 72, 155, 156 and 168; Wilcoxon rank-sum test to evaluate if patients mono-infected and HIV/HCV co-infected had different clinical and virological variables distributions. Results: The mutation rate observed in the different position were not different for HCV and HIV mono-infected patients. No difference in aa sequences were found between genotype 1a and genotype 1b patients. Diversity on the protease was more frequently observed in position : 71, 72 whereas the position 155, 156 and 168 were well conserved whatever the subtype 1 nor the HIV coinfection status. Conclusions: In this cohort of HCV and HIV/HCV coinfected patients, the natural strains of the NS3 protease domain related to resistance to HCVPI were well conserved. There is no influence of the anti-HIV PI therapy on the mutation rate in the NS3 protease. This finding could have implication for monitoring of the patients receiving anti-HCV PIs.

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1205 MICROENVIRONMENTAL REGULATION OF SINOISODAL ENDOTHELIAL CELL PHENOTYPE

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Liver Sinusoidal Endothelial Cells (LSEC) are distinct from other vascular endothelial cells (EC) present in other tissues in their structural and functional phenotypic characteristics. For example, in contrast to other EC, LSEC display fenestrations, have low or absent expression of PECAM-1, and in rat tissue, they distinctively express the specific surface marker SE-1. Interestingly, these phenotypic characteristics are lost over time when LSEC are placed in culture. Since phenotypic maintenance is
critical to the development of accurate in vitro models and tissue engineered constructs, this study set out to examine the effect of various microenvironmental stimuli, such as tailoring of the extracellular matrix (ECM) and co-culture with supportive cell types, on LSEC phenotype. Methods: Immunohistochemistry and Western blotting were used to characterize expression of the specific EC markers RECA, SE-1, PECAM and AcLDL in isolated primary rat LSECs cultured under the following conditions: a) on the different ECM proteins including, Collagen-I, Fibronectin, Laminin, and Collagen-IV; b) with various combinations of supportive cells, using the micromechanical reconfigurable culture method (PNAS 104:5722;2007) to enable tracking of individual cell types, separation into pure populations for analysis, and deconvolution of contact-mediated versus soluble signals; and c) in the presence of the tyrosine phosphatase inhibitor, orthovanadate (OV). Results: We documented a decrease in the expression of SE-1 and increase expression of PECAM-1 when LSEC are placed in culture; and an effect of specific ECM components on the levels of expression of SE-1 was observed, suggesting a role of ECM in modulating LSEC phenotype. Significantly, SE-1 expression could be maintained for longer periods through co-culture—up to 14 days in the optimal configuration involving co-cultivation with both hepatocytes and fibroblasts. The data also suggest that direct contact between LSECs and support cells is not necessary. To begin to gain a mechanistic insight into these observations, we investigated the role of tyrosine phosphorylation of cellular proteins in maintaining LSEC phenotype since it has previously demonstrated that OV inhibited LSEC apoptosis (AJP 168:1097; 2006). We found that SE-1 expression was strongly maintaining at day 3 when they were cultured in the presence of OV, suggesting that a decrease in protein phosphorylation is involved also in the loss of the phenotype. Conclusions: This study has identified novel microenvironmental regulators of the LSEC phenotype, which should be important for the development of better in vitro models of liver tissue.

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1206 DEVELOPMENT AND CHARACTERIZATION OF MICROSCALE MODELS OF RAT AND HUMAN LIVERS
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Tissue function depends on hierarchical structures that extend from single cells to functional subunits. Conventional cell culture disperses tissues into single cells while neglecting higher-order processes. The convergence of semiconductor-driven microtechnology with the biomedical arena now allows fabrication of microscale tissue subunits towards functionally improving in vitro models. In the case of the liver, isolated hepatocytes are typically randomly distributed onto extracellular matrix without precise control over architecture and cell-cell interactions, which are known to impact hepatic functions. We sought to use microtechnology and heterotypic interactions between hepatocytes and nonparenchymal cells to create functionally robust, microscale models of rat and human livers. Methods: Primary rat hepatocytes were isolated from Lewis rats via collagenase perfusion while human hepatocytes were purchased from registered vendors. Soft lithography was used to generate micropatterned collagen domains in multi-well plates. The dimensions of collagen domains (500um circular diameter, 1200um center-to-center spacing) were empirically optimized to provide maximal hepatic functions via the balance of homotypic (hepatocyte-hepatocyte) and heterotypic (hepatocyte-fibroblast) interactions. Hepatocytes formed confluent clusters on collagen domains and were surrounded with fibroblasts shown to induce liver-specific functions. Micropatterned co-cultures were characterized for liver-specific gene expression (Affymetrix) and measurement of surrogate markers for diverse classes of liver functions (protein synthesis, nitrogen metabolism, detoxification). Results: Hepatocytes in our system retained in vivo-like morphology, expressed liver genes at levels highly similar to fresh hepatocyte controls, metabolized compounds using active Phase I/II enzymes, secreted diverse liver-specific products (albumin, urea), and displayed functional bile canaliculi for several weeks (4-6 for human, 10-12 for rat) in vitro. Micropatterned co-cultures outperformed conventional models (collagen sandwich, Matrigel overlay, random co-cultures) with regards to both magnitude of functions and longevity of cultures. We also quantified drug-drug interactions and toxicity of several clinical hepatotoxins in our system and found good qualitative correlation with in vivo findings. Conclusions: We utilized microtechnology to develop models of rat and human livers which remain functional for several weeks. In the future, improved models of liver tissue may be used for fundamental investigations of liver physiology and disease, cell-based therapies and toxicity screening in drug development.

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1207 SWELLING-INDED TRAFFICKING OF ACTIN-COATED VESICLES AND CONTROL OF HEPATOCYTOPLIAL VOLUME REGULATION BY MYOSIN LIGHT CHAIN KINASE
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Physiological hepatocellular swelling stimulates vesicular exocytosis, which is exemplified by delivery to the plasma membrane of bile salt and other organic anion transporters to promote bile flow and volume recovery. Such vesicular trafficking, in part, requires an organized actin cytoskeleton and is regulated by myosin motors. However, since cell swelling evokes substantial dismantling of the actin cytoskeleton, it is unknown how such trafficking is regulated under these conditions. To address this, we performed live imaging of HTC hepatoma cells that expressed a green fluorescent protein (GFP) fusion of actin. In response to swelling produced by hypotonic exposure, the cortical actin cytoskeleton became less organized with fewer microfilaments, and actin puncta appeared from tubulovesicular structures in the cell interior and moved outward. Some puncta moved along filaments and then by apparent actin dynamics, we imaged cells expressing a red fluorescent protein (RFP) actin fusion with GFP fusions of other proteins. The fusion of actin-coated vesicles associated with known regulators of trafficking and actin dynamics, we imaged cells expressing a red fluorescent protein (RFP) actin fusion with GFP fusions of other proteins. The
RFP–actin-labeled puncta colocalized and moved with GFP fusions of cortactin, the Rho GTPase Cdc42, as well as with the Cdc42 activator Vav2. Because myosin light chain kinase (MLCK) has been implicated as a regulator of vesicular trafficking and of cell volume control, we tested whether it influenced these processes in HTC cells. Hypotonic swelling in the presence of the selective MLCK inhibitor ML7 (10 uM) produced enlarged intracellular actin structures that appeared to result from vesicle fusion and trapping, and ML7 attenuated cell volume recovery by approximately 60%. These data support the hypothesis that MLCK and, by implication, myosin motors regulate swelling-induced trafficking of actin-coated vesicles. Regulation of trafficking of such vesicles by MLCK is likely to be critical for targeting key signaling proteins, such as cortactin, Vav2, and Cdc42 to the cell cortex to coordinate cytoskeletal reorganization during volume recovery.

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1208
OVEREXPRESSION OF CYP2E1 IN LIVER INCREASES INSULIN RESISTANCE AND INHIBITS INSULIN SIGNALING IN AN ANIMAL MODEL OF NASH
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BACKGROUND: Down regulation of insulin signaling, characterized by increased serine phosphorylation of IRS-1 and decreased phosphorylation of Akt and of FoxO1a, has been reported in hepatocytes overexpressing CYP2E1 in vitro. AIM of our study was to evaluate insulin signaling pathways in an animal model of CYP2E1 overexpression and insulin resistance/NASH. METHODS: Rodent chow containing high fat (20% of calories from fat) was fed to male CYP2E1 transgenic (Tg) and to non-transgenic (n-Tg) mice for 9 months (n=7 in each group). Tg mice contain human CYP2E1 cDNA under control of the mouse albumin enhancer-promoter and overexpress CYP2E1 in the liver. Fasting serum insulin and glucose were measured a week prior to euthanasia. Mice were allowed free access to food until euthanasia. Liver pathology was evaluated and mRNA expression of IRS-1, IRS-2, Akt and phosphorylated IRS-1, IRS-2, Akt, GSK3 and FoxO1a protein content were measured by immunoprecipitation and western blotting. RESULTS: Tg mice had increased liver injury (fat, pathology, ALT) and insulin resistance as measured by significantly increased fasting serum insulin and glucose levels. mRNA expression of IRS-1 was increased 13-fold in Tg mice although the total amount of IRS-1 protein was similar in both groups. Tyrosine phosphorylation of IRS-1 was decreased by 40% in Tg mice although serine phosphorylation at 307 and 635 was unchanged. mRNA expression and protein content of total IRS-2 and total Akt were similar in the two groups, while p-Akt was 30% lower in Tg than in n-Tg mice. Total GSK3 was similar in both groups, while p-GSK3 was decreased by 40% in Tg mice. We found a 2-fold decrease in the amount of phosphorylated (inactivated) FoxO1a in Tg mice, associated with a 2-fold increase in the mRNA expression of PEPCK. CONCLUSIONS: In an animal model of CYP2E1 overexpression and NASH, we found increased insulin resistance, as demonstrated by increased fasting insulin and glucose. Insulin signal transduction was decreased in CYP2E1 Tg mice, as shown by decreased tyrosine phosphorylation of IRS-1, Akt and GSK3. Importantly, in Tg mice phosphorylation (inactivation) of FoxO1a was decreased and expression of PEPCK was increased, consistent with increased gluconeogenesis. This experiment demonstrates several defects in insulin signal transduction in vivo in the setting of increased CYP2E1 expression, and further supports a role for CYP2E1 in insulin resistance and NASH.

Disclosures:
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1209
POST-TRANSLATIONAL MODIFICATION OF HEPATIC SCAVENGER RECEPTOR CLASS B, TYPE I AND CHYLOMICRON METABOLISM IN A MOUSE MODEL
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BACKGROUND: The liver plays a pivotal role in post-prandial lipoprotein metabolism. Remnants of chylomicrons are cleared from circulation exclusively by the liver by receptor-mediated mechanisms that are both apoE-dependent and apoE-independent. Recent work with mice showed that: a) the hepatic scavenger receptor class B type I (SR-BI), which functions as a receptor for high-density lipoproteins, is also a receptor for chylomicron remnants in a manner independent of apoE; b) activation of peroxisome proliferator-activated receptor alpha (PPARalpha) elicits post-transcriptional or post-translational down-regulation of SR-BI protein in wild-type and apoE-deficient mice; c) treatment of apoE-deficient mice with PPARalpha agonists inhibits the SR-BI-mediated uptake of remnants by the liver. We investigated the mechanism underlying PPARalpha-mediated post-translational regulation of hepatic SR-BI which affects chylomicron metabolism. Methods: Homozygous female apoE-deficient mice (5 mice/group) on C57BL/6J background (8-12-week-old) were treated with or without 0.05% (w/w) ciprofibrate for 30 days. The plasma was then collected for the lipoprotein profile analysis with FPLC separation, measurement of mass contents of cholesterol and triglycerides, and Western blot assay of apolipoproteins B100/B48. Livers were harvested for histochemistry analysis to detect the distribution of SR-BI; for Western blot assay for SR-BI protein expression; and for biochemical analysis of post-translational modification of SR-BI by glycosylation. Results: Treatment of apoE-deficient mice with ciprofibrate produced a 3-4-fold increase of total cholesterol content and apoB48-rich chylomicron remnants in the plasma. Western blot analysis with an antibody against the c-terminal of murine SR-BI revealed that the fibrate treatment produced multiple bands (from 82 kDa to 54 kDa) of SR-BI in liver homogenate samples. N-glycosidase F treatment of liver homogenate from apoE-deficient mice and Western blot assay for SR-BI demonstrated that fibrate treatment inhibited N-glycosylation of SR-BI. Immunohistochemistry analysis showed that fibrate induced an increased expression of SR-BI protein in the cytosol of hepatocytes, but decreased its expression in the plasma membrane. Cessation of the fibrate treatment reversed both accumulation of chylomicron remnants and reduction of 82 kDa SR-BI in the membrane fraction. Conclusion: PPARalpha agonist-induced post-translational modification of hepatic SR-BI decreases the presence of SR-BI on the surface of hepatocytes, and therefore, interferes with chylomicron remnant metabolism.

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Hepatocarcinogenesis.

We found upregulation of Wnt3 expression in human hepatocellular carcinoma (HCC) tissues compared to adjacent peritumoral tissues, whereas the expression level of Wnt11 was downregulated in HCC. Activation of the canonical Wnt signaling is often to be observed in HCC and Wnt3 is a major Wnt ligand to activate the canonical pathway in HCC. In contrast, the role of Wnt11, which is an activator for the non-canonical Wnt signaling pathway in hepatocarcinogenesis is completely unknown. In the present study, we investigated the effects of Wnt11 on the canonical Wnt signaling through the activation of the non-canonical pathway in HCC. Methods: We used FOCUS and Huh7 HCC cells overexpressing Wnt11 by transient and stable transfection. The canonical Wnt signaling was examined by Westernblotting, immunofluorescencstaining, and β-catenin-mediated T-cell factor (TCF) reporter assays. The non-canonical signaling was evaluated by Protein Kinase C (PKC) kinase assay, Rho pull down assay, and immunoblot analysis for CamKII and cJUN activity. A MTS assay was used for evaluation of cell proliferation rate. Results: Overexpression of Wnt11 in HCC cells decreased the total expression and nuclear localization of β-catenin compared to control cells. The levels of phospho-β-catenin, leading to β-catenin proteasomal degradation, increased in Wnt11 overexpressing cells. The β-catenin-mediated TCF transcriptional activity was also reduced by 50% in Wnt11 overexpressing cells. Knockdown of Wnt11 using RNA interference in Wnt11 overexpressing FOCUS cells (FOCUSWnt11) restored β-catenin expression and TCF transcriptional activity confirming that Wnt11 antagonized the Wnt/β-catenin pathway. In the non-canonical Wnt signaling pathway, overexpression of Wnt11 activated PKC kinase and Rho kinase activities and a PKC specific inhibitor, not a Rho inhibitor, reduced phosphorylation of β-catenin indicating that PKC was responsible for β-catenin phosphorylation in FOCUSWnt11 cells. Finally HCC cell proliferation rate was reduced in FOCUSWnt11 compared to control cells. Conclusion: Wnt11 stimulated PKC and Rho activity, and the activation of PKC was involved in the inhibition of the canonical Wnt signaling pathway, leading to the growth suppression in HCC cell lines, suggesting that distinct Wnt signaling pathways may be capable of crosstalk, leading to functional antagonism in hepatocarcinogenesis.

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The following people have nothing to disclose: Takashi Toyama, Han Chu Lee, Jack R. Wands, Miran Kim

Wnt11 inhibits the canonical Wnt pathway through the PKC-mediated β-catenin phosphor-ylation in human hepatoma cells

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Background and Aims: Wnt signaling plays an important role in early embryonic development and carcinogenesis. Recently we identified Wnt3 and Wnt11 as major Wnt ligands among 19 Wnt family members in human hepatoma cells. Furthermore, we found upregulation of Wnt3 expression in human hepatocellular carcinoma (HCC) tissues compared to adjacent peritumoral tissues, whereas the expression level of Wnt11 was downregulated in HCC. Activation of the canonical Wnt signaling is often to be observed in HCC and Wnt3 is a major Wnt ligand to activate the canonical pathway in HCC. In contrast, the role of Wnt11, which is an activator for the non-canonical Wnt signaling pathway in hepatocarcinogenesis is completely unknown. In the present study, we investigated the effects of Wnt11 on the canonical Wnt signaling through the activation of the non-canonical pathway in HCC. Methods: We used FOCUS and Huh7 HCC cells overexpressing Wnt11 by transient and stable transfection. The canonical Wnt signaling was examined by Westernblotting, immunofluorescencstaining, and β-catenin-mediated T-cell factor (TCF) reporter assays. The non-canonical signaling was evaluated by Protein Kinase C (PKC) kinase assay, Rho pull down assay, and immunoblot analysis for CamKII and cJUN activity. A MTS assay was used for evaluation of cell proliferation rate. Results: Overexpression of Wnt11 in HCC cells decreased the total expression and nuclear localization of β-catenin compared to control cells. The levels of phospho-β-catenin, leading to β-catenin proteasomal degradation, increased in Wnt11 overexpressing cells. The β-catenin-mediated TCF transcriptional activity was also reduced by 50% in Wnt11 overexpressing cells. Knockdown of Wnt11 using RNA interference in Wnt11 overexpressing FOCUS cells (FOCUSWnt11) restored β-catenin expression and TCF transcriptional activity confirming that Wnt11 antagonized the Wnt/β-catenin pathway. In the non-canonical Wnt signaling pathway, overexpression of Wnt11 activated PKC kinase and Rho kinase activities and a PKC specific inhibitor, not a Rho inhibitor, reduced phosphorylation of β-catenin indicating that PKC was responsible for β-catenin phosphorylation in FOCUSWnt11 cells. Finally HCC cell proliferation rate was reduced in FOCUSWnt11 compared to control cells. Conclusion: Wnt11 stimulated PKC and Rho activity, and the activation of PKC was involved in the inhibition of the canonical Wnt signaling pathway, leading to the growth suppression in HCC cell lines, suggesting that distinct Wnt signaling pathways may be capable of crosstalk, leading to functional antagonism in hepatocarcinogenesis.

Disclosures:
The following people have nothing to disclose: Takashi Toyama, Han Chu Lee, Jack R. Wands, Miran Kim

1211 Negative feedback regulation of β-catenin signaling by the microenvironment through a frizzled-like module in the extracellular matrix of hepatocellular carcinoma

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Collagen XVIII (C18) is an extracellular matrix component and a plasma protein secreted by hepatocytes. Of its variants contains a protein module of unknown function homologous to secreted frizzled-related proteins (SFRPs), which are inhibitors of the Wnt/β-catenin pathway, hence named frizzled (FZC18). The expression of the C18 variant containing FZC18 was assessed in 19 normal livers, 54 hepatocellular carcinomas (HCCs) and 49 matching non tumor areas. Increased FZC18 mRNA expression was associated with liver fibrosis (p<0.0001) and small (≤2 cm), well-differentiated HCCs (p=0.02). Immunohistochemistry was performed on serial sections of normal, cirrhotic liver and HCCs with anti-FZC18, anti-β-catenin and anti-glutamine synthetase (GS) antibodies. GS is a downstream target of β-catenin and a marker of activating β-catenin mutations in HCC (Oncogene, 26:7774). In normal liver, FZC18 epitopes were predominantly periportal, contrasting with the well-characterized pericentral vein localization of GS (Developmental Cell, 10: 759), indicating that FZC18 is associated with liver zones of low β-catenin pathway activation. In HCCs, FZC18 was negatively correlated with GS (r=−0.42; p=0.02; n=24) and cytoplasmic β-catenin staining (r=−0.47; p=0.02; n=23). Conversely, FZC18 was positively correlated with membranous β-catenin staining (r=0.67; p<0.001; n=23). As expected, nuclear and cytoplasmic β-catenin staining were highly correlated (r=0.89; p<0.001; n=23), as well as GS and nuclear (r=0.74; p<0.001; n=23) or cytoplasmic β-catenin (r=0.63; p<0.001; n=23). Moreover, the human HCC cell line Huh-7 (wild-type β-catenin and basal pathway activity) was transfected with a constitutively activated form of β-catenin (Δ29-48), which increased β-catenin pathway activity by 10 folds in a β-catenin/TCF reporter assay, as well as total and non phosphorylated β-catenin, GS and cyclin D1 protein levels. Cotransfection of FZC18 cDNA decreased β-catenin activity, including total and non phosphorylated β-catenin, together with GS and cyclin D1 proteins to normal levels. These findings indicate that the FZC18 module of collagen XVIII down-regulates β-catenin pathway activity in HCC. Thus, the microenvironment, which contains frizzled-like modules entrapped within the extracellular matrix, such as FZC18 and SFRPs, may negatively regulate the Wnt/β-catenin pathway and thus impede the progression of hepatocellular carcinoma.

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1212 Inactivation of caspase-8 affects hepatocyte proliferation after partial hepatectomy in mice

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The cytokine TNF plays a crucial role during early liver regeneration after partial hepatectomy (PH) triggering signaling path-
ways for cell proliferation (via NF-kB), activation of cJun N-terminal kinases (JNK) and apoptosis through caspase-8 activation. The aim of this study was to investigate the potential role of caspase-8 during liver regeneration. PH was performed in hepatocyte-specific caspase-8 knock-out (casp8Δhepa) mice and wildtype (WT) controls. Analysis of hepatocyte proliferation was determined by BrdU-staining from liver cryosections. Expression of cell cycle markers for G1- (cyclin D), G1/S- (cyclin E) and S-phase (cyclin A) were analysed by real-time PCR and immunoblotting. Histone H3 phosphorylation was used as a marker for M-phase progression. Phosphorylation and nuclear translocation of c-jun in hepatocytes was displayed using as a marker for M-phase progression. Phosphorylation and nuclear translocation of c-jun in hepatocytes was displayed through immunofluorescence microscopy. Apoptosis in the regenerating liver was monitored by TUNEL assay. Following PH, cyclin D1 expression in casp8Δhepa mice reached maximum levels 10 hours (h) earlier compared to controls with a peak already 30h after surgery. BrdU incorporation exhibited a 4 fold higher increase in casp8Δhepa mice 30h after PH and a prolonged S-phase compared to controls. Additionally, first expression of cyclin E and A was detected earlier in casp8Δhepa mice (30h post PH) compared to WT animals and reached its peak 12h before the control group (36h compared to 48h after PH). At time points 60h and later post surgery proliferation decreased within both groups to a similar level. M-phase analysis revealed higher number of mitotic cells in casp8Δhepa mice compared to WT animals in a time frame between 30h and 60h after surgery. Seven days after PH relevant levels of apoptosis were detected in WT mice, but not in casp8Δhepa animals indicating that apoptotic mechanisms are relevant for terminating liver regeneration. To further investigate the mechanism leading to earlier onset of cell cycle progression in casp8Δhepa mice, we analysed activation of c-jun, which is a known transactivator of cyclin D expression. Interestingly, already 30 minutes after surgery we observed phosphorylation and nuclear translocation of c-jun in casp8Δhepa mice, whereas first detection in WT animals was evident after 2 hours. Our data demonstrate that depletion of caspase-8 leads to an accelerated onset of liver regeneration after PH. We conclude that rapid activation of c-jun in casp8Δhepa mice contributes to earlier induction of cyclin D and subsequent cell cycle progression.

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1213

IGF-II IS A GROWTH FACTOR TO HEPATOBLASTOMA AND HEPATOCELLULAR CARCINOMA VIA PHOSPHATIDYL INOSITOL 3 KINASE AS A TARGET OF MOLECULAR THERAPY

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Hepatoblastoma (HBL) arises in livers of infants younger than 2 years of age, while hepatocellular carcinoma (HCC) in adults. Insulin-like growth factor (IGF-II), deeply involved in proliferation and differentiation, is more expressed in tumor than surrounding non-tumor of HBL and HCC. IGF-II is highly expressed in the blood of HBL patients. IGF-II binds IGF-I receptor (IGF-IR), and its stimulation is transferred via phosphatidyl inositol (PI) 3 kinase or mitogen activated protein (MAP) kinase. We addressed the possibility that IGF-II is a growth factor of HBL and HCC and its signaling pathway. Growth of HCC cell lines [HLE, HLF, PLC Huh-7, Hep3B] and HBL cell lines [Huh-6, HepG2] were analyzed with 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium inner salt (MTS) assay. MTS is bioreduced by cells into a colored formazan product that reduces absorbance at 490nm. MTS assay was performed after 72 hours culture with IGF-II (0, 2, 20, 200 ng/ml) following 24 hours of serum free culture. Protein was isolated after 48 hours serum free culture for Western blot analysis with IGF-IR antibody. LY294002 (50 μM), a specific inhibitor of PI3 kinase or PD98059 (20 μM), a specific inhibitor of MAP kinase, was added with IGF-II (200 ng/ml) for 72 hours after 24 hours serum free culture, and cell growth was analyzed with MTS assay. P value <0.05 was accepted as statistical significance with one-factor ANOVA. Cell growth was not changed between 200 ng/ml of IGF-II and 0 ng/ml in HLE (1.03) and HLF (1.09), poorly differentiated HCC (n=9). In well differentiated HCC, cell number was not changed in PLC (1.07) and Huh-7 (1.00), while elevated in Hep3B (1.47, P<0.05) (n=9). Cell number was elevated in Huh-6 (1.39, P<0.05) and HepG2 (2.00, P<0.05), well differentiated HBL cell lines (n=9). IGF-IR was expressed in all cell lines. When LY294002 was added, cell growth was suppressed for all cell lines compared as with IGF-II only (0.22-0.43) (n=3). Cell growth was suppressed with PD98059 with IGF-II as compared with IGF-II only in HepG2 (0.35, P<0.05) while it was not changed significantly in the other cell lines (n=3). It was clarified that IGF-II activated proliferative potential of well differentiated HBL and HCC cell lines via IGF-IR followed by PI3 kinase. It was hypothesized that poorly differentiated HCC proliferate independently of a growth factor. Phosphorylation of IGF-IR might differ among cell lines, which varied the proliferative response to IGF-II. In the future, we will focus on phosphorylation of IGF-IR. It was suggested that PI3 kinase was a suitable candidate for molecular therapy to HCC as well as HBL.

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1214

PTEN ASSOCIATION WITH PI3K85α SUBUNIT IS A NEGATIVE REGULATORY MECHANISM FOR INSULIN SIGNALING IN THE LIVER

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Background: Insulin is an important growth factor for the normal liver. In hepatocellular carcinoma (HCC) there is often constitutive activation of insulin/IGF-1 signaling through insulin receptor substrate-1 (IRS-1). In this context, signals are sent via the insulin/IRS-1/PI3K/Akt/GSK3β pathway thus promoting hepatocyte survival since the functional consequences are inhibition of apoptosis. The mechanisms of how insulin signaling is regulated with respect to liver growth is not clear. Recent preliminary studies suggest that PTEN may associate with the PI3K85α subunit which would negatively regulate PI3K/Akt signaling leading to insulin resistance both in vivo and in vitro. The present study was designed to evaluate how the insulin signal is attenuated as it relates to downstream growth effects and survival signals in liver. Methods: HuH 7 cells were cultured in DMEM in 10% fetal calf serum. After an overnight period of serum starvation, insulin stimulation (0.075U/ml) was provided for 40 min. We assessed Akt phosphorylation/dephosphorylation at different time points as a downstream indicator of insulin signaling through PI3. In addition, we evaluated the physical association between PTEN and the PI3K85α subunit by co-immunoprecipitation experiments. To further clarify the role of PTEN, siRNA knockdown experiments of PTEN gene expression
were performed to enhance insulin signaling through PI3K. Finally, in vivo experiments were performed in mice acutely treated with an I.P. injection of insulin followed by euthanization. Liver samples were tested for p-Akt activity and the association of PTEN with PI3K85α. Results: We observed that insulin stimulation of HuH7 cells rapidly activated the PI3K/Akt signal transduction cascade as revealed by a time dependent increase in p-Akt. Within 5 - 10 min of insulin exposure, a negative regulatory effect of PTEN was evident as shown by a rapid association with PI3K85α both in vitro and in vivo. The association of PTEN with PI3K85α revealed a similar time course to the activation and deactivation of p-Akt. Therefore, this rapid association of PTEN with PI3K85α represents a negative regulatory mechanism to attenuate the insulin signal. More important, silencing of the PTEN gene by siRNA prolonged the activation of p-Akt which further demonstrated its important role in insulin homeostasis. Conclusions: These studies suggest that the PI3K85α subunit is a natural substrate for PTEN and the interaction between these two proteins provide an important negative regulator function for insulin signaling in the liver.

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1215
FARNESOID X RECEPTOR (FXR) REGULATES RECK EXPRESSION
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Elevated expression of RECK (reversion-inducing cysteine-rich protein with Kazal motifs) is a favorable prognostic marker in hepatocellular carcinoma, potentially due to the ability of RECK to inhibit tumor cell invasion and metastatic activities. Here we demonstrate that the farnesoid X receptor (FXR) induces RECK expression. Oral administration of a potent synthetic FXR agonist, FXR-450, for seven days elevated RECK expression several fold in the liver of multiple mouse strains. The basal liver expression of RECK was reduced in FXRKO mice, and FXR-450 did not increase RECK expression in FXRKO mice. A single oral dose of FXR-450 significantly increased RECK expression in the liver within 2 hours, suggesting a direct induction. RECK expression was also rapidly induced in cultured primary mouse hepatocytes and in AML-12 hepatocytes by the FXR agonists GW4064 and FXR-450. The selective FXR antagonist WAY-396551 blocked these inductions and reduced basal RECK expression. An active FXR response element was localized within the first intron of the RECK gene. Finally, FXR-450 also induced RECK expression in human primary hepatocytes. Together these results suggest FXR is a novel regulator of RECK and point to the potential therapeutic use of FXR agonists in tumors expressing FXR such as hepatocellular carcinoma.

Disclosures:
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1216
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Aims: Hepatocellular carcinoma (HCC) is one of the most lethal malignancies worldwide and no effective chemotherapeutic or -preventive treatments are currently available. We previously showed that specific cyclooxygenase (COX)-2 inhibition activates intrinsic as well as extrinsic apoptotic pathways in HCC cells and induces apoptosis in an in vivo model of HCC [1-3]. We also showed that the neoplastic effects of COX-2 overexpression can predominantly be ascribed to PGE2, which acts via differentially regulated EP-receptors (EP1-4). This study was designed to analyze the expression of potential novel targets downstream of COX-2 (PGE2-synthases (mPGES-1 and -2) and EP1-4) in HCCs and to evaluate the potential of EP-receptor-antagonism as a new approach to the treatment of HCC. Methods: Using tissue micro-arrays (TMAs) comprising a total of 14 control livers, 17 liver cirrhoses, 22 premalignant dysplastic nodules (DNs) and 162 HCCs with different histological grades, the expression of COX-2, mPGES-1 and -2 and EP1-4 were analyzed. Western immunoblot analyses were performed to confirm the expression in HCC cell lines. The effects of EP1-4-receptor antagonism on cell viability and apoptosis were investigated using MTT-assays and FACS-analyses of propidium iodide-stained nuclei. Results: COX-2 and EP1-4 were expressed in all HCC cell lines and tissues. COX-2 expression was highest in DNs and declined with loss of HCC-differentiation, whereas a converse expression of EP1-3, respectively an inverse expression of mPGES-1 and -2 with high expression in poorly-differentiated HCCs was found in comparison to COX-2. Selective EP1- and EP3-antagonists significantly reduced the viability of HCC cells in a dose-dependent manner (up to 80% reduction), which was associated with apoptosis induction. In contrast, selective EP2- and EP4-receptor antagonists elicited no marked effects. Conclusion: Our TMA-results indicate that mPGES-1 and -2 as well as EP1-3 might represent potential new targets. Furthermore, our data suggest that PGE2-induced neoplastic effects are, at least in part, mediated via EP1- and EP3-receptors in HCC cells, implying that selective antagonists of these EP-receptors may provide a novel therapeutic approach to the treatment of HCCs. EP-receptor signaling depends largely on the cellular context. Thus, selective modulation of EP-receptors might exert fewer side-effects compared to a general abrogation of prostaglandin synthesis with COX-inhibitors. 1. Kern MA, Haugg AM, et al. (2006) Cancer Res 66: 7059-66.; 2. Kern MA, Schoneweiss MM, et al. (2004) Carcinogenesis 25: 1193-9.; 3. Kern MA, Schubert D, et al. (2002) Hepatology 36: 885-94.

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1217 CONDITIONAL ABLATION OF C-FLIP RENDERS HEPATOCYTE MORE SENSITIVE TOWARDS CD95 MEDIATED APOPTOSIS IN VIVO

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Hepatocytes are highly susceptible to CD95 induced apoptosis and receptor mediated apoptosis is of particular importance in many liver diseases. CD95 (Fas) and CD120a (TNF-R1) are the most prominent cell death receptors in this context. Receptor aggregation upon ligand binding and subsequent recruitment of the adapter molecules FADD is a prerequisite for DISC formation, caspase-8 activation and subsequent initiation of the apoptotic machinery. The caspase-8 homologue FLIP exerts antiapoptotic function and was shown to be involved in maturation and CD95 mediated apoptosis of T-cells (Zhang and He, JEM, 2005). Here, we demonstrate that FLIP is involved in regulation of CD95 induced liver injury in vivo. Method: We generated mice conditionally lacking c-FLIP by crossing mice harboring a loxP flanked exon 1 of flip with transgenic mice expressing Cre recombinase under the control of the albumin promoter. Results: AlbCre-flip-/- mice were significantly more sensitive towards CD95 induced liver failure. Liver function tests of 8-week-old wildtype (n=12) and albCre-flip-/- (n=7) mice were evaluated 3 hours after intra venous injection of 0.15 ug/g of anti-CD95 (Jo2) antibody: The flip-/- mice showed significantly higher numbers for ALT (2237 vs. 259) and AST (2015 vs. 119 IU/L). This was accompanied by higher relative activities for caspase-3 (33840 vs. 2980), caspase-8 (4675 vs. 3507) and caspase-9 (1534 vs. 756 rel. Units) in whole liver lysates. In line with these observations, histology in H/E staining showed more apoptotic cells in the anti-CD95 treated albcre-flip-/- mice. In conclusion, our data demonstrate a specific role for c-FLIP in the regulation of CD95 mediated apoptosis of hepatocytes in vivo.

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1218 MICRORNA-370 REGULATES INTERLEUKIN-6 DEPENDENT P38 MAP KINASE SIGNALING IN HUMAN CHOLANGIOCARCINOMA

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Background & Aims: The inflammatory cytokine Interleukin-6 (IL-6) is over-expressed in cholangiocarcinoma and contributes to tumor growth by aberrant p38 MAP kinase signaling. Enforced expression of IL-6 can alter the expression of microRNAs (miRNAs), non-coding RNAs that reduce the expression of target genes. Thus, our aims were to characterize the role of aberrantly expressed miRNA in the modulation of p38 MAP kinase signaling by IL-6 in human cholangiocarcinoma cells. Methods: MzChA-1 and KMCH-1 cholangiocarcinoma cells were stably transfected to over-express IL-6. miRNA expression profiling was performed in normal and malignant cholangiocytes using a hybridization based microarray. The expression of selected mature miRNA was verified by real-time PCR assay. The effect of selected miRNA on cell growth was assessed using precursor miRNA to increase cellular expression, or miRNA specific antisense oligonucleotides to decrease miRNA expression. Cell proliferation was quantitated using MTS assay and protein expression measured by quantitative immunoblot analysis. Results: The expression of MAP3K8, an upstream kinase regulator of p38 MAPK was increased in MzChA-1 cells by 62 ± 12% and in KMCH-1 cells by 51 ± 7% relative to normal human cholangiocytes. Using target prediction algorithms, MAP3K8 was identified as a potential target for microRNA mir-370. MAP3K8 was experimentally verified as a direct target of mir-370 by dual luciferase reporter assay. The expression of mature mir-370 was decreased in both Mz-ChA-1 and KMCH-1 cells compared to normal human cholangiocytes. In IL-6 over-expressing cells, expression of mir-370 was decreased by 85 ± 7%, whereas that of MAP3K8 was increased by 105 ± 19%. Cell growth was not altered in normal human cholangiocytes transfected with mir-370 precursor. However, over-expression of mir-370 in Mz-ChA-1 cells decreased tumor cell proliferation by 31 ± 3% and moreover decreased expression of both MAP3K8 by 0.34 ± 0.11-fold and phosphorylated p38 MAP kinase by 0.45 ± 0.13-fold. In IL-6 over-expressing tumor cell xenografts, mir-370 expression was reduced by 87 ± 16%, while MAP3K8 and phospho-p38 MAPK were increased by 153 ± 22% and 87 ± 15%, respectively. Summary and Conclusions: These results identify for the first time that MAP3K8 is a biological target gene of miR-370. The modulation of expression of mir-370 and MAP3K8 provides a direct mechanism by which IL-6 over-expression can result in aberrant p38 MAPK signaling. These findings identify a novel mechanism by which oncogenic kinases can be aberrantly expressed and contribute to tumor growth in cholangiocarcinoma.

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1219 IL-22 MAY BE INVOLVED IN LIVER REGENERATION VIA A STAT-3 SIGNALING PATHWAY AFTER 70% HEPATECTOMY

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Objective: Hepatocyte proliferation, triggered by partial hepatectomy, is an important component of liver regeneration. Recent studies have shown that IL-22 mediates inflammatory processes through IL-10 receptor β and/or IL-22 receptor α. In vitro studies have also demonstrated that IL-22 is also involved in cellular proliferation in other cell types. The goal of this study was to investigate the potential role of IL-22 in mouse liver regeneration after 70% hepatectomy. Methods: Partial hepatectomy or sham laparotomy was performed in C57BL/6 mice, animals were sacrificed in a kinetic fashion, and real-time PCR was used to measure hepatic levels of IL-22 and IL-22R after hepatic resection. Hepatocyte proliferation, as estimated by BrdU staining, and measurement of p-stat-3 levels by Western blot, was investigated in mice undergoing 70% hepatectomy or sham laparotomy after treatment with IL-22 antibody or IL-22 antigen. Results: These data show that IL-22 is upregulated within 6 hours of 70% hepatectomy, and significant increases in IL-22R are seen as early as 1 hour, with peak levels occurring 3 hours post-hepatectomy. Administration of exogenous IL-22 antibody 30 minutes before hepatectomy decreased hepatocyte proliferation at 48 hours post-hepatectomy and reduced stat-3 activation within 30 minutes of resection, as compared to mice receiving control IgG prior to hepatic resection. While exogenous administration of IL-22 antigen immediately before liver resection did not significantly change hepatocyte proliferation, this treatment did increase stat-3 acti-
Differential expression of discoid domain receptors in epithelial-mesenchymal transition

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Repressors of E-cadherin, such as Snail, Slug, and Twist, cause "epithelial-mesenchymal transition (EMT)"; however, acquired features of the transformed cells are not fully understood. The aim of this study was to assess alterations in both the proliferative potential and the responsiveness to extracellular matrices (ECMs) in EMT. Methods: MDCK cells, the immortalized human hepatocyte cell line OUMS-29, and the clonally-identified sister hepatoma cell lines HAK-1A and HAK-1B were used in this study. EMT was induced by transfection of the Slug cDNA (FLAG-tagged), which was a kind gift from Professor Eric R. Fearon (University of Michigan Medical School). Cell proliferation was analyzed by flow cytometry and Western blot of the cell-cycle associated proteins. ECM-stimulated cell proliferation was examined by counting cells cultured on the following ECMs: type I collagen, type IV collagen, fibronectin, and laminin. Protein phosphorylation was detected by immunoprecipitation-Western using anti-tyrosine antibody. Results: Both the G1 and the G2/M arrest were found in the Slug-transfected cells, and the responsible molecules for the cell-cycle arrest were, at least in part, p21 and Wee1. Once in contact with type I collagen and fibronectin, Slug-transfected cells dramatically started to proliferate up to six-fold in cell number at day 5, in contrast to only two-fold increase in cell number in parental cells. Thus, we focused on the expression status of the collagen receptors including those of non-integrin types, and found a remarkable increase in the discoid domain receptor (DDR) 2 expression, in contrast to a significant decrease in the DDR1 expression, in Slug-transfected cells. The expression levels in β1 and αV/β3 integrins were not changed. The constitutive expression of DDR2 was predominantly found in HAK-1B, in comparison with HAK-1A, which resembled well differentiated hepatocellular carcinoma cells. Immunoprecipitated DDR2 protein in the lysate of the collagen-stimulated Slug-MDCK cells was phosphorylated, indicating that the Slug-induced DDR2 mediated "outside-in" signal. Conclusion: We found a cell-cycle arrest in Slug-transfected cells; however, contact with type I collagen clearly abolished the arrest and even promoted cell proliferation in these cells. The Slug-transfected cells predominantly expressed the non-integrin collagen receptor DDR2, which was also profoundly expressed in aggressive hepatoma cells. DDR2 may be a marker for EMT and play a role in mediating signal from type I collagen.

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P53 is a specific molecular target of ursodeoxycholic acid in regulating apoptosis

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p53 plays an important role in regulating expression of genes that mediate cell cycle progression and/or apoptosis. In addition, we have previously shown that the hydrophilic bile acid ursodeoxycholic acid (UDCA) prevents TGF-β1-induced p53 stabilization and apoptosis in primary rat hepatocytes. Therefore, we hypothesized that p53 may represent an important target in bile acid-induced modulation of apoptosis and cell survival. Primary rat hepatocytes were cotransfected with plasmid DNA encoding wild-type or mutant p53 and a reporter gene construct that utilized the bax gene promoter to drive transcription of chloramphenicol acetyltransferase (CAT). Twelve hours prior to transfection, hepatocytes were pre-treated with either vehicle or 100 μM of UDCA. At 48 h post-transfection, hepatocytes were harvested for CAT ELISA and luciferase control assays. Cultures were also scored for apoptosis following Hoechst staining, and culture media used for viability assays. General caspase-3-like activity was determined in cytosolic protein extracts by enzymatic cleavage of the substrate. Nuclear, mitochondrial, and cytosolic fractions, or total proteins were also prepared and analyzed by immunoblotting. p53 DNA binding activity was evaluated in parallel experiments. Finally, immunofluorescence and immunoblotting were used to detect p53 cellular distribution. The results showed that UDCA reduced p53 transcriptional activity, thereby preventing its ability to induce Bax expression, mitochondrial translocation, cytochrome c release and apoptosis in primary rat hepatocytes. More importantly, bile acid inhibition of p53-induced apoptosis was associated with decreased p53 DNA-binding activity. Subcellular localization of p53 was also altered by UDCA. Both events appear to be related with increased association between p53 and its direct repressor, Mdm-2, as detected by immunoprecipitation analysis. Posttranscriptional silencing of the mdm-2 gene confirmed its involvement in the antiapoptotic function of UDCA. In conclusion, these results further clarify the antiapoptotic mechanism of UDCA and suggest that modulation of Mdm-2/p53 interaction is a prime target for this bile acid.

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HEPATIC STAT3 ATTENUATES SYSTEMIC INFLAMMATORY RESPONSE AND LETALITY IN SEPTIC MICE

Ryotaro Sakamori, Tetsuo Takehara, Hayato Hikita, Akira Sasaki, Keisuke Kohga, Akio Uemura, Shinjiro Yamaguchi, Tomohide Tatsumi, Kazuyoshi Ohkawa, Norio Hayashi

Background: IL-6 is an important modulator of pathophysiological changes in sepsis. STAT3 is a key signaling molecule of the IL-6 response during inflammatory response. However, the significance of hepatic STAT3 in the process of sepsis is unclear. In the present study, we used a murine model of septic shock and compared outcomes between hepatocyte-specific STAT3 KO (L-STAT3) mice and wild-type littermates. Materials and Methods: We generated L-STAT3 mice by the Cre/loxP recombinant system. CLP-induced sepsis was performed using cecal ligation with a single 23-gauge puncture (CLP). STAT3 activation in the liver was evaluated by western-blotting. The concentration of TNF-α, IL-6, IFN-γ and IL-10 in the serum was determined with multi-analyte profiles in sera. Furthermore, we isolated hepatocytes from L-STAT3 KO mice and control mice, collecting the conditional medium of hepatocytes. Next, we cultured murine macrophage cell line RAW264.7 in the culture supernatants of hepatocytes with or without LPS, following the measurement of TNF-α, IL-6, and IL-10. Results: STAT3 was rapidly phosphorylated in the liver of wild-type mice after CLP but was not in that of L-STAT3 mice. The levels of serum acute phase proteins including haptoglobin and fibrinogen increased in wild-type mice 24 hours after CLP but only to lesser extents in L-STAT3 mice. Of interest is the finding that the rate of CLP-induced mortality was significantly higher in L-STAT3 mice than in wild-type mice. The levels of pro-inflammatory cytokines including TNF-α, IL-6 and IFN-γ which are known to be associated with sepsis-induced mortality was significantly higher in L-STAT3 mice than those in wild-type mice. We injected 4 mg/kg of LPS into LSTAT3 and wild-type mice and confirmed that fixed dose of endotoxin also induced hyper-inflammatory responses in LSTAT3 mice. The wild-type hepatocytes produced more haptoglobin than KO hepatocytes, and hepatocyte culture supernatants suppressed the levels of cytokines produced from RAW cells. Suppressive activity of KO hepatocytes supernatant was significantly weaker than that of wild-type hepatocyte supernatant. Conclusion: Liver STAT3 deficiency increased inflammatory cytokine production and mortality in a murine model of septic shock. Hepatic STAT3 represses systemic hyper-inflammatory response by stimulating hepatic production of soluble substance(s) that can attenuate immune cell overactivation and also improves host survival during septic condition.

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gp130-dependent pathways. To study this mechanism we combined conditional knockout and knockin technology for gp130 and generated hepatocyte-specific gp130 knockout (hepaΔgp130) mice that are either deficient for hepatocyte-specific gp130-dependent Ras/Erk (hepaΔgp130RAS) or STAT (hepaΔgp130STAT) activation. We thus performed PH in these mice and hepatocyte proliferation, cell cycle regulation, Erk1/2 and STAT3 activation, expression of its negative regulator SOCS3 and the acute phase response was analyzed in all mouse strains. According to the major impact of gp130-dependent signalling on acute phase gene (APG) regulation after PH it was blocked in hepaΔgp130STAT animals, while hepaΔgp130RAS mice showed an enhanced APG response. As the STAT3 regulating feedback loop is blocked in hepαΔgp130RAS mice prolonged STAT3 activation and significantly higher SOCS3 mRNA levels were evident and associated with a later start of hepatocyte proliferation 48 h after PH. More detailed analysis of cell cycle parameters demonstrated that G1-S-phase transition was delayed in these animals. To define the role of SOCS3 during hepatocyte proliferation, primary hepatocytes were co-stimulated with IL-6 and HGF. Higher SOCS3 expression in gp130-ΔRas hepatocytes correlated with delayed hepatocyte proliferation in vivo and in vitro suggesting that the strength of SOCS3 expression is involved in controlling DNA synthesis. Next, we tested the impact of LPS - mimicking bacterial infection - on liver regeneration. LPS and PH induced SOCS3 in all animal strains and delayed cell cycle progression. Additionally, IL-6/gp130-dependent STAT3 activation in hepatocytes was essential in mediating protection and thus required for maximal proliferation. In summary, our results implicate that gp130-dependent STAT signalling during liver regeneration in hepatocytes via SOCS3 controls timing of G1/S-phase transition thereby providing protective signals that are important to allow hepatocyte proliferation during stress conditions.

Disclosures: The following people have nothing to disclose: Uta Dierssen, Naiara Beraza, Matthias Ernst, Christian Trautwein

1225 LIVER TETRAPLOIDIZATION IS TRIGGERED BY WEANING UNDER AN INSULIN SIGNAL

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Hepatocytes polyploidization is an important feature of mammalian liver growth, correlated with ageing and senescence. Whereas hepatocytes of newborn are exclusively diploid, during post-natal growth, the liver parenchyma undergoes progressive polyploidization, with the appearance of tetraploid and octoploid cells classes with one or two nuclei. In adults, liver polyploidization is differently regulated upon loss of liver mass and liver damage. Interestingly, partial hepa-tectomy induces marked cell proliferation, followed by an increase in liver cell ploidy. In contrast, in different liver pathologies, hepa-tocarcinome for example, growth shifts to a non-polyploidizing pattern and expansion of the diploid hepatocytes population has been found to take place. In a previous study using time-lapse videomicroscopy, our group has clearly established the lineage existing between hepatocytes of different ploidy. We have demonstrated that the appearance of binucleated 2x2n and mononucleated 4n hepatocytes is chronologically controlled during development, binucleated hepatocytes being generated by a process of incomplete cytokinesis. We have now deciphered the molecular mechanism regulating this event.

Indeed, mitotic hepatocytes perform karyogenesis without cleavage plane specification (absence of actin cytoskeleton rearrangement associated with unattached microtubules to the equatorial cortex). Consequently, RhoA pathway, orchestrator of cytokinesis, is not activated leading to the genesis of tetraploid cells. Furthermore, we also demonstrated that weaning triggers this process. Indeed, if animals are weaned at 15 days, we clearly observed that the incomplete cytokinesis process is largely initiated at 19 days in contrast with animals that stay with the mother with no access to nutriment. Weaning period is clearly associated with physiological changes, such as modification of nutritional diet, hormonal variations and circadian clock appearance. We are now trying to define which cell signalling pathways trigger incomplete cytokinesis. By modifying the nutrient supply at the weaning period (high/low fat diet), we have demonstrated that glucids are not involved in the induction of binucleation process. Moreover, we are currently assessing the effect of modifying the level of insulin around the time of weaning. Our preliminary results indicate that when insulin signalling is impaired, binucleation process is not initiated. Insulin regulates several pathways involved in distinct cell cycle events and we are now in the process to define which of them could control the genesis of tetraploid progenies.

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1226 MOLECULAR MECHANISMS OF ANTI-APOTOTIC AND ANTI-OXIDANT EFFECTS OF GRAPE AND ITS COMPOUNDS

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Background & Aims: Apoptosis plays a critical role in variety of liver diseases such as hepatitis, cirrhosis, and hepatocellular carcinoma. We reported that freeze-dried grape powder (FDGP) attenuates mitochondria-induced apoptosis. However, the underlying mechanisms remained unknown. Aim of this study was to analyze the mechanism responsible for anti-apoptotic effect of FDGP or its active components. Methods & Results: We examined signaling pathways induced by FDGP and its active components such as epicatechin, cyanidin, quercetin and resveratrol in human hepatoma cells, Huh7 using Western blot analysis. After titration, 0.8 mM H2O2 was used to generate the oxidative stress and 400µM taurodeoxycholic acid (TDCA) for mitochondria-induced apoptosis as analyzed by MTT assay. FDGP (300µg/ml) dramatically decreases TDCA-induced activation of caspase-3, caspase-7, caspase-9 and Bax and restores levels of PCNA, p53 and NF-kB. The reduction of TDCA-induced apoptosis with FDGP treatment was confirmed by flow cytometry. Interestingly, both epicatechin (50-150µM) and cyanidin (30-100µM) reduced TDCA-induced apoptosis in a dose-dependent manner. No effects were seen with resveratrol and quercetin at the similar concentrations present in FDGP. No anti-apoptotic effects were seen even with higher doses although resveratrol increased expression of Sir1, an important modulator of apoptosis/cell survival and lifespan expansion. H2O2-induced stress resulted in downregulation of Akt and PCNA expression levels and induced phosphorylation of c-Jun in a dose- and time-dependent manner. Pretreatment with FDGP restored the level of Akt and PCNA expression and inhibited c-Jun phosphorylation. Similarly, both epicatechin and cyanidin reduced H2O2-induced apoptosis whereas no effects were seen with quercetin or resveratrol. Conclusion: FDGP...
reduces the oxidative stress and mitochondria-induced apoptosis. The mechanisms of anti-apoptotic effects of FDGP were associated with prevention of mitochondrial damage, inhibition of c-Jun/AP-1, and upregulation of Akt expression. We speculate that epicatechin and cyanidin are the active compounds of FDGP involved in attenuation of mitochondria- and H2O2-induced apoptosis. No effects were seen resveratrol and quercetin, although high concentrations of resveratrol induced Sirt1 expression. These findings may contribute to our understanding of the molecular mechanisms of anti-apoptotic and anti-oxidant effects of grape and its components and assist in developing clinical protocols to treat a variety of stress-mediated liver conditions.

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The following people have nothing to disclose: Jing Yu, Yumin Xu, Zhongying Bao, Vladimir Khaoustov, Boris Yoffe

1227 EXPRESSION AND LOCALIZATION OF ATYPICAL PKC ISOFORMS IN LIVER PARENCHYMAL CELLS
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Members of the protein kinase C (PKC) family transduce extracellular stimuli to the cellular signalling networks. The family comprises 10 isoforms that can be subdivided into three classes: the classical PKCs (α, β, γ and δ), the novel PKCs (ε, η and θ) and the atypical PKCs (ζ and η/ζ), which share 72% amino acid (aa) identity. Expression of PKCs and localization of atypical PKCs in hepatocytes is only partially characterized. Methods: Full-length PKCζeta and iota were cloned from total RNA of human liver and fused to a yellow fluorescent protein (YFP-tag). PKCζeta-YFP and PKCiota-YFP were overexpressed in HEK293 cells in order to test the specificity of commercially available aPKC antibodies by immunofluorescence and Western blot analysis. Subcellular localization was analyzed by differential centrifugation with separation of cytosol, basolateral and canalicular membranes and by immunofluorescence in isolated rat hepatocytes and liver sections. Results: Expression of PKCζeta, βζ, δζ, εζ and ηζ in isolated hepatocytes was confirmed by western blot analysis. Additionally, PKCζeta-mRNA and protein were detected in hepatocytes. A PKCζeta antibody directed against the 20 aa from the N-terminus was specific for PKCζeta in Western blot. Two PKC iota antibodies (immunogens: aa 168-243 and 404-587) were specific for PKCiota and did not react with PKCζeta. The antibody H-1 (immunogen: aa 542-592 from PKCζeta) detected PKCζeta as well as PKCiota. Using the specific antibodies a strong canalicular staining of PKCζeta in immunofluorescence studies of hepatocytes and liver sections was found. Analyzing the subcellular localization by cell fractionation and Western blotting, PKCζeta was faintly detectable in the cytoplasmic fraction, and showed a strong signal in the basolateral and canalicular membrane fractions. PKCζeta was exclusively localized in the canalicular fraction. Discussion/Conclusion: This study demonstrates the expression of PKCiota in hepatocytes. Both atypical PKC isoforms exhibit a prominent canalicular localization in liver parenchymal cells proposing a role in the regulation of canalicular transporters. The exact topology of atypical PKCs needs to be considered in the functional evaluation PKCζeta and PKCiota in hepatocytes.

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The following people have nothing to disclose: Claudia Stross, Verena Keitel, Ralf Kubitz, Dieter Häussinger

1228 ESTROGEN RECEPTORS ACTIVATE THE EXPRESSION OF THE HUMAN ENTEROHEPATIC TRANSPORTER GENES SLC10A2 AND SLCO1B1 BY ENHANCING THE DNA-BINDING OF HNF-1α
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Background: Estrogens are steroid hormones that elicit complex transcriptional programmes in many metabolic pathways. Most actions by estrogens take place through their functions as ligands for the estrogen receptor-α (ERα) and -β (ERβ), which bind to the response elements in the regulatory regions of their target genes. Additionally, ERs possess nongenomic functions, independent of their DNA-binding. Estrogenic effects on enterohepatic transporter gene expression have been widely studied in rodents, but little is known about regulation of transporter genes by estrogens in the human liver and intestine. Aim: To investigate the mechanism of regulation of the human transporter genes SLC10A2 (encoding the apical intestinal sodium-dependent bile acid transporter ASBT) and SLCO1B1 (encoding the hepatic organic anion transporting polypeptide OATP1B1) by ERs and estrogens. Methods: Regulatory regions of the human SLC10A2 and SLCO1B1 genes were cloned, linked to the luciferase reporter gene and analyzed in transient transfections in human intestine- and liver-derived cell lines. In vitro and in vivo protein binding to DNA were studied in EMSAs and ChIP assays, respectively. Results: Exogenous expression of ERα and ERβ increased the promoter activities of the human SLC10A2 and SLCO1B1 genes. Treatment of cells with 17β-estradiol enhanced ER-mediated activation of the two genes. No ER binding sites could be identified within either promoter. Promoter mutagenesis showed that in the context of both transporter genes the ER response was mediated through the binding sites for hepatocyte nuclear factor-1α (HNF-1α). The ER-responsive HNF-1α binding sites are located in the 5′-UTR of the human SLC10A2 gene (+305/+317) and in the proximal region of the human SLCO1B1 promoter (51′-39). In EMSA and ChIP assays, DNA-binding of HNF-1α and recruitment of HNF-1α to the transporter promoters were increased by ERs and 17β-estradiol. ERs did not increase HNF-1α mRNA or protein levels or HNF-1α promoter activity, but enhanced the interaction between the HNF-1α protein and its DNA response elements by post-translational mechanisms. Conclusions: We have revealed a novel signalling cascade, by which ERs and estrogens activate the expression of the human enterohepatic transporter genes SLC10A2 and SLCO1B1 via HNF-1α response elements in their promoter regions. ERs do not affect HNF-1α expression levels, but mediate enhanced association of HNF-1α with its target genes. By this mechanism, elevated estrogen levels may increase bile acid uptake into human enteroocytes by ASBT and enhance extraction of organic anions and drugs from portal blood into human hepatocytes by OATP1B1.

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The following people have nothing to disclose: Jyrki J. Eloranta, Christian Hiller, Gerd A. Kullak-Ublick

1229 S6K1 INHIBITION BY A NOVEL CLASS OF DITHIOLETHIONES ABROGATES INSULIN RESISTANCE INDUCED BY HYPEROSMOTIC STRESS
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The development of insulin resistance in the clinical situations associated with hyperosmotic dehydration is suggested to
 impair the metabolic insulin effects in liver and peripheral tissues. Inhibition of insulin resistance recovers the ability of insulin to suppress hepatic glucose production, and to promote glucose uptake in peripheral tissues, therefore, pharmaceutical interventions to prevent insulin resistance are of therapeutic interest. Oltipraz, a prototype diithiolethione, inhibits TGF-β1 and has the ability to regenerate cirrhotic liver, which provided a basis for its clinical trial. We previously found that novel synthetic diithiolethiones including oltipraz prevent TNFα-induced hepatic insulin resistance via AMP-Activated protein kinase (AMPK)-dependent p70S6 kinase (S6K1) inhibition. Although hyperosmotic stress blocks insulin-dependent PI3K-Akt signal by mTOR pathway, in a certain cellular context, it improves glucose homeostasis via AMPK activation. This study investigates whether synthetic diithiolethiones affect hyperosmolarity-induced insulin resistance and altered cell signaling. In HepG2 cells, oltipraz decreased hyperosmolar sorbitol-induced AMPK activation although oltipraz alone also activated AMPK. The reduced intracellular ATP level by sorbitol, which could be responsible for its AMPK activation, was completely restored to control level by oltipraz pretreatment. Moreover, oltipraz abolished sorbitol-induced S6K1 phosphorylation. Oltipraz inhibited the ability of sorbitol to induce insulin receptor substrate (IRS)1 serine phosphorylation at Ser312. An experiment using dominant negative S6K1 supports the essential role of S6K1 in the hyperosmolarity-stimulated phosphorylation of IRS1. Oltipraz treatment recovered insulin signal impaired by hyperosmotic stress, whereas constitutively active S6K1 transfection eliminated the protective effects of oltipraz on IRS1 and glycogen synthase kinase (GSK)3β phosphorylations, indicating that oltipraz protects insulin responses by inhibiting S6K1 activation. Protection of insulin signal by oltipraz from hyperosmotic stress promoted glucose uptake in C2C12 myotubes, but not in 3T3-L1 adipocytes. Synthetic diithiolethiones comparably inhibited S6K1 activity and insulin resistance. Among the diithiolethiones examined, 4,5,6,7-tetrahydrobenzo-1,2-dithiole-3-thione and 5-(6-hydroxy-3,4-dimethyl-2-thioxo-1,3-dihydro-1H-imidazol-2-yl)thiophene enhanced insulin-dependent glucose uptake in C2C12 myotubes against hyperosmotic stress. The results of this study demonstrate that diithiolethiones prevent insulin resistance induced by hyperosmolarity, and thereby improve insulin responses, which result from S6K1 inhibition.

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1230 ADAM17 PROMOTES ERK-MEDIATED PHOSPHORYLATION OF SER112 IN BAD
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Background: ADAM17, which belongs to the ADAMs family of transmembrane protein, participates in cytokine and growth factor shedding and cell proliferation. Although ADAM17 has been implicated in disease pathologies and development of cancer, a few studies have been reported about liver cancer. ADAM17 also mediates shedding of both cytokines and cytokine receptors and plays important roles in cell signaling. Recently, an association between ADAM17 and ERK has been reported. However, the influence of ADAM17 on the downstream signaling of ERK has not been confirmed. Aims: 1. To examine whether ADAM17 is expressed in liver cancer cell lines. 2. To determine whether ADAM17 in liver cancer cell lines could regulate the activation of BAD which is located downstream in ERK signaling pathways and plays an important role in apoptosis. Materials and Methods: Expression of ADAM17 was confirmed in human liver cancer cell lines (HuH7, PLC/PRF/5, SKHep) by western blot analysis. Human liver cancer cells were transfected with either small-interfering RNA (siRNA) targeting ADAM17 to inhibit ADAM17 expression or control siRNA using lipofectamine. Phosphorylation of ERK, AKT, and BAD under ADAM17 expressed or unexpressed condition were analyzed by western blot. U0126 was used to inhibit Erk/MEK activation. Results: ADAM17 was expressed in HuH7, PLC/PRF/5, SKHep cells and the expression was inhibited by ADAM17 specific siRNAs in all cell lines. Although phosphorylation of ERK was enhanced in liver cancer cells with time after EGF stimulation, siRNA to ADAM17 inhibited ERK phosphorylation by EGF stimulation. We examined whether ADAM17 could affect AKT phosphorylation because BAD can be activated by both ERK and AKT pathways. However, the activation of AKT was unrelated to the presence of ADAM17 expression. The phosphorylation of BAD at Ser155 was not observed and the phosphorylation at Ser136 showed very weakly. ADAM17 didn’t have an effect on the phosphorylation at both Ser136 and Ser155. On the other hand, the phosphorylation of BAD at Ser112 was boosted by EGF stimulation and was inhibited by siRNA targeting ADAM17. Moreover, the phosphorylation of BAD at Ser112 disappeared with U0126, MEK/ERK inhibitor. Conclusion: We confirmed the expression of ADAM17 in human liver cancer cell lines. ADAM17 enhanced the activation of ERK and the phosphorylation of BAD at Ser112 by EGF stimulation in human hepatocellular carcinoma cells.

Disclosures: The following people have nothing to disclose: Katsuhiro Senda, Haruhisa Nakao, Shunsuke nojiri, Takashi Joh, Tomokatsu Miyaki

1231 OVEREXPRESSION OF THE LIVER TRANSCRIPTION FACTOR HNF6 ACCELERATES HEPATOCYTE PROLIFERATION DURING MOUSE LIVER REGENERATION
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Background and Aims: Hepatocyte nuclear factor 6 (Hnf6, also known as Oc1) belongs to ONECUT transcription factor family whose members play essential roles in development, cellular differentiation, and metabolism. Mouse genetic analysis of Hnf6 null embryos demonstrated that Hnf6 is essential for development of the pancreas, gall bladder and both intrahepatic and extrahepatic bile ducts. Published promoter microarrays combined with chromatin immunoprecipitation (ChIP) from hepatoma cells shows that Hnf6 binds to several endogenous promoters involved in proliferation such as Cdc25A, E2F and Cdk2, suggesting that Hnf6 might be involved in hepatocyte proliferation during liver regeneration. To test our hypothesis, we investigated whether overexpression of Hnf6 affects stimulate hepatocyte proliferation following mouse partial hepatectomy. Methods: CD-1 mice infected with replication defective adenovirus expressing either the mouse Hnf6 cDNA (AdHnf6) or the control bacterial LacZ (β-Galactosidase; AdLacZ) gene were subjected to partial hepatectomy (PHx). The regenerating livers were harvested at several time points between 32hr and 48hr after PHx. Hepatic DNA synthesis was
assessed by immunohistochemical staining of bromodeoxyuridine (BrdU) incorporation. Furthermore, cotransfection and ChIP assays were used to determine transcriptional target promoters of HNF6 and its target genes. Results: Overexpression of HNF6 during mouse liver regeneration caused a significant increase in the number of hepatocytes entering DNA replication. This accelerated proliferation in AdHNF6 infected mouse livers was associated with increased expression of the hepatocyte mitogen Transforming Growth Factor α (TGFα), the cell cycle regulators Cyclin D1, Cdk2 and Forkhead Box m1 (Foxm1) transcription factor. Cotransfection and ChIP assays demonstrated that TGFα, Cyclin D1 and HNF6 promoters were direct transcriptional targets of HNF6 and that combining HNF6 and Foxm1 further enhanced transcriptional activities of the TGFα promoter. We also showed that HNF6 associated with Foxm1 protein in the regenerating livers and this complex formation resulted in activation of Foxm1 transcription. Furthermore, cotransfection and ChIP assays demonstrated that both Foxm1 and HNF6 proteins activated transcription of the endogenous HNF6 promoter region. Conclusion: Our current studies demonstrate that the liver-enriched HNF6 transcription factor could contribute to liver regeneration through transcriptional activation of cell cycle regulatory genes.

Disclosures: The following people have nothing to disclose: Yuichi Yoshida, Yongjun Tan, Douglas E. Hughes, Shintaro Tamura, Norio Hayashi

1232
THE HUMAN SMALL HETERO DIMER PARTNER (SHP) PROMOTER CONTAINS INDEPENDENT AND UNCONVENTIONAL 9-CIS RETINOIC ACID- AND BILE SALT-RESPONSIVE ELEMENTS

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The small heterodimer partner (SHP) is an important transcriptional regulator of bile salt homeostasis. It represses expression of key genes in liver and intestine involved in bile salt biosynthesis and transmembrane bile salt transport. SHP itself is positively regulated by the Farnesoid X Receptor (FXR), the mammalian bile salt sensor. FXR functions as a heterodimer with the Retinoid X Receptor α (RXRα). FXR is activated by the bile salt chenodeoxycholic acid (CDCA) and RXRα by the vitamin A derivative 9-cis retinoic acid (9cRA). Previously, a FXR-responsive element (FXRE) has been identified at position -291/-279 in the hSHP promoter (Goodwin et al., 2000). Recently, we found that 9cRA differentially affects CDCA-induced expression of FXR target genes. 9cRA stimulates and fully represses CDCA-induced expression of SHP and the bile salt export pump (BSEP), respectively. In vitro binding studies revealed that 9cRA inhibits binding of FXR/RXRα to the FXRE. Here, we studied the molecular mechanism of the co-stimulatory effect of CDCA and 9cRA on SHP expression in a human intestinal cell line. The human colon carcinoma cell line DLD-1 was transiently transfected with hFXR- and hRXRα-expression plasmids and cultured in the presence or absence of CDCA and/or 9cRA. Deletion mutants of a 579-bp human SHP promoter element in a luciferase reporter plasmid were generated to determine the location of CDCA and/or 9cRA responsive elements. Transcription of FXR target genes was quantified by Q-PCR and the promoter activity of hSHP was determined by luciferase reporter assays. CDCA and 9cRA induce transcription of hSHP in DLD-1 cells 39- and 12-fold, respectively. Maximal SHP expression (98-fold) was observed when DLD-1 cells are co-exposed to both ligands. Similar regulation was observed for the -569/+10 hSHP promoter element. SHP promoter fragments that were 5' truncated up to position -122 still showed CDCA-induced luciferase expression. This DNA fragment does not contain a sequence similar to the previously identified FXREs. 9cRA dependent activation was lost when truncating the hSHP promoter to -278. Induction of transcription by CDCA and 9cRA were dependent of FXR and RXRα. The hSHP promoter contains a novel bile salt-responsive element, which is different from the previously suggested FXRE at position -291/-279. The hSHP promoter contains a separate RXRα/9cRA response element at position -303/-279. Independent regulation of hSHP by bile salts and 9cRA may be important to maintain adequate levels of bile salt synthesis and transport during cholestatic conditions that are associated with reduced vitamin A levels.

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1233
ROLE OF FARNESOID X RECEPTOR IN REGULATING HEPATIC LIPID HOMEOSTASIS

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Nuclear receptor farnesoid X receptor (FXR) plays an important role in cholesterol metabolism and bile-acid homeostasis. A number of studies have shown that FXR is critical in the regulation of fatty acid metabolism and triglyceride homeostasis. But the mechanism by which and to what extent FXR regulates the lipid metabolism remains to be elucidated. In order to clarify the roles of FXR in liver lipid metabolism, new mouse models were developed with hepatocyte-specific or enterocyte-specific deletion of the FXR gene (AFXR, FXR albumin Cre (+/-) mice; and VFXR, FXR villin Cre (+/-) mice). Comparing these with the whole-body FXR knockout mice (FXR KO), the serum lipid profiles of FXR KO, AFXR and VFXR Cre (+) or Cre (-) mice were determined after fed either a chow diet or high-fat diet for 4 weeks. The hepatic lipid content was determined by oil-red O staining. Northern blot was employed to measure the expression of hepatic and intestinal genes involved in fatty acid uptake, intracellular transport, lipogenesis and lipoprotein assembly, in mice on the chow diet or high-fat diet for 7 days. Our data showed that the expression of FXR in both the liver and intestine is important in regulating triglyceride and fatty acid homeostasis; in addition, using the tissue-specific FXR knockout mice, our results suggest that the expression of FXR in extra enterohepatic sites is also involved in lipid regulation. These data provide a basis for further understanding of FXR function in lipid metabolism.

Disclosures: The following people have nothing to disclose: Bo Kong, Noriko Esterly, Manimaran Rengasamy, Grace Guo

1234
HOMOCYSTEINE-INDUCED INHIBITORY EFFECTS ON ADIPONECTIN PRODUCTION IN ALCOHOLIC LIVER DISEASE

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Although recent evidence suggests that the down-regulation of the adipocyte hormone adiponectin has pathophysiological importance in the development of alcoholic liver
disease (ALD), the underlying mechanisms are still elusive. Abnormal hepatic methionine metabolism and hyperhomocysteinemia induced by prolonged alcohol exposure has been reported both in clinical and experimental studies of ALD. Here, we conducted both in vivo and in vitro experiments to examine the effects of prolonged alcohol exposure on homocysteine levels in adipose tissue and its potential involvement in regulating adiponectin production in ALD. C57BL/6 male mice fed a Lieber-DeCarli ethanol-containing liquid diet were utilized as the animal model of ALD. Our results demonstrated that chronic alcohol exposure decreased the circulating adiponectin concentration and adiponectin mRNA and protein levels in epididymal fat pads. Alcohol feeding induced modest hyperhomocysteinemia and increased homocysteine levels in the epididymal fat pad, which was associated with decreased mRNA levels of cystathionine-β-synthase (CBS). Betaine supplementation (1.5%, m/v) in the alcohol fed mice reduced homocysteine accumulation in the adipose tissue, and was accompanied by improved adiponectin levels. Moreover, in our in vitro study, we found that exogenous homocysteine administration reduced gene expression, protein production, and secretion of adiponectin in primary adipocytes. Furthermore, CBS/- mice had lower circulating adiponectin levels. Mechanistic studies revealed that both inactivation of the extra-cellular signal regulated kinase 1/2 (ERK1/2) pathway and induction of the endoplasmic reticulum (ER) stress response, specifically CHOP expression, may contribute to the inhibitory impact exerted by homocysteine. In conclusion, chronic alcohol feeding caused abnormal accumulation of homocysteine in adipocytes, which contributes to decreased adiponectin production in ALD. Our findings provide a novel mechanism by which betaine supplementation may have therapeutic efficacy in ALD.

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The following people have nothing to disclose: Zhenyuan Song, Zhanxiang Zhou, Ion V. Deaciuc, Theresa S. Chen, Craig J. McClain

2135 PROTEIN KINASE C REGULATES H2O2-INDUCED HEPATOCYTES NECROSIS BY SUPPRESSION OF PROTECTIVE SIGNALING VIA AMP ACTIVATED KINASE AND AKT

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Reactive oxygen species such as H2O2 have been suggested to mediate liver damage during inflammation, and drug- and alcohol-induced liver injury. Cell death induced by H2O2 has been traditionally attributed to oxidation and damage of cellular macromolecules (e.g. proteins, DNA, lipids) by H2O2. However now it is known that reactive oxygen species can affect many signal transduction pathways including Protein kinase C (PKC; family of serine/threonine kinases) to modulate cell death. Treatment of primary cultured hepatocytes with PKC inhibitors (Ro-31-8425 or Bisindolylmaleimide I) significantly decreased necrosis caused by H2O2 (80% decrease at 300 μM H2O2). Ro-31-8425 and Bisindolylmaleimide I, caused translocation of PKCα and PKCε to the membrane in an inactive form. Knocking down PKCα and/or PKCε with antisense in hepatocytes did not protect against necrosis induced by H2O2 suggesting that these two isoforms of PKC are not the only isoforms that explain protective effect of PKC inhibitors. PKC inhibitor treatment was also found to activate Akt (Protein kinase B), an anti-apoptotic serine/threonine kinase important in insulin signaling, and AMPK (AMP-activated protein kinase), an important regulator of metabolism in cells. PKC inhibitor treatment increased levels of phospho-Akt (serine 473), which were reduced following H2O2 treatment in hepatocytes. Akt inhibitors partially inhibited the protective effect of PKC inhibitor, suggesting that the protective effects of PKC inhibitor was partially mediated through Akt activation. PKC inhibitor treatment was also found to increase AMPK phosphorylation (threonine172) in hepatocytes. Compound C, a selective AMPK inhibitor, significantly reduced the protective effect of PKC inhibitors against necrosis induced by H2O2. Similarly, AICAR and metformin, AMPK activators that increase energy generation in cells, significantly inhibited H2O2-induced necrosis (45% decrease at 300 μM H2O2). These results suggest that activation of AMPK, possibly through activation of energy generating pathways, plays a protective role against H2O2-induced necrosis in hepatocytes. Taken together, our data suggests that PKC regulates Akt and AMPK signaling pathways linked to energy and metabolism, to modulate H2O2-induced necrosis in hepatocytes. H2O2 activates PKC which suppresses activation of AMP kinase and Akt and consequently inhibition of PKC unmasked signaling pathways which lead to resistance to necrosis.

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The following people have nothing to disclose: Saberi Behnam, Mie Shinohara, Naoko Hanawa, Derick Han, Neil Kaplowitz

1236 ALTERING TRANSPLANTED CELL ENGRAFTMENT AND PROLIFERATION IN THE LIVER THROUGH PARACRINE SIGNALING WITH COTRANSPLANTATION OF LIVER SINUSOIDAL ENDOTHELIAL CELLS (LSEC) AND HEPATOCYTES IN MICE

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After cell transplantation in liver, multiple cell compartments, including endothelial, stellate and Kupffer cells are activated and cell-cell interactions regulate transplanted cell engraftment and proliferation. To develop strategies for liver-directed cell and gene therapy, insights into such cell-cell interactions will be helpful, particularly for improving transplanted cell engraftment, survival and proliferation. Recently, we demonstrated that under suitable circumstances transplanted LSEC can engraft, function and reconstitute significant portions of the hepatic endothelium. Similarly, transplanted hepatocytes can repopulate the liver and replace deficient functions. To establish potential roles of LSEC in engraftment of transplanted hepatocytes, we optimized protocols in mice preconditioned with the pyrrolizidine alkaloid, monocrotaline (MCT), which causes significant endothelial injury and improves transplanted cell engraftment. We used transgenic GFP mice as LSEC donors and wild-type C57Bl/6 mice as hepatocyte donors. To deposit cells in hepatic sinusoids, we transplanted LSEC intraportally and hepatocytes intrasplenicly in syngeneic DPPIV- mice, followed by cell engraftment analysis after 7 d. GFP-positive transplanted LSEC were integrated in the sinusoidal lining and DPPIV-positive transplanted hepatocytes were in liver parenchyma. Comparison of recipients where only LSEC, only hepatocytes, or both LSEC and hepatocytes were transplanted, showed that cell cotransplantation improved hepatocyte engraftment. Next, to establish whether genetic manipulation of LSEC will offer further opportunities for cotransplantation studies, we used lentiviral vectors (LV) to transduce primary LSEC and hepatocytes. We built a bidirectional LV construct to express hHGF and GFP and a
bicistronic LV construct to express hVEGF and GFP. Transgene expression was verified in primary mouse LSEC and hepatocytes in vitro. When LV-modified cells were transplanted into MCT-conditioned mice, LSEC engrafted and proliferated, similar to control LSEC from GFP transgenic mice throughout the 1 month study duration. Conclusions: Cotransplantation of primary LSEC improved engraftment of transplanted hepatocytes. As LV-transduced LSEC survived, proliferated and expressed introduced transgenes over the long-term, this offers paradigms to establish the effects of multispecific paracrine factors, i.e., hHGF and VEGF, on transplanted cell engraftment, proliferation and function. The ability to reconstitute the liver with genetically-modified LSEC and/or hepatocytes will offer potent ways to investigate biological mechanisms and to pursue cell and gene therapy in suitable disorders.

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1237 USEFULNESS OF ONCOSTATIN M GENE THERAPY ON LIVER DAMAGE INDUCED BY DIMETHYLNITROSAMINE IN RATS
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The liver has a remarkable ability to respond to injuries inflicted by various causes, such as partial hepatectomy, toxic exposure, and virus infection. Hepatocytes, that are liver parenchymal cells and normally in the quiescent G0 phase, reenter the cell cycle following injury to restore its mass, architecture, and function. In this process, a number of growth factors and cytokines have been reported to be involved. Oncostatin M (OSM) is a member of the interleukin (IL)-6 cytokine family that includes IL-6, IL-11, leukemia inhibitory factor, ciliary neurotrophic factor, cardiotrophin-1, and novel neurotrophin-1/B-cell-stimulating factor-3. Because it has been recently reported that liver regeneration is impaired in OSM-specific receptor deficiency (-/-) mice and CCl4-induced acute liver failure is ameliorated by the administration of OSM in mice, it is likely that OSM plays a crucial role in liver regeneration. To assess the usefulness of OSM gene therapy in liver regeneration, we here examined whether the introduction of rat OSM cDNA enhances the regeneration of livers damaged by dimethylnitrosamine (DMN) in rats. Repeated injection of rat OSM cDNA in hemagglutinating virus of Japan envelope into the spleen resulted in the exclusive expression of OSM protein in Kupffer cells of the livers, which accompanied by the increase of body weight, liver weight, and serum albumin levels and the reduction of serum liver injury parameters, such as bilirubin, aspartate aminotransferase, and alanine aminotransferase, and a serum fibrosis parameter, hyaluronic acid. Histological examination showed that OSM gene therapy reduced centrilobular necrosis and infiltration of inflammatory cells and augmented hepatocyte proliferation. The apoptosis of hepatocytes identified by TUNEL staining and the fibrosis identified by Azan stain were also suppressed by OSM gene therapy. In addition, time-course studies on OSM gene therapy prior to or after DMN treatment showed that this therapy was effective not only in enhancing regeneration of hepatocytes damaged by DMN but also in preventing hepatic cytotoxicity caused by subsequent treatment with DMN. These results indicate that OSM is a key mediator for proliferation and antianapoptosis of hepatocytes in liver regeneration and suggest that OSM gene therapy is useful, as preventive and curative means, for the treatment of patients with liver damage.

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1238 SEROTONIN 2B RECEPTOR SIGNALING: A NOVEL NEGATIVE REGULATOR OF LIVER GROWTH
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Background/Aim: Tight regulation of liver cell proliferation is a key event to control organ size during development and hepatic regeneration. Platelet-derived serotonin (5-HT) has recently been described as a stimulator of liver regeneration. A role for 5-HT receptors as regulators of 5-HT-stimulated regeneration has been suggested but is not yet well defined. There are seven distinct classes of 5-HT receptor (5-HT1R to 5-HT7R) and within each class there are multiple subclass members (e.g. 5-HT2A, 5-HT2B and 5-HT2C). The aim of this study was to understand how 5-HT2B receptors contribute to serotonin dependant liver regeneration. Methods: A two-thirds partial hepatectomy or sham laparotomy was performed in wild-type or 5HT2B receptor knockout mice and livers were harvested at 2, 4, 36, 48 and 72 hours post-operatively. Cell proliferation was assessed by staining for Ki67 and BrdU incorporation. Expression of 5-HT2B, 5-HT2A, IL-6, TNF-α, HGF, TGF-α, TGF-β, cyclin E, p21 (Waf1/Cip1) were quantified at the messenger RNA. Results: Gene expressions of hepatic 5-HT2 receptors increase following partial hepatectomy. Maximal liver regeneration in control wild type mice peaked at 36-48 hours post-operatively. Conversely 5-HT2B receptor knockout mice demonstrated increased numbers of proliferating liver cells and prolonged regenerative period compared to wild type mice. Cytokine priming by IL-6 and TNF-α gene expression was reduced in knockout mice. No significant difference in growth factors was observed, however, the anti-proliferative cytokine TGF-β gene was significantly lower in knockout mice at 36-48 hours post-operatively. Additionally P21, a potent inhibitor of cell cycle, was increased in wild type mice 36-72 hours post partial hepatectomy, but interestingly, this increase was not observed in the knockout mice. Conclusion: In conclusion 5-HT2B receptors are negative regulators of hepatic growth and function. We suggest that 5-HT2B functions as a “brake” on hepatocyte proliferation and limits the regenerative capacity of the liver, possibly via a P21-dependent mechanism. These data highlight the potential to exploit serotonin signaling to treat liver disease; 5-HT2B receptors antagonists may enable liver regeneration in end stage liver disease, whilst, agonists will limit the proliferative potential of hepatocytes in hepatocellular cancer.

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The sodium-coupled neutral amino acid transporter (SNAT) 4 is expressed in developing mouse liver and regulated by hepatocyte nuclear factor (HNF) 4α.

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Hepatocyte nuclear factor (Hnf) 4α plays a pivotal role in liver organogenesis. However, the underlying molecular mechanisms by which Hnf4α regulates the fetal liver development remain to be elucidated. In the current study, we have established the organ culture system of mouse fetal liver, and overexpression of Hnf4α was achieved using the adenoviral gene expression system. By applying suppression subtractive hybridization (SSH) to this system, we identified the genes regulated by Hnf4α. Among the identified genes to be up-regulated by Hnf4α over-expression, solute carrier family 38, member 4 (Slc38a4) was included. This gene encodes the sodium-coupled neutral amino acid transporter (SNAT) 4, which is a member of system A amino acid transporters. The expression of SNAT4 in liver organogenesis is unclear. We examined the expression pattern of SNAT4 during liver development, and verified the regulation of its gene expression by Hnf4α. Real-time PCR analyses showed that SNAT4 mRNA was increased in parallel with increase of endogenous Hnf4α mRNA, and SNAT4 mRNA level was predominant among system A amino acid transporters. Whole-mount in situ hybridization revealed that SNAT4 mRNA was expressed in liver bud at E9.5. In situ hybridization using tissue sections from E12.5 adult mice demonstrated that both hepatoblasts and mature hepatocytes expressed SNAT4. Western blot analyses confirmed that SNAT4 protein was detected in fetal liver after E16.5, and also in adult liver. Next, to examine whether Hnf4α regulates SNAT4 expression at transcriptional level, we performed a series of promoter analyses. The 3.7-kbp promoter fragment of the mouse SNAT4 gene was obtained by PCR, and various length of fragments (-3676, -959, -554, -153, -108, and -28/+104 bp from the transcription start site) were inserted into the luciferase reporter vector pGV2 containing neither promoter nor enhancer. Those promoter analyses showed that -153/+104 bp region exhibited maximal responses in HepG2 cells (Hnf4α expressing cells). Furthermore, the transactivation of this promoter was up-regulated by Hnf4α in Hela cells (Hnf4α is not expressed). In conclusion, taking advantage of fetal liver organ culture system and SSH, we identified the SNAT4 as a downstream target of Hnf4α. The expression of SNAT4 increased during the embryonic liver development both in mRNA and protein levels. These results suggest that the development of amino acid transport system is a critical event during liver morphogenesis.

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DIFFERENTIAL FUNCTIONS OF CYCLIN E1 AND E2 FOR CELL CYCLE CONTROL AND ENDOREPLICATION DURING LIVER REGENERATION IN MICE

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Background and aims: Recent data indicated that cyclin E1 and E2 are dispensable for continuously dividing cells, but are essential for both endoreplication and cell cycle re-entry of quiescent cells. The aim of this study was to explore the role of E-type cyclins during liver regeneration. Methods: 70% partial hepatectomy (PH) was performed in cyclin E1 and E2 knockout mice (KO) and wildtype (WT) controls. Liver regeneration was monitored by measuring the liver/body weight ratio after PH and cell cycle markers for G1/S-Phase (PCNA, cyclin E1, E2), S-phase (Brdu, cyclin A) and M-phase (histone H3, cyclin B). DNA content of hepatocytes was determined by FACS analysis. Activity of cyclinE/cdk2 complexes were analysed through histone H1 kinase assays. Results: Both E1 and E2KO mice performed liver regeneration after PH, but displayed different proliferation kinetics compared to WT controls. E1KO mice revealed a slight delay of G1/S phase transition but stronger hepatocyte proliferation at later time points (48-72h post PH) and a prolonged cdk2 kinase activity compared to WT animals. In E2KO mice we found a contrary effect. Hepatocyte proliferation started earlier and was significantly higher in course of regeneration. Cdk2 kinase activity was observed from 36 to 96 hours post PH whereas in WT mice cdk2 kinase activity was only found from 40 to 48 hours after PH. Accordingly, E2KO mice showed a 40% higher liver/body weight ratio in comparison to control animals 7 days after PH. In E1KO mice the cyclin E2 expression started significantly later after PH with an overall lower expression level compared to WT animals. In contrast, cyclin E1 expression in E2KO was higher than in the control group. Surprisingly, we found only minor differences in M-phase between E1KO, E2KO and control animals. Therefore the ploidy of hepatocytes post PH was analysed by FACS. In E1KO mice we observed a prevalence of diploid cells and only a low number of cells with higher polyploidy index. In contrast, E2KO mice showed much higher polyploidy levels (>4n) from 72h to 96h post PH indicating that the strong DNA synthesis in these animals after PH predominantly results in higher endoreplication. Conclusion: Our data demonstrates that neither cyclin E1 nor E2 alone is essential for liver regeneration. Depletion of cyclin E1 can largely be compensated presumably by cyclin E2. However, as cyclin E2 depletion leads to earlier and stronger onset of S-phase, higher polyploidy and hepatomegaly, we conclude that cyclin E2 acts as a negative regulator of cyclin E1, whereas our data suggests cyclin E1 as the key player for G1-S phase transition and endoreplication in hepatocytes.

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S-ADENOSYL METHIONINE AND METHYLTHIOADENOSINE INHIBIT LIPOPOLYSACCHARIDE-INDUCED TUMOR NECROSIS FACTOR α (TNFα) EXPRESSION VIA MODULATION OF HISTONE METHYLATION

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Background and aim: SAMe has been shown to reduce the expression of pro-inflammatory cytokines such as tumor necrosis factor α (TNFα), which plays a key role in the development of many liver injuries. SAMe and its metabolite methylthioadenosine (MTA) can block lipopolysaccharide (LPS)-induced TNFα expression in RAW cells and Kupffer cells at the transcriptional level without affecting NFκB nuclear binding. However, the exact molecular mechanism(s) of the inhibitory effect remains unclear. While SAMe is a methyl donor, MTA is an inhibitor of methylation and has been shown to inhibit histone 3 lysine 4 (H3K4) methylation. SAMe can convert to MTA spontaneously so that the effect of exogenous SAMe may be mediated by MTA. The aim of our work is to examine whether the mechanism of SAMe and MTA’s inhibitory effect might involve modulation of histone methylation. Methods: RAW cells were pretreated in serum-free medium with 0.75 mM SAMe or 0.5 mM MTA for 16 h. They were then stimulated with LPS (500 ng/ml) or vehicle alone for 4 h, and processed for TNFα gene expression analysis using real-time PCR. The effect of SAMe and MTA on LPS-induced TNFα promoter activity was determined using a luciferase reporter gene assay. In order to study the endogenous chromatin organization of the TNFα promoter, chromatin immunoprecipitation (ChIP) assay was carried out. High-performance liquid chromatography (HPLC) was used to quantify the levels of SAMe and MTA. Results: Consistent with previous studies, we found that LPS induced the TNFα expression by 75.8 fold (±8.0) and the pretreatment with either SAMe or MTA decreased this expression to 18.4 fold (±1.5) or 24.5 fold (±3.7), respectively. The inhibitory effect of SAMe and MTA lies at the transcriptional level as they completely blocked the LPS-induced TNFα promoter activity. Using ChIP assay, we found that LPS increased the binding of trimethylated H3K4 (linked to transcriptional activation) to the TNFα promoter containing two key NFκB sites and this was completely blocked by either SAMe or MTA pretreatment. Exogenous SAMe is unstable and converts spontaneously to MTA, which is stable and more cell permeant. Treatment with both SAMe (0.75 mM) or MTA (0.5 mM) doubled intracellular MTA level, suggesting that the mechanism of SAMe’s inhibitory effect on histone methylation and TNFα expression is likely mediated by MTA. Conclusions: LPS treatment promotes the binding of trimethylated H3K4 to the TNFα promoter. Therapeutic use of SAMe can inhibit LPS-induced gene expression via MTA-mediated inhibition of histone methylation. This aspect of SAMe has not been described and is important in understanding its therapeutic effect.

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ADENO VIRAL TRANSFER OF SIRNA AGAINST POLO-LIKE KINASE 1 INHIBITS TUMOR PROGRESSION OF HEPATOCELLULAR CARCINOMA IN VITRO AND IN VIVO

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Background and Aims: Effective systemic therapy of the hepatocellular carcinoma (HCC) is not available. Polo-kinase 1 (Plk1), a mitosis-associated serine/threonine kinase, is a prime target in carcinomas, in which it is overexpressed. The present
study examined if down-modulation of Plk1 by RNA interference might be a suitable strategy to treat patients with HCC. Methods: Plk1 expression was studied in 7 HCCs and normal liver tissue from the same patient undergoing liver resection by immunoblotting and real-time PCR. Small interfering (si) RNAs raised against Plk1 as well as adenoviral vectors (AdV) encoding short hairpin (sh) RNA against Plk1 (AdV-Plk1), were constructed and examined for their effects on Plk1 expression by immunoblotting, viable cell mass by the MITT test and apoptosis by Hoechst staining in different HCC cell lines and primary hepatocytes. The efficacy of infection of cultured cells as well as of HuH-7 xenografts with AdV upon intravenous injection of the recombinant adenoviral vectors was determined using an AdV encoding β-galactosidase (AdV-LacZ). In addition, the effect of Plk1 down-modulation on HCC progression was studied by intravenous injection of AdV-Plk1 or a control virus in HuH-7 tumors bearing nude mice. Results: Plk1 was strongly expressed in 6/7 HCCs as compared to normal liver tissue from the same patient (average 28.9-fold at the mRNA level). Suppression of Plk1 expression by transfection of siRNAs directed against Plk1 or infection with AdV-Plk1 strongly decreased the viability and increased apoptosis of several cultured HCC cell lines, whereas primary human hepatocytes remained unaffected. Intrahepatic administration of AdV-LacZ into HuH-7 tumor bearing nude mice revealed a high degree of β-galactosidase expression in the tumors. Intravenous administration of AdV-Plk1 caused down-modulation of Plk1 expression and strongly inhibited tumor progression. Conclusions: Plk1 appears to be a suitable target in the treatment of HCC. Systemic adenoviral delivered RNAi-induced down-modulation of Plk1 might be a novel strategy for an effective HCC therapy.

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1244 GENE EXPRESSION BASED RECURRENCE PREDICTION OF HBV-RELATED HUMAN HEPATOCELLULAR CARCINOMA

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Poor prognosis of hepatocellular carcinoma (HCC) is in part due to high rate of recurrence even after “curative resection” of tumors. Therefore, it is axiomatic that development of an effective prognostic prediction model for HCC patients after surgery would at minimum help to identify in advance those who most benefit from the treatment and at best provide new therapeutic strategies for the patients with high risk of recurrence. For the prediction of the risk of recurrence in HCC patients, gene expression profiles were generated in 65 HCC patients with hepatitis B infection. Microarray hybridization was carried out with Human Genome U133A 2.0 chips (Affymetrix, Santa Clara, CA). For the validation of the prognostic reproducibility of this recurrence signature, we applied our recurrence signature directly to an independent gene expression dataset of HCC patients (n=139) [data from Laboratory of Experimental Carcinogenesis (LEC), National Cancer Institute, NIH]. Recurrence associated gene expression signature successfully discriminated between high- and low-risk patients (log-rank test, p=1.9e-6). Comparison of this recurrence signature in independent HCC microarray datasets showed robustness and consistency. Kaplan-Meier plots of HCC subtypes showed a significant difference of recurrence between the subgroups of each individual dataset [SNU data set: p=0.007, LEC dataset: p=0.005, respectively, log-rank test]. Kaplan-Meier analysis on the overall integrated dataset successfully dissected subgroups based on the recurrence rate (p=0.0003). The multivariate analysis, including all the clinicopathological variables and the molecular subtype, showed that only the molecular subtype was significantly associated with tumor recurrence (hazard rate=12.54, 95% CI:3.59-43.76, p<7.30e-5). Genetic network analyses identified SP1 and peroxisome proliferator-activated receptor α (PPARα) as common regulators in the high- and low-risk groups, respectively, suggesting their functional significances for HCC recurrence. In conclusion, we have identified gene expression signature that effectively identifies recurrence subtypes in HCC and our recurrence signature is consistent, robust, and independent of cohorts and experimental platforms of individual studies. The molecular subtype generated by recurrence signature is the most successful predictor even after the clinicopathological features have been considered. Our results might provide novel biological insights into the mechanisms of tumor recurrence.

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1245 MITOCHONDRIAL CHOLESTEROL IN HEPATOMA CELLS MODULATES APOPTOSIS SUSCEPTIBILITY AND CANCER THERAPY IN TUMOR XENOGRAFTS

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Cholesterol metabolism is dysregulated in many malignancies, including hepatocarcinoma and stathis have been proposed for cancer therapy. However, experimental data indicate that antitumor properties of statins are primarily due to their blockade of protein isoprenylation and independent of sterol-synthesis inhibition. Mitochondrial cholesterol levels in hepatoma cells correlate with the degree of malignancy. We have previously shown that in vitro cholesterol enrichment of rat liver mitochondria impairs the mitochondrial permeability transition induced by apoptotic stimuli. The AIM of this study was to examine the role of high mitochondrial cholesterol in hepatoma cells in chemotherapy-induced apoptosis and tumor growth. Methods: Rat hepatocarcinoma H35 cells and human hepatoblastoma HepG2 cells were treated with lovastatin or YM-53601, a squaraine synthase inhibitor, for 24 hours examining their susceptibility to thapsigargin, arsine trioxide (ATO), londamine or doxorubicin. Cytochrome c and Smac/DIABLO release from mitochondria was examined by Western Blot. Doxorubicin therapy in vivo was evaluated in tumor xenografts in nude mice by co-treatment or not with atorvastatin (10 mg/kg) or YM-53601 (15 mg/kg). Results: Mitochondria from H35 and HepG2 cells exhibit high mitochondrial free cholesterol levels (5-12 fold) and reduced susceptibility to londamine, ATO, thapsigargin or doxorubicin. Incubation with lovastatin significantly reduced the cholesterol content of mitochondria from H35 cells (6.3 ± 3.5 vs. 13.1 ± 4.4 µg/mg prot.) and HepG2 cells (8.5 ± 2.6 vs. 25 ± 6.4 µg/mg prot.), and increased susceptibility to cell
Moreover, HGF transduction suppresses mice as the result of H-E staining and Azan-Mallory staining. The establishment of liver fibrosis was found in all of AAV5-HGF received mice. AAV5-HGF reached stable HGF expression both in serum and situ zymography were performed. (Results) Mice that received HGF injection, mice were underwent BDL, and then sacrificed 3, 6, 9, and 12 weeks after AAV5-HGF establishment of liver fibrosis, AAV5-HGF was injected once into the portal vein through the splenic hilum. AAV5-LacZ was used as a MOCK. Mice were killed 3, 6, 9, and 12 weeks after treatment. In the present study, we constructed HGF expressing AAV (AAV5-HGF) and examined its effect in two kind of mouse liver fibrosis model. (Method) Two kinds of liver fibrosis model were used in this study. One model was established by carbon tetrachloride (CCl4) administration. CCl4 was administered in Balb/c mice weekly during our experimental schedule. After establishment of liver fibrosis, AAV5-HGF was injected once into the portal vein through the splenic hilum. AAV5-LacZ was used as a MOCK. Mice were killed 3, 6, 9, and 12 weeks after injection to assess therapeutic efficacy. Another model was established by bile duct ligation (BDL). Seven weeks after AAV5-HGF injection, mice were underwent BDL and then sacrificed two weeks after BDL. Liver was removed and underwent histological analysis in both models. Expression of Ets-1, a responsive transcriptional factor of HGF, was also analyzed by Western blotting, and the binding activity of Ets-1 to liver was determined by gel mobility shift assay. To investigate the role of HGF on liver fibrogenesis by CCl4 or BDL, real-time PCR and in situ zymography were performed. (Results) Mice that received AAV5-HGF reached stable HGF expression both in serum and liver for at least 12 weeks. In CCl4 model, significant improvement of liver fibrosis was found in all of AAV5-HGF received mice as the result of HE staining and Azan-Mallory staining. Moreover, HGF transduction suppresses α-SMA expression in mouse livers. These findings were also found in BDL model. In addition, necrotic change was significantly improved by HGF gene transduction. Expression of Ets-1 protein and increased Ets-1 binding ability were found in the liver of mice that were transduced HGF gene. TGF-β, Collagenα1 and TIMP-1 mRNA were significantly suppressed in the liver of AAV5-HGF transduced mice both CCl4 mice and BDL mice. MMP-13 mRNA was significantly increased in the liver of AAV5-HGF transduced mice both CCl4 mice and BDL mice. In situ zymography showed extensive gelatin degradation in liver section of HGF transduced mice compared with control. (Conclusions) Single injection of AAV vector containing HGF gene achieved long term expression of HGF and resolution of mouse liver fibrosis. HGF gene therapy mediated AAV is feasible for the treatment liver fibrosis.

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1247

SUBCUTANEOUS VACCINATION WITH DENDRITIC CELLS ENGINEERED TO EXPRESS MAFF AND IL-12 INDUCES INHIBITION OF AFP-EXPRESSING SUBCUTANEOUS HEPATOCELLULAR TUMOR GROWTH IN MICE

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Background: Dendritic cells (DC) are professional antigen presenting cells able to prime strongly T-cells against tumor-associated antigens (TAA). However, their potential to induce HCC regression is still limited. AFP is the most relevant TAA of HCC, which can be used as a target for immunological approaches of HCC. IL-12 is the main cytokine for the development of a Th1 immune response. In this study, DC co-transduced with adenoviruses encoding AFP and IL-12 enhanced inhibition of the growth of AFP-expressing HCC in mice. Methods: Four adenoviruses were generated: Ad-mAFP [encoding murine AFP], Ad-IL-12, Ad-GFP-luc (GFP and luciferase) and Ad-LacZ (as control). DC were obtained from bone marrow of C3H-mice and cultured with GM-CSF and IL-4. DC were co-transduced with adenoviruses (MOI 500) on day 6. AFP-expression was detected in Western Blot and CD80, CD86 and CD40 expressions were assayed by flow cytometry. IL-12-expression was detected by ELISA. DC were injected in the left flank of mice s.c. twice at weekly intervals. One week after the last vaccination, a HCC was induced by inoculation of 106 AFP-positive or -negative Hepa129 cells into the right flank of mice. Assessment of transgene expression by DC in vivo was followed using bioluminescence imaging. Results: Ad-mAFP-transduced DC showed a dose-dependent AFP-expression detected already at MOI 100. IL-12-secretion of DC, which were transduced with Ad-IL-12 was significantly enhanced compared to control or Ad-AFP-transduced DC. CD80, CD86 or CD40 expressions were not reduced by adenoviral transduction. Vaccination with AFP-expressing DC could inhibit significantly the growth of AFP-expressing HCC, but not of AFP-negative tumors, indicating AFP-specificity of the immune response. Vaccination with Ad-IL-12/Ad-mAFP co-transduced DC inhibited significantly the tumor volume compared to controls, vaccinated with Ad-AFP or Ad-LacZ-transduced DC. Two weeks after tumor induction, the hepatocellular carcinoma was significantly (p<0.05). Using Ad-GFP-luc-transduced DC, a delivery of transgene expression of adenoviral transduced DC into the ipsilateral lymph nodes was observed in vivo. Conclusions: Our data show that s.c.-vaccination with Ad-IL-12/Ad-AFP-transduced DC can significantly enhance the inhibitory effects of Ad-AFP-transduced DC against AFP-positive HCC in mice. Moreover, we observe a migration and delivery of transgene expression of adenoviral transduced DC into secondary lymphoid tissues, suggesting that
interaction of TAA-expressing DC with Th1-lymphocytes takes place in the lymph nodes.

Disclosures:
The following people have nothing to disclose: Maria A. Gonzalez-Carmona, Carlo Schneider, Ingo G. Schmidt-Wolf, Volker Schmitz, Tilman Sauerbruch, Wolfgang H. Caselmann

**1248**
TRANSFORMING GROWTH FACTOR-β GENE EXPRESSION SIGNATURE PREDICTS CLINICAL OUTCOME IN CANCER

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Hepatocellular carcinoma (HCC) is one of the most common cancers in the world. The clinical heterogeneity of HCC and the lack of good diagnostic markers and treatment strategies have rendered the disease a major challenge. Patients with HCC have a highly variable clinical course indicating that HCC comprises several biologically distinctive subgroups. We hypothesized that the prognostic variability likely reflects a molecular heterogeneity of tumors. In the process of testing this hypothesis, we have asked if a gene expression signature specific for TGF-β signaling pathway could refine the diagnosis and/or prognostic predictions of HCC patients. Since TGF-β exhibits tumor stage dependent suppressive (i.e. growth inhibition) and oncogenic (i.e. invasiveness) properties, a TGF-β gene expression signature may contain gene sets characteristic for these properties and thus be relevant for the molecular classification of the tumors. Applying a comparative functional genomic approach we demonstrated that temporal TGF-β gene expression signatures established in mouse primary hepatocytes successfully discriminated distinct subgroups of HCC. The TGF-β positive cluster highlighted two independent, early (1-2 hours) and late (4-24 hours), TGF-β signatures. To evaluate the clinical significance of TGF-β signature in the molecular classification of HCC, we then compared the distribution of several clinical and pathological variables between HCC harboring early or late TGF-β signatures. Kaplan-Meier plots and log-rank statistics indicated that the patients with a late TGF-β signature showed a significantly shorter mean survival time (16.2 ± 5.3 months) compared to the patients with an early (60.7 ± 16.1 months) TGF-β signature. Also, tumors expressing late TGF-β responsive genes displayed an invasive phenotype and an increased tumor recurrence. Furthermore, we demonstrated that the TGF-β gene expression signature possess a predictive value for tumors other than HCC and therefore open new avenues for novel TGF-β based therapeutic approaches.

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The following people have nothing to disclose: Cedric Coulouarn, Valentina M. Factor, Snorri S. Thorgeirsson

**1249**
A UNIQUE METASTASIS-RELATED MICRORNA EXPRESSION SIGNATURE IS A PROGNOSTIC INDICATOR OF SURVIVAL AND RECURRENTENCE IN HEPATOCELLULAR CARCINOMA

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Background: MicroRNAs (miRNAs) have been utilized as biomarkers to distinguish cancers from their normal counterparts. Hepatocellular carcinoma (HCC) is an aggressive cancer with a dismal outcome largely due to metastasis and post-surgical recurrence. Objectives: We investigated whether the expression of unique miRNAs are associated with metastatic HCC and correlate with prognosis and recurrence. Methods: We examined the miRNA expression profiles of 482 cancerous and non-cancerous specimens from radical resection of 241 HCC patients. Using a supervised algorithm, we built a miRNA metastasis signature based on 131 clinically well-defined metastatic and non-metastatic HCC specimens, which was then used to predict the prognostic outcomes of the remaining 110 independent HCC specimens. Results: A unique 20-miRNA signature could significantly predict (p<0.001) 29 primary HCC tissues with venous metastases from 102 metastasis-free solitary tumors with 10-fold cross validation which significantly correlated with survival (p=0.005). However, significant miRNAs could not be identified from the corresponding non-cancerous hepatic tissues. The tumor miRNA signature was a significant independent predictor of patient survival (p=0.009) in 110 additional independent HCC specimens. The signature was also significantly associated with both survival and relapse in 89 early stage HCC (p=0.022 and 0.002, respectively). This signature was associated with a two-fold increase in death for cases predicted to be metastatic. Conclusions: Specific miRNAs exceed the prognostic capacity of current staging systems for HCC. We suggest that a simple method to profile certain miRNAs may have clinical utility for advanced identification of HCC patients who are likely to develop metastases/recurrence and subsequent selection of candidates for appropriate treatment.

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**1250**
HUGL-2, THE HUMAN HOMOLOGUE OF THE DROSOPHILA TUMOR SUPPRESSOR LGL, IS REGULATED BY EGF AND SNAIL, A PRIME REGULATOR OF EPITHELIAL-MESENCHYMAL TRANSITION (EMT)

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Background: Hugl-1 and Hugl-2, the human homologues of Drosophila lethal giant larvae (lgl), are highly conserved tumor suppressor genes encoding cytoskeletal proteins. The human lgl
polarity gene, Hugl-2, is involved in development of normal epithelial differentiation and maintenance of cell integrity and adhesion. Reduced expression of Hugl contributes to tumor progression in various tissues. The aim of this study was to identify regulatory elements in the Hugl-2 promoter to better understand the transcriptional regulation of Hugl-2 during tumor progression especially in human hepatocellular carcinoma. Here we report the regulation of Hugl-2 by EGF and the transcription factor Snail. Methods: Hugl-2 promoter region was identified by using the MatInspector program and cloned in pGL3-basic luciferase vector. We examined the promoter activity and activities of truncated forms in transiently transfected HepG2 cells and primary hepatocytes after treatment with different agents. Regulating transcription factors and their sequence binding sites were identified. We co-transfected Snail cDNA expression plasmids with the Hugl-2 promoter. Snail binding sites were mutated. To detect direct binding of Snail on the promoter Chromatin immunoprecipitation was performed. Results: Northern Blot analyses of Hugl-2 showed that the gene is mainly expressed in the liver and pancreas, whereas Hugl-1 is mainly expressed in brain with absent expression in liver. Hugl-2 might be implicated in hepatic and pancreatic processes. Promoter analyses showed high basal Hugl-2 promoter activity in cell lines and primary hepatocytes. Searching for conserved transcription factor binding sites and regulatory pathways, we identified EGF and Snail as suppressors of Hugl-2 promoter activity, suggesting that Hugl-2 is regulated by EGF and Snail. Down-regulation of Hugl-2 by these factors might result in a reduction of cell adhesion and therefore in an acceleration of tumor progression. Co-transfection experiments with Snail cDNA expression plasmids and the Hugl-2 promoter construct revealed a strong and dose dependent repression of the Hugl-2 promoter by Snail. Using Hek-293 cell lines stably expressing Snail, we could show not only repression of the Hugl-2 promoter plasmid but also of the endogenous Hugl-2 promoter. Chromatin immunoprecipitation assays revealed a direct binding of Snail on the Hugl-2 promoter. Conclusions: The tumor suppressor gene Hugl-2 is transcriptionally regulated by EGF and Snail. We identified molecular targets in the Hugl-2 promoter which may play a role in the downregulation of the Hugl-2 tumor suppressor gene during progression of human hepatocellular carcinoma.

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1251 ENHANCED RETINOIC ACID-INDUCED APOPTOSIS IN HEPATOCELLULAR CARCINOMA CELLS VIA ER STRESS-RELATED JNK ACTIVATION IS ATTENUATED BY HYPOXIA INDUCTION OF CRABP-II
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Background/Aims: Hepatocellular carcinoma (HCC) is characterized by hypervascularization and its growth is tightly linked to the neovascularization. However, advanced infiltrative HCCs seldom show hypervascularity, though they are more rapidly growing than mass-forming types. Therefore, hypoxia is most likely to activate survival signaling in HCC cells. In this study, we attempted to find an agent which can induce cell death more efficiently in hypoxic HCC cells than in normoxic cells.

Methods: Human HCC cell lines were used in this study. RNAs extracted from cells cultured either in a normoxic or hypoxic condition were used for cDNA microarray analysis. Cell growth was assessed using the MTS assay and the apoptotic cell death was measured by DAPI staining. Apoptotic signaling cascades and kinase signaling were explored by immunoblot analysis. The occurrence of ER stress was evaluated with immunoblot for phospho-eIF2α.

Results: Out of many molecules up-regulated in hypoxic cells, we found that cellular retinoic acid binding protein II (CRABP-II) signal was significantly increased. Hypoxia induction of CRABP-II protein expression was confirmed by immunoblot analysis. Since CRABP-II is a binding partner of all-trans-RA (RA) and RA can induce cellular apoptosis, we next treated HCC cells with RA. HCC cell growth was suppressed following RA treatment. In particular, this growth suppression was significantly enhanced in hypoxic cells as compared to normoxic cells. RA treatment induced HCC cell apoptosis by activating caspase 9, and this was more enhanced in hypoxic cells than in normoxic cells. This enhanced caspase 9 activation was associated with enhanced Jun N-terminal kinase (JNK) activation in these hypoxic cells. ER stress, which can lead to pro-apoptotic JNK activation, was more activated in hypoxic cells following RA treatment as compared to normoxic cells. To clarify the role of CRABP-II induction by hypoxia in RA-induced apoptosis, cells were then transfected with CRABP-II-specific siRNA. Cells with reduced CRABP-II expression showed more enhanced RA-induced JNK activation and cellular apoptosis than control cells.

Conclusions: These results demonstrate that RA induces cellular apoptosis more efficiently in hypoxic cells than in normoxic cells via enhanced ER stress-related JNK activation, and that CRABP-II induction by hypoxia attenuates RA-induced apoptosis in hypoxic HCC cells. Therefore, this RA/CRABP-II signaling may therapeutically be useful in the treatment of advanced hypovascular HCCs, which are exposed to hypoxic environment.

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1252 INVESTIGATION OF THE ROLE OF GLYPICAN-3 IN RAT HEPATOCYTE GROWTH AND LIVER REGENERATION
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Glypicans are heparan sulfate proteoglycans (HSPGs) that are bound to the cell surface through a glycosylphosphatidylinositol (GPI) anchor. Five members of the glypican family have been identified in mammals, which share different levels of homology and expression patterns. Among these glypicans, it has been reported that mutations in the human glypican 3 (GPC3) gene are associated with the Simpson-Golabi-Behmel (SGB) syndrome, which is an X-linked disorder characterized by pre- and postnatal overgrowth. GPC3 is reported to be over-expressed in human hepatocellular carcinoma (HCC) and several hepatoma cell lines. Whether GPC3 is involved in liver regeneration and hepatocyte proliferation is still unknown. We investigated its role in regenerative liver using the 2/3rd partial hepatectomy models in rats. Results from RT-PCR showed that expression of GPC3 is upregulated in liver starting from 3 hours after partial hepatectomy. However, GPC3 protein on the membrane significantly decreases after hepatectomy through day 2 and then returns to normal levels by day 5. Examination of GPC3 levels in primary hepatocyte cultures revealed that GPC3 is upregu-
lated from day 7, when the process of proliferation slows down. Yeast two hybrid assay was performed to investigate its potential interacting protein and pathway. We found several interesting target proteins such as CD81 and Hrs. The interaction test assay and pathway study has been carried out for CD81, a member of tetraspan family and reported to involve in cell proliferation. RT-PCR result showed that in regenerative liver, CD81 RNA level decreases after heptectomy through day 2 and then returns to normal levels by day 5, which corresponds to the changes of GPC3 protein level. Immunofluorescence and immunohistochemistry study showed that both CD81 and GPC3 localize on the cell membrane of hepatocytes. Further study will include GPC3 transgenic mice screening and study, and in vivo and in vitro growth study. We hypothesize that GPC3 negatively regulate cell growth and may interact with CD81.

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The following people have nothing to disclose: Bowen Liu, William C. Bowen, Aaron W. Bell, Shrish Paranjape, Wendy M. Mars, Kari N. Nejaj-Bowen, Jianhua Luo, George Michalopolous

1253
THE ROLE OF NF-κB ACTIVITY IN THE ANTIVIRAL ACTION OF INTERFERON
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The role of NF-κB activity in the induction of IFN's antiviral action was examined in MEFs that either had normal NF-κB function (WT MEFs), or had a germline disruption of both p50 and p65 NF-κB proteins (p50/p65-DKO MEFs). It was very interesting that p50/p65-DKO MEFs were more sensitive to the antiviral action of IFN against influenza virus as compared to WT MEFs. For example while IFN (100 U/ml) only results in a ~8-fold reduction in influenza virus titer in WT MEFs, a 100-fold lower IFN concentration (1 U/ml) induced an equivalent antiviral effect in DKO MEFs. Thus the IFN-activated NF-κB pathway may attenuate the induction of antiviral activity by IFN. To characterize the role of NF-κB in regulating IFN-stimulated gene (ISG) expression, we compared gene expression in WT and p50/p65-DKO MEFs treated in the presence or absence of IFN by bioinformatic approaches. The induction by IFN of a subset of genes encoded GTP-binding and antigen presentation proteins was regulated by NF-κB. The NF-κB-regulated GTP-binding ISGs included the 65-67 kDa guanylate-binding proteins (Gbp 1 and Gbp2), the Mx proteins (Mx1 and Mx2), and the 47-kDa GTPase Ifi47 [also called lrg47]. GBP proteins play important roles in resistance to viruses, as well as intracellular protozoa and bacteria. The NF-κB-regulated antigen presentation ISGs (Tap1, Tap2, Psmb9/Lmp2, and Psmb8/Lmp7) are involved in degrading intracellular proteins into antigenic peptides, and contribute to the transport of these peptides to endoplasmic reticulum where they bind to the assembled MHC class I molecules. We found that Gbp1, Ifi47, Mx1, Mx2, Tap1, Psmb9/Lmp2 and Psmb8/Lmp7 were induced by IFN to a greater extent in NF-κBKO MEFs than in WT MEFs, while Gbp2 and Tap2 were induced to a greater extent in WT MEFs than in p50/p65-DKO MEFs. These findings suggested that NF-κB differentially regulates the expression of a subset of ISGs that play important roles in innate immunity. To further investigate the induction of NF-κB-regulated ISGs, qPCR was performed for Ifi47, Tap1 and Mx1 using RNA from WT and p50/p65-DKO MEFs and confirmed the microarray results. The effect of NF-κB on regulation of these ISGs is specific since the expression profile of another ISG, IRF1, was not regulated by NF-κB, and did not reflect altered JAK-STAT signaling since STAT2 activation was similar in WT and p50/p65-DKO MEFs. Our results indicate that NF-κB selectively attenuated the transcription of a subset of ISGs.

Disclosures:
The following people have nothing to disclose: Lawrence Pfeffer

1254
GANGLIOSIDE GD3 SYNTHASE OVEREXPRESSION SENSITIZES HUMAN HEPATOMA CELLS TO HYPOXIA BY SUPPRESSING THE NUCLEAR FACTOR-κB-DEPENDENT SURVIVAL PATHWAY
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Hypoxia is a common feature of solid tumors which regulates malignant progression and responsiveness to therapy. The adaptation to hypoxia is mediated by mitochondrial reactive oxygen species (ROS) generation that contributes to survival of cancer cells, angiogenesis, vascularization, glycolytic ATP and tumor invasion through NF-κB and HIF-1α activation. Ganglioside GD3 is a lipid death effector due to its interaction with mitochondria and inactivation of NF-κB-dependent survival pathways. We have recently shown that NF-κB plays a critical role in the survival of hepatoma cells during hypoxia. Thus, the aim of our work was to examine the survival of hepatoma cells that overexpress GD3 during hypoxia. Methods. GD3 synthase full-length cDNA (1.2 kb) was cloned into pcDNA3.1D/V5-His-TOPO®. Hep3B were transfected with pcDNA3M-GD3 (Hep3B-GD3) or pcDNA3 (no insert, Hep3Bwt) and selected during 3-4 weeks based on expression of GD3 synthase, its enzymatic activity and GD3 levels. Hypoxia (2%) was induced in Hep3B cells for 24-72 hours. NF-κB and HIF-1α transactivation were measured by luciferase reporters. Results. Immunostaining analyses indicated that GD3-expressing Hep3B clones accumulated GD3 both at the cell surface and in internal membranes, including mitochondria. These cells did not exhibit changes in morphology, growth and apoptosis as compared to Hep3Bwt during normoxia. However, the exposure of Hep3B-GD3 cells to 2%O2 elicited a time-dependent decrease in cell survival reaching 55% at 72 hours while in Hep3Bwt was 90%. This was accomplished by greater ROS generation (1.5-2 fold) in Hep3B-GD3 versus Hep3Bwt. Hypoxic NF-κB activation observed in Hep3Bwt (2.3 fold) was abrogated in Hep3B-GD3 cells (1.2-1.5 fold) whereas HIF-1α levels (6 fold versus normoxia) remained similar in both clones. We next, focused on the regulation of Mn-SOD, a known κB-controlled antioxidant enzyme. Interestingly, hypoxia induced Mn-SOD protein and mRNA levels (2-3 fold, respectively) in Hep3Bwt that were reduced upon NF-κB downregulation in Hep3B-GD3 (1.2-fold). Moreover, MnTBAP, a SOD mimetic, reduced ROS generation and protected Hep3B-GD3 cells against 2%O2 at 72 hours (viability of 75%). Conclusions. Endogenous expression of ganglioside GD3 sensitizes Hep3B cells to hypoxia through suppression of inducible κB-dependent antioxidant Mn-SOD resulting in cytotoxic ROS overgeneration. These findings suggest that ganglioside GD3 may be useful in cancer gene therapy as a sensitizing agent in hypoxic-resistant cancer cells.

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The following people have nothing to disclose: Josep M. Lluís, Albert Morales, Scott Welford, Amato Giaccia, Jose C. Fernandez-Checa
1255 TRANSLATIONAL CONTROL OF GENE EXPRESSION DURING LIVER DIFFERENTIATION

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Background: Our early studies defined expression of the asialoglycoprotein receptor (ASGR) as a biochemical marker of hepatocyte terminal differentiation. Unlike most differentiation-dependent genes whose expression is regulated at the transcriptional level, developmental control of ASGR expression is at the translational level. The AIM of the present study was to characterize the molecular elements that govern differentiation-dependent ASGR expression and to determine if common elements control both in vivo and in vitro expression.

Methods and Results: Insertion the ASGR mRNA 5' untranslated region (UTR) upstream of the firefly luciferase AUG start site reduced the level of firefly luciferase activity by almost 2.5 fold in proliferating less well defined HuH-7 cells as compared to well differentiated quiescent cells. Electrophoretic mobility shift assay (EMSA) was used to confirm the presence of RNA binding protein (RBP) or trans-acting factor responsive to the state of hepatocellular differentiation. The observed 4x decrease in the specific activity of a trans-acting factor in quiescent HuH-7 suggests a derepression pathway was responsible for enhanced ASGR translation during hepatocellular differentiation. A capped, cRNA transcript containing the full-length ASGR was transcribed and translated in vitro using a wheat germ lysate in the presence of extracts from HuH-7 cells grown to low or high density. Cytosolic protein isolated from cells at low density inhibited translation over 80% while protein from high density cells was nearly inactive. A 10x molar excess of the 5'UTR ASGR mRNA fragment added as a decoy to the low density cell lysate restored translation to nearly 80%, confirming the presence of the cis-acting element. Characterization of ASGR protein and RNA expression during mouse liver development indicates that while there is an increase in transcript number during the transition between 18 day embryos and newborn mice it does not result in a concomitant increase in ASGR protein, suggesting a repression of translation during this period of liver development. EMSA was used to confirm the presence of common RBPs responsive to the state of hepatocellular differentiation. Inhibited a protein directed gel shift by preincubation of human 5'UTR with mouse cytosol strongly supports the contention that common structural elements govern translational regulation of ASGR.

Conclusion: Taken together, the results obtained from cell culture and in vivo experiments provide support for the notion that common RBP(s) interact with the ASGR 5'UTR secondary structure to play an essential regulatory role in differentiation-dependent translation.

Disclosures:
The following people have nothing to disclose: Desiree M. Espiet, Richard Stockert.

1256 IN VIVO SUPPRESSION OF MAFA mRNA WITH SIRNA AND ALTERATION OF THE GENE EXPRESSION PROFILE, ESPECIALLY OF ADIPOCYTOKINE GENES, IN MOUSE LIVER ANALYZED BY THE MICROARRAY METHOD

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One of the large maf molecules, mafa, is a strong transactivator of insulin gene transcription in pancreatic β cells. We previously reported that suppression of the mafa mRNA level in the mouse pancreas by the RNA interference technique resulted in down-regulation of adipocytokine genes in addition to the genes encoding insulin and glucagon. Mafa is likely to be related not only to the formation of pancreatic islets but to adipocytokine regulation. On the other hand, there have been several reports that mafa contributes to liver cell transdifferentiation to insulin-producing cells in combination with several transcriptional factors, suggesting a potential role of mafa in cell differentiation in the liver. In view of the role of mafa in the pancreas, the present study investigated whether reduction of the mafa mRNA level in vivo liver would induce an alteration of the gene expression profile, including adipocytokine genes. An expression vector carrying synthetic mafa siRNA was rapidly injected via the tail vein of 8-week-old mice by the hydrodynamic method, and the gene profile was analyzed with a microarray system (Affymetrix). The level of each transcript was confirmed by real-time PCR. Interference with the mafa mRNA level in the liver by siRNA resulted in a 70% reduction. The microarray analysis showed that the suppression of mafa mRNA expression in the liver caused down-regulation of genes related to lipid metabolism or cell growth, including the genes coding insulin like growth factor binding protein-1, thyroid hormone responsive spot 14, and fatty acid synthase, and up-regulation of genes coding heat shock protein 68, Onecut1/HNF-6, and Slc25a30. Both Onecut1/HNF-6, a transcription factor activated during liver development and pancreatic duct formation, and Slc25a30/KMCP1, a mitochondrial carrier protein induced during fasting and cell regeneration in the kidney, were significantly up-regulated, 2.7-fold and 3.9-fold respectively, by real-time PCR. Real-time PCR also showed that expression of adipogenic genes, such as adiponectin and adiponectin, was significantly down-regulated. Thus, mafa may have potential roles in adipocytokine regulation in the liver, for example, stellate cell differentiation (dedifferentiation) may occur through adipogenic genes regulation, or working in tandem with other transcription factors, in which mafa may be involved. In summary, mafa may be implicated in cell differentiation and the regulation of expression of some adipocytokines in the liver, the same as in the pancreas.

Disclosures:
The following people have nothing to disclose: Mariko Tsujiya, Atsushi Maeda, Junko Tanaka, Ken Tsujiya.

1257 AUGMENTER OF LIVER REGENERATION (ALR): THE FACTOR THAT REGULATES HEPATOCYTE APOPTOSIS IN LIVER REGENERATION AFTER PARTIAL HEPATECTOMY

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Background: Hepatocyte apoptosis is an important process in the fine-tune recovery of liver mass after major resection. Still unknown are the factors that control this event among the GFs and cytokines upregulated during liver regeneration. Aims: To identify the factor/s that control hepatocyte apoptosis in the animal model of liver proliferation after 70% partial hepatectomy. Materials/Methods: All the animals used underwent 70% partial hepatectomy. Recombinant Hepatocyte Growth Factor (rHGF) and Augmenter of Liver Regeneration (rAlrp) were used. We first determined serum profiles of HGF and ALR, recording a peak, for both GFs, at the 18th hr after surgery. We then administered to the animals rHGF or rAlrp, every six hrs, starting at 18th hour until 48 hrs after PH. On liver samples, hepato-
Hepatocyte proliferation (L.I.), apoptosis (TUNEL) and apoptotic genes (Bcl-2, Bax, Casp-3) mRNA levels were determined. Results: Hepatocyte proliferation in control animals was: 24hrs L.I.=22±3.5, 36hrs L.I.=21±2.4 and 48hrs L.I.=13±2.5. rHGF injection increased hepatocyte proliferation at 24 hours (L.I.=33±4), 36 hours (L.I.=28±3) and at 48 hours (L.I.=18±2.7). rAlp injection had no effect on hepatocyte proliferation. Apoptotic profile of control animals was 7.5±1.8, 3.0±0.9 and 4.1±1.2 apoptotic hepatocytes/1000 hepatocytes respectively at 24, 36 and 48 hours. rHGF administration did not produce any significant effect on hepatocyte apoptosis, while rAlp injection reduced it at 24 hours (1.2±0.5) and at 48 hours (2.2±0.7). Apoptosis-related genes showed an up-regulation of the anti-apoptotic gene (Bcl-2) and a down-regulation of the pro-apoptosis genes (Bax and Casp 3) (Fig). Conclusions: The present data demonstrate that ALR is one/ the factor responsible of hepatocyte apoptosis control in liver regeneration after partial hepatectomy by inducing anti-apoptotic genes and down-regulating pro-apoptotic genes.

1258
COBALT PROTOPORPHRYN BINDS TO BACH1, A TRANSCRIPTIONAL REPRESSOR OF HEME OXYGENASE 1 GENE

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Background/Aims: Heme Oxygenase 1 (HO-1) catalyzes heme degradation, generating ferrous iron, carbon monoxide and biliverdin, which have anti-oxidant and anti-inflammatory activities in vivo. It has been reported that enhancement of HO-1 in the liver suppresses the development of fibrosis associated with recurrent liver injury, including chronic viral hepatitis, alcohol abuse, autoimmune diseases and hereditary metabolic disorders. We found previously that cobalt protoporphyrin (CoPP) markedly up-regulates HO-1 in human liver cells; however, little is known about the mechanism by which this occurs. Bach1 is a basic leucine zipper (bZip) mammalian transcriptional repressor of HO-1. Heme is known to bind Bach1C (the C-terminal heme binding region of Bach1 that possesses four dipeptide cysteine-proline (CP) motifs), leading to its transport from nuclei to cytoplasm and thus to up-regulation of HO-1 gene expression by Nr4f2 and other small Maf proteins. The Aim of this study was to understand the binding of CoPP and other selected metalloporphyrins to Bach1. Methods: pET SUMO expression system (Invitrogen) was used to express Bach1C as a fusion protein with a small ubiquitin-related modifier (SUMO), in E.coli. The fusion protein was purified by Ni-NTA affinity chromatography and cleaved by a SUMO-specific protease to obtain native Bach1C, which was further purified by Ni-NTA affinity chromatography. CoPP was incubated with Bach1C for 30 min, followed by removal of free CoPP by dialysis. The resulting mixtures were scanned using UV-visible spectroscopy to observe the typical Soret band of protein-bound metalloporphyrins. Results: The purified Bach1C-SUMO fusion proteins were a brownish color and displayed a typical hemoprotein spectrum with Soret bands, suggesting the presence of endogenous heme in the purified Bach1C-SUMO protein. Upon incubation of Bach1C with CoPP, a Soret band with a peak at 420 nm was observed. Mutations of all of the CP motifs in Bach1C abrogated its interaction with CoPP as measured by spectroscopy. These results suggest that CoPP binds to the four CP motifs as heme does. We did not detect the binding of Bach1C to zinc- or tin mesoporphyrin. Conclusion: CoPP directly binds to Bach1C, thus presumably regulating its DNA–binding activity. These and other recent results (Hou et al, Gastroenterology 2007; 132:A825) indicate that CoPP may hold promise as a potential therapeutic agent to prevent or ameliorate hepatic fibrogenesis. Acknowledgement: Supported by NIH grant RO1 DK38825

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POSSIBLE ROLE OF TYROSINE KINASE INHIBITOR AND PROTEASOME INHIBITOR FOR HCC TREATMENT

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Aim: It has been reported that proteasome inhibitors or tyrosine kinase inhibitors can inhibit proliferation of several cancers, suggesting their therapeutic possibility or efficacy. Here, we determined whether these two type of inhibitors could become effective therapeutic tools for human hepatocellular carcinoma (HCC) through induction of apoptosis or cell cycle arrest. Materials and Methods: Human HCC cell lines, HuH7, HepG2 and HuH2 cells, were treated with either MG132, a proteasome inhibitor, or PD166289 (PD), a tyrosine kinase inhibitor. The cell cycle profile was determined by FACS analysis at each time point, and cell population at sub-G1 phase was defined as the apoptotic cells. The expression level of proteins was analyzed by western blotting; in addition physical interaction of p21Cip1 and CDK family was confirmed by IP-western technique. In order to knock down p21Cip1 or Wee1 kinase, we transfected the plasmid vector which expressed the short interfering RNA (siRNA) directed against their gene. The immunostaining was performed by using anti-Wee1 kinase antibody in
human HCC tissues which were surgically resected and used under the informed consent. Results: MG132 induced G2/M cell cycle arrest within 8 h after addition of MG132 and in a dose dependent manner. The Wee1 kinase induction and phosphorylation of Tyr15 in CDC2 was associated with the G2/M cell cycle arrest. Pretreatment with Wee1 kinase inhibitor could release cells from G2/M cell cycle arrest. p21Cip1 was simultaneously up-regulated in MG132-treated HuH7 cells, but was not p27Kip1. This p21Cip1 could not physically bind to CDC2, moreover, HuH7 cells knocked down p21Cip1 still showed G2/M cell cycle arrest by MG132 treatment. PD treatment induced apoptosis in cells associated with de-phosphorylation of Tyr15 in CDC2 leading to apoptosis. The PD-mediated apoptosis was completely inhibited by pretreatment with Roscovitine or Alsterpaulone, CDC2 inhibitors. The immunostaining of Wee1 kinase revealed positive HCC cells in 36% of poorly to moderately-differentiated HCC tissues. Conclusion: Proteasome inhibitor induced G2/M cell cycle arrest, while PD treatment executed apoptosis in HCC cell lines. From the results of immunostaining, down regulation of Wee1 kinase might be a possible approach to growth suppression of HCC by induction of apoptosis.

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1260 GENE EXPRESSION PROFILE OF PERIPHERAL BLOOD MONONUCLEAR CELLS MAY REFLECT THOSE OF TUMOR-INFILTRATING LYMPHOCYTES IN HEPATOCELLULAR CARCINOMA

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Peripheral blood mononuclear cells (PBMCs) are circulating in the systemic blood flow and interacting with each organ. Infiltrating lymphocytes are often found in cancerous tissues including hepatocellular carcinoma (HCC). We have explored gene expression profile of infiltrating lymphocytes in HCC or non-cancerous liver tissues. Furthermore, we have examined whether gene expression profile of PBMCs of type C cirrhosis (LC) patients with HCC was different from those without HCC and assessed how gene expression of PBMCs in HCC patients correlated with that of HCC-infiltrating lymphocytes. [Methods] Enrolled were 32 patients of LC and 30 patients of LC with HCC (Age:64.9+/-10.3, 70.3+/-9.0 years old, male/female=13/19, 12/18, respectively). Another series of 12 cirrhotic patients underwent surgery for HCC, and infiltrating lymphocytes in HCC as well as non-cancerous liver tissues were isolated by laser capture microdissection. RNAs were extracted from obtained cells, amplified, labeled with Cy5 dye, mixed with Cy3-labeled reference RNA and hybridized to DNA microarrays (AceGeneHuman30K, HitachiSoft). Intensity was obtained, normalized, and analyzed using BRB-ArrayTools (NCBI) and MetaCore (GeneGo). Retrieved gene expression database for major leukocytes from Gene Expression Omnibus (NCBI) were also used for data analysis. [Results] Up-regulated genes in HCC-infiltrating lymphocytes were mostly involved in retrieved gene expression database of macrophages and helper T cells, and this constituent was confirmed by immunohistochemistry. Gene expression profiles of PBMCs were significantly discernable in context of HCC or LC patients by unsupervised clustering and supervised learning method. Comparison of gene expression for infiltrating lymphocytes in HCC and non-cancerous liver tissues identified 320 significant genes (p<0.01) and they discerned gene expression of PBMCs of patients relying on existence of HCC with 82% accuracy. Many biological processes were shared with significant genes of HCC-infiltrating lymphocytes and PBMCs in HCC patients, such as ubiquitin-proteasome, response to hypoxia and oxidative stress for up-regulated genes etc. The commonly up or down-regulated genes in HCC-infiltrating lymphocytes as well as PBMCs of HCC patients constructed gene network with 3 pivotal genes, nucleophosmine, SMAD3, and PCNA pertaining to proteasome, cell cycle, anti-oxidative genes. Interestingly, it involved FOXP3 as up-regulated and JAK3 as down-regulated genes, implying disturbed immunity. [Conclusion] Infiltrating lymphocytes in local HCC tissues might have influence on PBMCs and the usage of gene expression profiles of PBMCs could discern HCC in LC patients.

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1261 HUMAN GANKYRIN OVEREXPRESSION DOWNREGULATES P53 EXPRESSION AND PROMOTES CELL PROLIFERATION IN ZEBRAFISH

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Background and Aims: Gankyrin is an oncoprotein containing seven ankyrin repeats that is overexpressed in hepatocellular carcinoma. Gankyrin binds to Mdm2, which results in accelerated ubiquitylation via degradation of p53, and it also plays an important role in cell proliferation. However, little is known about the relationships between p53 levels, cell proliferation, and gankyrin over-expression. Therefore, we investigated the influence of gankyrin on p53 expression and cell proliferation in vitro and in vivo. Methods: We constructed human gankyrin (hgankyrin) expression vectors, pCS2-hgankyrin and pCS2-hgankyrin-EGFP. In order to investigate the influence of gankyrin protein on p53 and Mdm2 in a zebrafish model, we injected hgankyrin-containing expression vector or mock vector as control into zebrafish embryos. RTPCR, northern blot and in-situ hybridization methods were used to measure p53 and Mdm2 expression in hgankyrin-injected embryos and in controls. To observe the effect of gankyrin cell proliferation in vitro, we transfected hGankyrin-containing vector or mock vector into HEK 293 cell line, and trypan blue staining and MTT assay were performed. Following the injection of gankyrin into embryos, we observed cell proliferation in vivo using BrdU immunostaining. Results: We examined levels of p53 that resulted from gankyrin over-expression compared to controls in zebrafish. In vivo results indicated that RNA levels of p53 decreased but those of Mdm2 were not decreased. These results suggest that gankyrin downregulates p53 expression and not Mdm2 expression. In the study of cell proliferation, BrdU-positive cells were predominantly increased in the head and tail regions in hgankyrin-injected zebrafish. Additional in vitro studies using trypan blue staining and MTT assay showed that gankyrin-expressing HEK 293 cells proliferated at a faster rate, indicating that gankyrin promotes cell proliferation. Conclusion: Our results demonstrate that human gankyrin downregulates p53 expression and promotes cell proliferation in
zefrafish. Gankyrin may play an important role in hepatocarcinogenesis via its effects on p53 and cell proliferation.

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1262 IN VITRO VALIDATION THAT PHARMACOLOGIC INHIBITION OF FRIZZLED RECEPTORS CAN EXERT ANTI-ONCOGENIC PROPERTIES IN HUMAN CANCEROUS HEPATOCYTES

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Background and Aims: Hepatocellular carcinoma (HCC) is one of the most lethal cancers since therapeutic options are limited. Dysregulation of growth factors and their receptors is central to hepatocarcinogenesis. We previously demonstrated that the Frizzled-7 receptor (FZD7)-mediating the Wnt signaling activates the Wnt/β-catenin pathway and promotes malignancy in hepatitis B virus-related HCC. More recently, we showed that, in addition to FZD7, two other receptors (FZD3 and FZD6) could be activated by WNT ligands in most of human HCCs. Herein we aimed at establishing the proof of principle that pharmacological inhibition of the FZD3/6/7-mediated signalling could exert antitumour activity against HCC cells. Material and Methods: We disposed of human HCC cell lines overexpressing FZD3, 6 and/or 7 (Huh7, SK-Hep1, Focus, PLC/PRE/S, HepG2) and primary normal hepatocytes that do not (Cambrex), as measured by quantitative RT-PCR. We developed a rhodamine-tagged therapeutic peptide (TP) composed of 19 amino-acids containing the Dishevelled-binding domain of FZD3/6/7 linked to a protein transduction domain sequence allowing its penetration within cells, and a control peptide (CP). Antitumor cytotoxicity was assessed by cell counting and MTT test, and apoptosis by Annexin-V staining. Activity on three FZD-dependent pathways was assessed by TOP-Flash reporter gene assay (β-catenin), and western blot with anti-phospho-PKC (PKC) and anti-JNK (JNK). Results: Rhodamine-tagged peptides clearly entered within live hepatocytes (inversed fluorescence microscopy). By contrary to CP, TP exerted apoptosis-mediated cytotoxicity in all HCC cell lines overexpressing FZD3/6/7 while primary hepatocytes survived. When testing specifically Huh7 cells, CP-mediated apoptosis was associated with a substantial decrease in β-catenin and PKC activities, but not in JNK. Ectopic expression of the mutant dominant positive of β-catenin T41A (transient transfection) partially inhibited CP-induced apoptosis, and HepG2 cells (β-catenin mutants) were less sensitive to TP. Conclusions and perspectives: We have brought the proof of principle that in vitro pharmacological inhibition of some of the FZD receptors could engender antitumor effect in cancerous hepatocytes. This effect is mediated via at least inhibition of β-catenin, and maybe through PKC pathway repression too. These data underlined that FZD could be a very attractive target for therapeutic intervention in HCC.

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The following people have nothing to disclose: Sarah Beseme, Lydie Lefrancois, Pierre Jalinot, Christian Trepo, Ludmila Vitvitski, Philippe Merle

1263 HYPOXIA INDUCED GENE EXPRESSION SIGNATURE IN NORMAL HEPATOCYTES AND HUMAN HEPATOCELLULAR CARCINOMA

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Hepatocellular carcinoma (HCC) is the most common liver malignancy and among the five leading causes of cancer death in the world. HCC treatment options remain limited, and surgical resection is considered the only curative treatment. However, patients who undergo surgery, suffer frequent and often early recurrences. Also standard clinical pathological classification of HCC has limited usefulness for predicting the outcome of treatment. Therefore, molecular characterization of HCC into homogenous subtypes may provide an important step in improving the treatment options and success. HCC is considered a hypervascular tumor, and expression of the hypoxia inducible factor (HIF) and its target genes has been reported to be associated with a poor prognosis phenotype. Using a comparative genomics approach, we have characterized the hypoxic gene expression profile in freshly isolated mouse hepatocytes, with the aim of using this profile to explore the importance of the hypoxic response in HCC. Culture of mouse hepatocytes under hypoxic conditions over 24 hours revealed more than 1800 significant (p<0.001) regulated genes. In the first 12 hours the most important response to the hypoxic conditions is the upregulation of genes involved in angiogenesis, blood vessel formation and blood coagulation, while the transition to an anaerobic metabolism seems to be the most important response after 24 hours. Genes that showed at least 2 fold expression difference between hypoxic and normoxic conditions were selected to define the hypoxia gene expression signature. 504 orthologous genes derived from the hypoxic signature were used to perform hierarchical cluster analysis of 139 human HCC. As a result, two subsets of genes were identified. One subset of 104 genes implicated in cell cycle and apoptosis regulation (e.g. Gadd45a, Cdk4, Map4k4, Dusp1, Csk2), blood coagulation (e.g. Plg, F2, F9, F13b, Hc, Agt, Serpins1, Serpinf1) and immune response (e.g. C1r, C8a, C8b, C8g, C9, Rarres2) among other functions, was able to predict HCC with a good prognosis. On the other hand, a subset of 62 genes, some of them involved in cell cycle progression (e.g. Cdk6, Ches1, Ccn1, Nfgb), apoptosis regulation (e.g. Bcl6b, Pik3r1) and angiogenesis (e.g. Vegf, Id3), was able to predict HCC with a poor prognosis. Interestingly, among these 62 genes, we identified some that have already been found to predict poor prognosis in other human cancers (Chin et al. PLoS Medicine. 2006 Mar; 3[3]). Further studies are aimed at characterizing the mechanisms by which hypoxic conditions regulate these 2 sets of genes.

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The following people have nothing to disclose: Salvador Naranjo-Suarez, Valentina M. Factor, Cedric Coulourn, Snorri S. Thororgeirsson

1264 CHROMATIN-BASED ANALYSIS OF THE IFNα TRANSCRIPTOME REVEALS FUNCTIONALLY DISTINCT SUBSETS OF STAT2 DIRECT TARGET GENES

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Background/Purpose: Virus and host factors associated with responsiveness to IFN-based anti-HCV therapies have been identified but the underlying molecular mechanisms are still
unknown. Recently, the up-regulation of a specific set of IFN-responsive genes has been shown to predict non response to exogenous therapy. Aiming to understand the molecular mechanisms that differentiate responder and non-responder CHC patients we have developed innovative tools for systematic investigation of target gene regulation in response to IFNα and the classification of IFNα/STAT2 direct target genes into functionally homogeneous subsets according to transcription rules. Methods: We have used a Chip-on-chips approach, that couples chromatin immunoprecipitation (Chip) with custom-made oligonucleotides microarrays, to analyze IFNα/Stat2 direct target genes regulation at the chromatin level. Chip analysis were performed with anti-AcH3, anti-SS-PolII, anti-STAT2 and anti-P-STAT2 abs. Results: By in silico analysis and literature survey, 113 IFNα/β, but not IFNγ, direct target genes (i.e. modulated by IFNα/β and containing >80% conserved ISRE sequences in their promoter or first intron) have been selected. 34 genes were already confirmed by Chip and 79 genes were out of >300 genes modulated in previously published microarrays analysis. To build-up the STAT2 dedicated microchip slide 3 different 50mer oligos for each of the 113 target gene were designed (upstream promoter, proximal promoter, downstream of the TSS), spotted in triplicate and the resulting array printed 4 times on the same slide for a total of 4068 spots. Gene ontology analysis of the 113 selected genes revealed that the spectrum of direct IFNα/β target genes is far more varied than expected. Chip and Chip-on-chip analysis have revealed different classes of IFNα/β target genes in terms of STAT2 and PolII promoter occupancy before IFNα/β treatment and phospho-STAT2 recruitment (early vs late) after IFN stimulation. In particular, STAT2 is already bound to more than 50% out of the 113 direct target genes analyzed before IFN treatment, both in the group of genes upregulated and/or downregulated. Moreover, 37 new direct IFNα/β target genes have been identified, including Dnp73 (a p53/p73 antagonist that blocks apoptosis, induces cell proliferation and is upregulated in human HCCs), HIF1 (involved in hypoxia response) and the transcriptional repressor HDAC10. Conclusion: The Chip-on-chip reagents and approach we have developed represent a powerful tool to characterize the IFNα/Stat2 transcriptome in the liver in vivo in the perspective to develop to predict and modulate IFNa resistance in hepatitis C patients.

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The following people have nothing to disclose: Barbara Testoni, Christine Voellenke, Giovanni Blandino, Massimo Leverro

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COMPREHENSIVE GENE EXPRESSION ANALYSIS IN HEPATOCELLULAR CARCINOMA (HCC) BY NEWLY DEVELOPED 5'-END SAGE METHOD AND IDENTIFICATION OF NOVEL UP-REGULATED INTRONIC TRANSCRIPT

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Introduction: Gene profiling is a powerful technique for understanding the genomic and molecular alterations related to carcinogenesis. Gene expression analysis of hepatocellular carcinoma (HCC) has been performed extensively; however, most gene expression data has been derived from the 3’-end region of mRNA. Recent advances in molecular biology have revealed diverse transcriptional start sites (TSS) and the biological significance of intron initiated RNA. We recently developed a 5’-end SAGE method that combines the oligo-cap and original SAGE methods and quantitatively provides comprehensive tag sequences transcribed from the TSS (Nature biotechnology). In this study, we applied this 5’-end SAGE method to gene expression analysis in hepatocellular carcinoma (HCC) for the first time. Materials and Methods: Samples were obtained from a patient with HCC who had undergone surgical hepatic resection. Serological tests of HBs antigen and HCV antibody were negative. Tumor (T) and non-tumor (NT) tissue were separately obtained from either tumorous or non-tumor parts of the resected tissue. We also prepared 5 normal liver (NL) tissue samples from 5 patients who had undergone surgical hepatic resection because of metastatic liver cancer. Another 4 normal liver tissue samples, 42 HCC tissue samples and 42 NT tissue samples were used for real-time detection (RTD)-PCR. Results: We analyzed about 225,000 tags in 3 libraries and mapped them to the human genome. Gene profiles were made based on positional information of ref_seq genes. A total of 254 genes were up-regulated by more than 5-fold and 172 genes were down-regulated by more than 5-fold in the T library when compared with to NL library. The gene expression profiles agreed with previous reports. However, we also identified 5’-end SAGE tags corresponding to intronic region in 2179 genes. These genes were related to signal transduction, transport, energy pathways, and protein metabolism and so on. We successfully cloned full transcripts that initiated from the intronic region. Interestingly, we found that one of these intronic transcripts was significantly up-regulated in 43 T tissues when compared with NT tissues, as determined by RTD-PCR. Furthermore, expression of the overlapping defined mRNAs was significantly repressed in the 43 T tissues, thus suggesting repressive regulation of intronic RNA for overlapping defined mRNAs. Conclusion: We obtained comprehensive gene expression profiles of the 5’-end regions of mRNA in HCC. Identification and expression analysis of novel transcripts is useful for further elucidation of HCC pathogenesis, and detection of novel diagnostic and therapeutic targets.

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The following people have nothing to disclose: Yuji Hodo, Shin-ichi Hashimoto, Shungo Deshimaru, Masao Honda, Taro Yamashita, Teruyuki Ueda, Kouji Matsushima, Shuichi Kaneko

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HYDRODYNAMICS-BASED INJECTION OF A SHORT HAIRPIN RNA-PRODUCING VECTOR TARGETING C-JUN IN RAT LIVERS

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Background: RNA interference is a recently discovered mechanism for silencing gene expression. Hydrodynamics-based injection of si RNA into mice has been a useful model both for investigating functions of genes and for treating pathological conditions. However, reports of gene silencing in rats have been very scarce. In this study, we inhibited expression of c-Jun in rat livers by a hydrodynamics-based method and examined the function in stimulated conditions. Methods: A vector producing short hairpin RNA specific for rat c-Jun transcript under CMV promoter was constructed. Male Wistar rats weighing 100-120 g were injected with several different volume of Ringer solution containing either control- or c-Jun silencing vector via tail veins within 30 seconds. Large vessels around the liver were inspected by ultrasound images. One hour after...
1267 CLINICOPATHOLOGICAL SIGNIFICANCE OF EZH2 AND BMI1 EXPRESSION IN PATIENTS WITH HEPATOCELLULAR CARCINOMA

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Introduction/Aims: EZH2, a member of Polycomb repressive complex 2 and BMI1, a member of Polycomb repressive complex 1, play a crucial role in the regulation of embryonic development and have been associated with the regulation of cell cycle. Recently, several studies have shown that EZH2 is highly expressed in aggressive tumors, including breast and prostate cancer, and BMI1 is highly expressed in lymphomas. BMI1 represses the expression of P16INK4a and P19ARF tumor suppressor genes leading to carcinogenesis. We thus analysed EZH2 and BMI1 expression immunohistochemically, and correlated its expression status with various clinicopathological parameters in patients with hepatocellular carcinoma (HCC).

Materials and Methods: The expression of EZH2 and BMI1 was examined in surgically resected 85 HCC nodules and corresponding non-tumor tissues from 75 patients. The tissue sections were immunohistochemically stained with a primary antibody against EZH2 (Zymed) and BMI1 (Upstate), respectively. Clinicopathological factors examined were as follows: age, sex, liver function tests (levels of total bilirubin, albumin, AFP, PIVKA2 and platelet count, and so on), and tumor factors (tumor size, tumor number, invasion to portal vein, hepatic vein and bile duct and so on). Cumulative survival and recurrence rates were analyzed by Kaplan-Meier method. Results: EZH2 and BMI1 were exclusively detected as nuclear staining of HCC cells and non-tumor hepatocytes. The expression of EZH2 was found in 46/75 (61.3%) and 4/75 (5.3%) of tumor and non-tumor tissues, respectively. Both of EZH2 and BMI1 were stained in 43/75 (57%) tumor tissues and neither of them were stained in 21/75 (28%). The correlation between expression of EZH2 and various clinicopathological factors showed higher albumin level associated with the expression of EZH2 (P=0.01). The correlation between expression of BMI1 and various clinicopathological factors showed greater tumor numbers associated with the expression of BMI1 (P=0.03). There was no difference of cumulative survival rates between patients positive and negative for EZH2 staining, and between patients positive and negative for BMI1 staining. There were higher cumulative recurrent rates in patients positive for EZH2 or BMI1 staining than those negative for EZH2 or BMI1 staining (P=0.038 and P=0.048 respectively). Conclusion: The expression of EZH2 and BMI1 were very frequent and specific in tumor tissues of HCC, thus can be a close association with the development of HCC and might be associated with higher cumulative recurrent rate.

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1268 RNA INTERFERENCE MEDIATED SILENCING OF EGFR IN REGENERATING RAT LIVER FOLLOWING A PARTIAL HEPATECTOMY

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Liver regeneration after partial hepatectomy (PHx) is an extremely complex process requiring interaction and cooperation of many growth factors and cytokines and crosstalk between multiple pathways. Along with HGF-met, the EGFR signaling pathway is also activated within 60 minutes after a PHx. EGFR phosphorylation also increases over basal levels by 60 minutes after a PHx; exactly paralleling activation of c-met. Cross talk between these two pathways has been documented and integration of these two pathways may be required for regeneration to proceed efficiently. We therefore decided to use shRNA to knock out EGFR expression in regenerating normal rat liver by following a protocol we recently published (Hepatology, 45:1471-1477, 2007) The EGFR family contains four members; Erbb-1, Erbb-2, Erbb-3 and Erbb-4. Erbb-3 is not expressed in liver while Erbb-2 is expressed only in embryonic liver and not in adult liver. Only Erbb-3 is expressed in the adult liver but has no intrinsic kinase activity and relies on Erbb-1 for activity. The four Erbb receptors recognize 11 different but structurally related growth factors that mediate diverse process like development, cell proliferation, and cell survival. Two EGFR rat specific short hairpin sequences were designed and cloned under the control of human H1 promoter. JM1 cells were transfected with the plasmids singly or in combination and screened for their ability to suppress EGFR expression. Stable transfecants expressing GFP were isolated in presence of zeocin. Levels of EGFR mRNA were estimated by both semi quantitative and real time PCR. Suppression of EGFR mRNA was evident. The plasmids were then injected in rats by following protocols reported earlier. Significant suppression of EGFR mRNA by day one post PHx was observed following two shRNA injection. How ever in contrast to the effects observed following c-met silencing, no suppression of mitosis was evident, probably indicating that although the c-met and EGFR pathways are similar, the contributions made by c-met are unique which are not compensated by EGFR or other cytokines related to liver regeneration. We are currently investigating the effects of EGFR
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RNAi THERAPEUTICS FOR THE TREATMENT OF LIVER DISEASES
Akin Akinc1, Daniel Anderson2, Birgit Bramlage2, David Bumcro1, J. Robert Dorkin1, Kevin Fitzgerald1, Maria Frank-Kamenetsky1, Michael Goldberg3, Jens Harborth1, Jay D. Horton4, K. Narayanannair Jayaprakash1, Muthusamy Jayaraman1, Matthias John1, Kallanithattil Rajeev1, Victor Kotelianski1, Robert Langer1, Muthiah Manoharan1, Lubomir Nechev1, June Qin1, Timothy Racie1, Ingo Roh1, Dinah Sah1, Jürgen Soutschek2, Hans-Peter Vornlocher2, Tracy Zimmermann1, Andreas Zumbuehl1, George Michalopoulos1

Successful delivery of small interfering RNAs (siRNAs) in vivo, using clinically relevant modes of administration, is critical for the advancement of RNA interference (RNAi) therapeutics. Here we describe a new class of lipid-like delivery molecules, which we term lipidoids, as delivery agents for RNAi therapeutics. Chemical methods were developed to allow the rapid synthesis of a large library of over 1,200 structurally diverse lipidoids. From this library, lipidoids were identified that facilitate high levels of specific silencing of endogenous gene transcripts. Using these materials, we have developed a novel liposomal formulation optimized for delivery of systemically-administered siRNA to the liver. Numerous endogenous liver genes have been successfully silenced employing this formulation in both rodent and nonhuman primate models. Specifically, we describe the potent down-modulation of two non-druggable disease targets, apolipoprotein B (apoB) and propionate convertase subtilisin kexin 9 (PCSK9). In nonhuman primates, a single injection of liposomal siRNA resulted in up to 85% silencing of apoB mRNA expression in the liver 48 h after administration. PCSK9 down-modulation has been demonstrated in mouse, humanized mouse, rat, and nonhuman primate models. Silencing of PCSK9 resulted in significant lowering of cholesterol (20-40%) in all animal models tested with 60% lowering of total cholesterol in the rat model. These findings demonstrate clinically relevant RNAi-mediated gene silencing, thus supporting the potential of RNAi therapeutics as a new class of drug for liver diseases.

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INHIBITION OF TNF ALPHA STIMULATION BY PRE-TREATING HUMAN HEPATOCYTES WITH STAUROSPORINE AND LY294002 ON INTRINSIC APOPTOSIS PATHWAYS
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Introduction. In acute and chronic liver disease, hepatocytes are exposed to higher quantities of cytokines [TNF-alpha (tumor necrosis factor alpha), IL-1-beta (interleukin 1 beta), INF-gamma (interferon gamma)], oxidative stress and bile acids. Though hepatocytes have an enormous capacity to defend themselves against these agents, excessive exposure results in cell death. Apoptosis deregulation plays a pivotal part in the development of numerous liver diseases. The binding of death receptors expressed on the hepatocyte’s surface to their ligands can activate closely-linked extrinsic and intrinsic pathways. To characterize the variability of the apoptotic process in healthy human hepatocyte cultures, the aims of this study are: 1) to determine whether the main proinflammatory TNF-alpha cytokines abounding in inflammatory processes related to liver injury can regulate and modulate Fas/FasL mRNA expression; 2) to assess the effects of hepatocyte pre-treatment with staurosporine (STS), a potent protein tyrosine/serine kinase, and LY294002, a synthetic inhibitor of PI3K (phosphoinositide 3-kinase), on the intrinsic (Bcl-2, Bax, Bad, NF-kB) and extrinsic (IL-1beta, TGF-beta, Fas/FasL) apoptotic pathways. Materials. Human hepatocytes were separated from livers rejected for transplantation, 3 X 106 and viable hepatocytes were suspended in six-well plates coated with acellular liver matrix and stimulated in medium with no fetal calf serum using different doses of TNF-alpha, STS and LY294002 for different times (4, 8, 12, 24 and 48 h). mRNA levels were quantified by real-time PCR. Results. Up to 48 h, TNF-alpha induced a significant increase in IL-1beta, Bcl-2 and NF-kB mRNA transcripts in human hepatocytes (p<0.0001, p=0.0029, p=0.0035 by Kruskal-Wallis, respectively), whereas Bax and Bad mRNA levels were inhibited without however reach a statistical significance. No differences in Fas mRNAs were observed while FasL overexpression was observed only after 12h of stimulation with TNF-alpha (p=0.05). Pretreating hepatocytes with LY294002 and staurosporine blocked the effects of TNF-alpha on Bcl-2 and NF-kB mRNA transcripts, while inducing the overexpression of TGF-beta. Conclusions. TNF-alpha is a proinflammatory cytokine constantly found in the micro-environment in acute and chronic disease that activates both extrinsic (IL-1beta, Fas/FasL) and TGF-beta (mRNAs overexpression) and the intrinsic (NF-kB, Bcl-2) apoptosis processes. Pretreating hepatocytes with PI3K inhibitors activates the intrinsic pro-apoptotic pathways, increasing Bax and Bad mRNAs. Our results shed light on how extracellular mediators may be involved in controlling the survival and death of primary human hepatocytes.

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1271 MODULATION OF STEMNESS IN DYSFUNCTIONAL PROGENITOR/STEM CELLS ALTERS CANCER FORMATION IN THE LIVER

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It is thought that at least 40% of hepatocellular cancer (HCC) are clonal and arise from dysfunctional progenitor/stem cells. Functional mechanisms involved in generation and renewal of these “cancer stem cells”, such as those of liver, remain poorly defined and are vital for the development of new targeted therapeutics for this poor prognosis cancer. We therefore chose to investigate pathways involved both in renewal and maintenance of stem cells and tumor suppression such as the TGF-β signaling pathway. Signaling molecules of the TGF-β pathway such as Smad2, Smad3 and an adaptor protein, ELF, are known to play pivotal roles in liver development and HCC suppression. Lethal liver hypoplasia was observed in elf/- and smad2+/−/smad3+/−/elf+/− mice develop frequent and spontaneous HCC by 12-15 months of age. Through studies in human living donor transplants our group identified a small population of stem cells in fully regenerated human liver that express all TGF-β markers which are lost in human HCC. Aims: 1. To identify functional pathways which are involved in renewal of TGF-β regulated liver cancer stem cells 2. To modulate these pathways and decrease HCC formation. Methods and Results: 1. Broad microarray proteomic analysis and immunohistochemical staining (IHC) reveal marked activation of IL-6/Stat3 signaling as well as cell cycle regulators such as CDK4 in HCC tissues from elf−/− mice. 2. Genetic abrogation of IL-6/Stat3 signaling in elf−/−/itih4−/− mice dramatically decreases the incidence as well as the size of HCC when compared to elf+/− mice. 3. Abrogation of CDK4 in elf−/−/CDK4−/− mice also reduces HCC formation. 4. Immunoprecipitation demonstrates the interaction between CDK4 and ELF which leads to phosphorylation of ELF. 5. In vitro biochemical studies shows that restoration of normal ELF expression significantly increases the percentage of cells in G1 arrest, decreases the expression of CDK4, and restores TGF-β target gene activation. Conclusions: Genetic studies demonstrate a link between IL-6/Stat3 pathway, a major stem cell signaling pathway, and TGF-β signaling pathway in the modulation of HCC which derive from altered TGF-β phenotypes. Also interaction of CDK4, a major regulator of cell cycle progression, and ELF suggests that liver cancer stem cells have dysregulated cell cycles with a short G1/S phase when ELF is disrupted. These two signaling pathways are shown to be involved in HCC formation from altered TGF-β phenotypes thereby presenting important therapeutic targets in treating HCC.

Disclosures:


1272 BETA-CATENIN IS INDISPENSABLE FOR LIVER DEVELOPMENT AND SURVIVAL

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β-Catenin, the central component of the canonical Wnt pathway, plays important roles in the processes of liver regeneration, growth and cancer. Previously, we have identified temporal expression of β-catenin during liver development. Here we characterize the hepatic phenotype only, resulting from the successful deletion of β-catenin in the developing hepateoblasts during liver development utilizing Foxa3-Cre transgenic and floxed-β-catenin (Ex2-6) transgenic mice. β-Catenin loss in developing livers resulted in significantly underdeveloped livers after embryonic day 12 (E12) with lethality occurring at around E17 stages. Histology revealed an overall deficient hepatocyte compartment along with remnant hepatocytes possessing an immature phenotype as indicated by high nuclear to cytoplasmic ratio, poor polarity and absent glycogen. The decrease in the hepatocyte numbers were due to a) increased cell death due to oxidative stress and apoptosis, and b) diminished expansion secondary to impaired proliferation. A concomitant paucity of primitive bile ducts was also evident. While the stem cell assays demonstrated no intrinsic defect in hepatopoiesis, distorted hepatic architecture and deficient hepatocyte compartment resulted in a defect in the endothelial cell organization and homeostasis, leading to impaired hepatic blood flow, fetal pallor and lethality. Conclusion. β-Catenin regulates multiple, critical events during the process of hepatic morphogenesis including an important role in hepatoblast maturation, expansion and survival and its hepatic disruption is indispensable to survival.

Disclosures:
The following people have nothing to disclose: Xinping Tan, Sattarshan P. Monga

1273 HUMAN TGF-β-REGULATED LIVER STEM/PROGENITOR CELLS IN NORMAL POST TRANSPLANT LIVING DONOR RECIPIENTS ARE ALTERED IN HEPATOCELLULAR CANCERS

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β-Catenin, the central component of the canonical Wnt pathway, plays important roles in the processes of liver regeneration, growth and cancer. Previously, we have identified temporal expression of β-catenin during liver development. Here we characterize the hepatic phenotype only, resulting from the successful deletion of β-catenin in the developing hepateoblasts during liver development utilizing Foxa3-Cre transgenic and floxed-β-catenin (Ex2-6) transgenic mice. β-Catenin loss in developing livers resulted in significantly underdeveloped livers after embryonic day 12 (E12) with lethality occurring at around E17 stages. Histology revealed an overall deficient hepatocyte compartment along with remnant hepatocytes possessing an immature phenotype as indicated by high nuclear to cytoplasmic ratio, poor polarity and absent glycogen. The decrease in the hepatocyte numbers were due to a) increased cell death due to oxidative stress and apoptosis, and b) diminished expansion secondary to impaired proliferation. A concomitant paucity of primitive bile ducts was also evident. While the stem cell assays demonstrated no intrinsic defect in hepatopoiesis, distorted hepatic architecture and deficient hepatocyte compartment resulted in a defect in the endothelial cell organization and homeostasis, leading to impaired hepatic blood flow, fetal pallor and lethality. Conclusion. β-Catenin regulates multiple, critical events during the process of hepatic morphogenesis including an important role in hepatoblast maturation, expansion and survival and its hepatic disruption is indispensable to survival.

Disclosures:

Hepatocellular cancer (HCC) remains a poor prognosis cancer and a significant number of cases are thought to arise from dysfunctional stem/progenitor cells. In adult humans, these liver stem/progenitor cells are immature epithelial cells found residing in the canals of Hering. Long term label retention of these multi-potent cells with high proliferative potential has been one approach towards identifying them in the gut epithelium. However, to date, identification of such liver stem/progenitor cell populations has remained elusive. Recent studies have shown that TGF-β signaling members TGF-β receptor type II (TBRII), Smad2, Smad4 and Smad-adaptor ELF, α-spectrin, play crucial roles in liver development and function as tumor suppress-
sors in hepatocarcinogenesis. Elf-/- and smad2+-/-smad3+-/-mutant mice are embryonic lethal from hepatic hypoplasia. In addition, as many as 40% of elf-/- mice (17/40) spontaneously develop HCCs. Aims: 1. To identify TGF-β-regulated liver stem/progenitor cell population. 2. To investigate whether the TGF-β phenotype is altered in human HCCs. Methods and Results: 1. Immunohistochemical labeling (IHC) was performed in post living donor liver transplanted tissues taken from 5 different recipients at 3 months after surgery. We identified 2-3 cells out of 30,000-50,000 cells which express embryonic stem cell markers Oct4 and Nanog localized around portal tract areas. These cells are also stained positively for ELF and TBRII. 2. Confocal triple labeling confirms co-localization of Oct4 and ELF in these cells along with a proliferative marker p-Histone and another important embryonic stem cell marker Stat3. 3. The Oct4+- cells express both cholangiocyte cell lineage marker CK19 and hepatocytic cell lineage marker albumin. 4. IHC labeling of 10 human HCC specimens reveals a distinct cluster of Oct4+- cells. Interestingly, these regions no longer expressed TBRII and ELF in 9 out of 10 samples. Conclusions: Genetic studies through elf knockout mice reveal a functional role of the TGF-β signaling pathway in HCC suppression and liver development. Prominent expression of TGF-β signaling proteins observed in the Oct4+/-Nanog+/-Stat3+ cells indicates that these cells are under TGF-β regulation and could represent a TGF-β-regulated stem/progenitor cell population. The identification of Oct4+ cells in human HCC specimens coupled with absence of TGF-β proteins in those areas suggests that the liver stem/progenitor cells with altered TGF-β phenotype could develop into cancer stem/progenitor cells. Understanding regulation of such “cancer stem cells” could provide novel therapeutic approaches in treating this cancer.

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1274 EXPRESSION OF NEIGHBOR OF PUNC E11 (NOPE) DURING MURINE LIVER DEVELOPMENT

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We have previously shown that murine fetal liver epithelial cells at embryonic day (ED) 12.5 are a homogeneous population of hepatoblasts that can be purified using the specific surface markers E-cadherin, Liv2 or Dlk. In a DNA microarray analysis comparing purified hepatoblasts isolated from ED 13.5 mouse fetal liver with adult liver (AL), we identified the membrane protein Neighbor of Punc E11 (Nope) to be significantly higher expressed in hepatoblasts than in AL. In the current project, we focused on the differential expression of Nope as a potential marker for bipotential hepatoblasts during liver development. Methods: We isolated livers at different stages of development (fetal, postnatal and adult livers) starting at ED 11.5. RNA and protein fractions were extracted and quantitative RT-PCR and Western blot studies were performed to quantify the expression level of Nope with Gapdh and β-Actin as internal controls. Immunohistochemistry and confocal microscopy were performed on cryosections. Stainings for Nope were combined with stainings for epithelial-specific markers (pancytokeratin, E-cadherin), markers of early hepatoblasts (Afp, Liv2) and hepatocytic (albumin) or biliary marker proteins (CK 19) at different stages of liver development. Results: Quantitative RT-PCR showed that Nope is highly expressed in fetal liver starting at ED 11.5, but is rapidly down regulated postnatally until 2 weeks after birth and no longer detectable in AL. Western Blot studies confirmed the expression of Nope in the membrane fraction of liver extracts until 2 days after birth. Immunohistochemistry demonstrated the specific coexpression of Nope with epithelial-specific markers as well as with markers of bipotential hepatoblasts in early fetal liver development. After commitment of hepatoblasts to cholangiocyte/hepatocytic differentiation, Nope was continuously expressed on both cell types until 2 days after birth as identified by co stainings with hepatocytic and bilary markers. Nope was not detected above background level in AL. Conclusion: Nope is a membrane protein specifically expressed by epithelial cells within the liver at all stages of fetal liver development and is rapidly down regulated after birth. While Nope is initially expressed on the surface of bipotential hepatoblasts, it continues to be expressed on fetal hepatocytes and cholangiocytes until 2 days after birth and is no longer detectable in AL. Further studies will focus on the potential use of Nope as a surface marker for stem/progenitor cells in the adult liver.

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The following people have nothing to disclose: Dirk Nierhoff, Sigrid Schulte, Jens Marquardt, Gisela Holz, Tobias Goeser

1275 SELECTIVE PROLIFERATION OF HUMAN HEPATOCYTE PROGENITOR CELLS IN COMBINATION WITH HYALURONIC ACID AND SERUM-FREE MEDIUM

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Background&Aim: Small hepatocytes (SHs) were first identified as a subpopulation of rat hepatocytes that had high replication potential in culture. Therefore, SHs may be ‘committed progenitor cells’ that can further differentiate into mature hepatocytes (MHs). We have recently reported that CD44, one of the hyaluronan receptors, is a specific marker for rat SHs and that rat SHs can selectively proliferate on a hyaluronic acid (HA)-coated dish in a serum-free medium. The aim of the present study is to isolate human SHs from normal adult livers and to culture them in serum-free medium for a long term. Methods: Normal liver tissues were obtained from resected specimens from patients who underwent hepatic resections at Sapporo Medical University under informed consent and with the approval of the Sapporo Medical University Ethics Committee. All tissues were obtained from the patients who had never had any hepatitis viral infections. Hepatic cells were isolated and then plated on HA-coated dishes. The cells were cultured in serum-free DMEM/F12 medium supplemented with nicotinamide, EGF, HGF, ITS, and Dexamethasone. Immunocytochemistry, ELISA, and RT-PCR were carried out. Results: When the isolated cells were plated on HA-coated dishes, some small-sized hepatocytes proliferated to form colonies. Many colonies continued growing for more than 3 weeks. The average number of cells in a colony was 38.6±18.0 at day 14, 79.0±54.0 at day 14, and 101.5±115.7 at day 21. About 0.04% of plated cells could form an SH colony. Immunocytochemistry showed that the cells were positive for albumin, transferrin, CK8 and CD44. Most cells showed CK19 positivity. The results of RT-PCR showed that colony-forming cells expressed not only albumin, transferrin, α1AT, fibrinogen, and glutamin synthetase but also liver-enriched transcription factors such as HNF3α, HNF4α,
C/EBPα, and C/EBPβ. In addition, cytochrome P450 isozymes such as 2A6, 2B6, 2C9, 2D6, 2E1, 3A4, and 3A5 were also expressed. Furthermore, the cells expressed Thy1.1, EpCAM, and AFP, which are expressed in hepatic stem cells, as well as CD44 and D6.1A, which are SH markers. Albumin was secreted into culture medium. The results indicate that human hepatocyte progenitor cells may be isolated and can grow on HA-coated dishes cultured in a serum-free medium. The human cells show characteristics similar to rat SHs. Conclusion: Human SHs may be a useful source for pharmacological examinations and cell transplantation.

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1276 REGULATION OF HEPATIC LINEAGE ADVANCEMENT IN HESC THROUGH A MESO-ENDODERMAL INTERMEDIATE RECAPITULATES FETAL STAGE-SPECIFIC PROCESSES

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To advance applications of stem cells, mechanisms driving cell differentiation into specific lineages need to be defined. As one way to understand lineage advancement mechanisms is to study stage-specific processes, differentiation of human embryonic stem cells (hESC) along hepatic lineages should benefit from analysis of lineage advancement in the context of organ development. In freshly isolated fetal human epithelial cells from the liver or pancreas at 20-24 week gestation, we demonstrated multilineage gene expression, including presence of epithelial, mesenchymal and stem cell genes. These cells underwent genetic reprogramming in culture suggesting reversion to more primitive meso-endodermal stages. To determine whether such a stage was recapitulated in hESC-derived epithelial lineages, we studied mesenchymal stem cells obtained from the WiCell H1 cell line, where spontaneously originating cells during fetal liver development in vivo and during hESC differentiation in vitro. The efficacy of hESC-derived cells in liver failure will eventually help advance clinical applications.

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1277 ANALYSIS OF DIFFERENTIATION OF HEPATIC PROGENITOR CELLS DERIVED FROM SEVERELY INJURED RAT LIVERS

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Background & Aim: Small hepatocytes (SHs) are a subpopulation of hepatocytes that have high growth potential in culture. SHs can clonally proliferate to form colonies and differentiate into mature hepatocytes (MHs) by interacting with hepatic nonparenchymal cells (NPCs). Thus, we consider that SHs may be hepatic progenitor cells that can further differentiate into MHs. Recently, we identified CD44 as a specific gene that expressed in proliferating SHs. On the other hand, oval cells is known as hepatic stem cells that appear in severely injured livers. The aim of the present study is to analyze the mechanism of differentiation and the fate of hepatic stem/progenitor cells in vivo and in vitro. Methods: Male F344 (DPPIV+) rats were treated with 75 mg/100g body weight D-galactosamine (GalN) and frozen section were made from the liver for immunohistochemistry. Thy1+ or CD44+cells were sorted by using MACS. The sorted cells were cultured, transplanted, or used for RTPCR. Female DPPIV- rats were treated twice with 30 mg/100g body weight retorsine following 2/3PH and used for cell transplantation. Results: Immunohistochemistry showed that Thy1+ oval cells were observed in the livers at day 2 after GalN-treatment (GalND2) and day 3 after GalN-treatment (GalND3). Some Thy1+-cells in the livers of GalND3 also showed CD44-positivity. CD44+ hepatocytes were also observed in the perportal area of liver lobules at day 4 after GalN-treatment (GalND4). The sorted CD44+-cells from the liver of GalND4 could proliferate and form large CD44+colonies. Although Thy1+-cells from GalND2 formed few colonies, those from GalND3 effectively formed CD44+colonies. RT-PCR revealed that Thy1+-cells from GalND3 expressed CD44, Dlk-1, C/EBPα. In addition, they were cultured between collagen gels in the medium without dexamethasone, many Thy1+-cells from GalND3 could differentiate into biliary duct cells, whereas most Thy1+-cells from GalND2 could not. At 30 days after cell transplantation the number and area of DPPIV+ cells in the livers were shown in Table 1. From these results, Thy1+/CD44- cells could not form SH colonies and Thy1+/CD44+ could form SH colonies. Therefore, Thy1+/CD44+ cells may differentiate into Thy1-/CD44- SHs, and then the SHs may differentiate to Thy1-/CD44- MHs. Conclusion: A part of oval cells may differentiate into hepatocytes via SHs or into BECs.

Table 1. The number and area of DPPIV+ cells from transplanted liver at 30 days after transplantation

<table>
<thead>
<tr>
<th>Days after GalN administration</th>
<th>Used Ab</th>
<th>Average Number of DPPIV+ foci</th>
<th>Average of DPPIV+ area (x 103 mm2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>Thy1</td>
<td>3.2</td>
<td>50.4±10.2</td>
</tr>
<tr>
<td>3</td>
<td>Thy1</td>
<td>2.6</td>
<td>118.9±54.2</td>
</tr>
<tr>
<td>3</td>
<td>CD44</td>
<td>77.2</td>
<td>209.5±313.6</td>
</tr>
</tbody>
</table>

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1278 INDUCTION OF PANCREATIC BETA-CELL PHENOTYPE IN HEPATIC PROGENITOR CELLS (HPC) ISOLATED FROM ADULT DIABETIC MICE

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Various studies have shown that liver cells constitute a promising cell source for induction of insulin production and secretion following different cell procedures. However, the potential of diabetic-derived adult stem cells to induce pancreatic beta cell phenotype has not been elucidated. Here we present our in-vitro study on induction of beta cell phenotype in hepatic progenitor cells (HPC) obtained from intact and diabetic mice. Our hypothesis was that hepatic progenitors, isolated from diabetic liver may be more committed to differentiate into beta cell phenotype, than cells derived from non-diabetic animals. HPC were obtained from livers of intact, control HPC (cHPC) and STZ-induced diabetic male mice (dHPC) 12 weeks old, undergoing hypoxic conditions. Cell expansion was performed by exposure of cells to conditioning culture medium able to induce a high number of HPC (1X10^7/mouse). Differentiation of HPC toward insulin producing cells was shown by RT-PCR and immunohistochemical analysis using a series of different markers including HNF-3β, PDX-1, NGN-3, insulin-1 and insulin-2. Insulin production in differentiated cells was evident by immunocytochemistry, as well as by ELISA determination of insulin content and secretion. Following hypoxic selection a higher expression level of insulin producing cells differentiation markers, such as HNF-3β and PDX-1 were found in dHPC when compared to cHPC. After expansion procedure HPC progenitors expressed known oval-cells markers such as alpha-fetoprotein and cytokeratine-19, as well as beta cell differentiation transcripts in both cHPC and dHPC. Insulin presence was detected in cell extracts and culture medium after 2wks expansion. Interestingly, dHPC showed about 4-fold higher insulin-content and insulin secretion when compared to cHPC. Our preliminary results suggest that hepatic progenitor cells derived from diabetic animals have a potential to express beta-cell like phenotype.

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1279 DIFFERENTIATION OF RAT MULTIPOTENT ADULT PROGENITOR CELLS TOWARDS THE HEPATIC LINEAGE BY RECAPITULATING LIVER EMBRYOLOGY

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Background/Aims. Bone marrow derived Multipotent Adult Progenitor cells (MAPCs) might represent a unique in vitro system to explore early lineage commitment and mammalian development. The aim of this study was to check whether the current knowledge on liver development could be applied to optimize in vitro the differentiation of rat MAPCs towards hepatic-like cells. Methods. The differentiation protocol was divided into 4 consecutive steps. First, Wnt3a and ActivinA guide the cells towards a mesendodermal fate. Second, specification of the ventral foregut endoderm to hepatic endoderm was mimicked by adding bFGF and BMP4, which are known to be secreted by the cardiac mesoderm and septum transversum mesenchyme in vivo. Next, aFGF, FGF4 and FGF8b were used as they enrich embryonic liver cultures for bipotent and unipotent hepatocyte progenitors. Finally, further maturation was induced by culturing the cells in the presence of HGF and Follistatin, the latter to inhibit cholangiocyte differentiation. Aside from testing these cytokines, we varied basic media components, such as insulin, serum, glucose and dexamethasone. As a control, cells were cultured in the same medium and at the same cell density, but in the absence of cytokines. Results. Using this cytokine combination, a mixture of immature and mature hepatocyte-like cells was gradually obtained. Mesendodermal markers such as Goxosecoid, CXC4, 1m42f2 were transiently expressed, suggesting the formation of definitive endoderm. Low percentage of serum, high concentration of dexamethasone and glucose favoured expression of mature liver transcripts. Cells displayed hepatic morphology on electronic microscopy. Besides hepatic-like cells, the culture also contained mesodermal-derived cell types, which might exert a positive effect on the hepatic maturation. Albumin, CK18, HNF1 and alpha-fetoprotein were seen on the protein level by immunohistochemistry. Differentiated cells secreted albumin, conjugated bilirubin and stored glycogen. In the absence of cytokines, liver markers were also expressed, but at a lower level and at a later time point during differentiation as compared to the multipotent protocol. Conclusion. Liver development is a modular process, requiring multiple cytokines and cell interactions, each playing a significant role at distinct time points during embryogenesis. We found that differentiation of rat MAPCs towards the hepatocyte lineage mimics liver embryology, making them a useful tool to study mammalian development in vitro. In the future, this strategy will also be applied to generate hepatic-like cells from other types of pluripotent stem cells.

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1280 DIFFERENTIAL ACETAMINOPHEN-INDUCED HEPATOXICITY BETWEEN TWO TYPES OF DIETARY STEATOSIS OF THE LIVER IN MICE

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Background: Nonalcoholic fatty liver disease (NAFLD) is a common disease recognized as a major health problem. Acetaminophen (APAP) is an often used analgesic and antipyretic that can cause intoxication through overdosing. However, whether livers with steatosis are more susceptible to APAP-induced liver injury has not been clarified. Aim: To generate different fatty liver models by feeding a fat rich and fructose rich diets and then evaluate APAP-induced hepatotoxicity. Methods: Seven week old C3H/HetJc/Jc male mice were divided into 3 groups by different diets: low protein diet (LPD, protein: 52g/kg), low protein+high-fat diet (LP-HFD, soybean oil: 312g/kg), and low protein+high fructose diet (LP-HF5D, fructose: 682g/kg). After feeding for 3 weeks, the mice were
treated with APAP (120 mg/kg) by intraperitoneal injection to induce liver injury. After 6 hours mice were sacrificed and blood and liver specimens collected. The plasma levels of ALT, AST, triglyceride (TG), total cholesterol (TC), glucose, insulin, total protein, and albumin were determined. The hepatic contents of TG and TC in each group of pretreated mice were measured. Real time RT-PCR was carried out to evaluate the pretreated hepatic expression of fatty acid synthase (FAS), phosphoenolpyruvate carboxykinase (PEPCK), glucose 6-phosphatase (G6Pase), CYP1A1, and CYP2E1 in pretreated mice. Results: The liver tissue specimens of the LP-HFD and LP-HFrD-fed mice revealed significant fatty infiltration. The hepatic contents of TG and TC in both these mouse groups were significantly higher than those in LPD-fed mice. Real time RT-PCR analysis demonstrated a higher expression of FAS in mice fed LP-HFrD than those fed LPD and LP-HFD (1.8-fold and 2.0-fold, respectively). A higher expression of G6Pase in mice fed LP-HFD than in those fed LPD and LP-HFD (1.7-fold and 3.0-fold, respectively) was also revealed. There was no significant difference in basal levels of CYP2E1 in the livers among the three groups. By contrast, CYP1A1 expression in mice fed LP-HFD was higher than LPD and LP-HFrD (2.5-fold and 1.8-fold, respectively) fed mice. The levels of ALT 6 hours after APAP gavage in the LP-HFD group was significantly higher than in LPD (8093 ± 1862 IU/L, n=5) than those in LPD (12±2 IU/L, n=4) (p<0.0001) and LP-HFrD (40±25 IU/L, n=5) (p<0.0001) groups. Conclusions: We succeeded in generating two types of mouse fatty liver model with different glucose and fatty acid metabolisms. Our results showed that hepatic steatosis elicited by low protein and high fat diets significantly increased APAP-induced hepatotoxicity. This effect was independent of CYP2E1 expression, which stimulated APAP bioactivation.

Disclosures:
The following people have nothing to disclose: Yuqing Wang, Makoto Oketani, Masahisa Haruiuchi, Yasushi Imamura, Akihito Maruiichi, Susumu Hasegawa, Hirofumi Uto, Aki Ida, Hirohito Tsubouchi

1281
REPEATED EXPOSURE TO ACETAMINOPHEN PROTECTS AGAINST A SUBSEQUENT LETHAL DOSE THROUGH SELECTIVE DEPLETION OF CYTOSOLIC GLUTATHIONE

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Mechanisms to account for unintentional death and hepatotoxicity from acetaminophen (APAP) exposure are not understood. Our laboratory has established an animal model of adaptive tolerance to APAP exposure in order to identify protective mechanisms that when disabled through genetic variation or environmental conditions, subject the individual to increased risk of hepatotoxicity. In this model, male BALB/C mice are divided into four groups of 5 animals each: tolerated, tolerated-plus-lethal dose (tolerated LD), naive and naive-lethal dose (naïve LD). Both the tolerated and tolerated LD groups were treated with increasing doses of APAP intraperitoneally as follows: 50 mg/kg for two days, 100 mg/kg for two days, 250 mg/kg for two days. On the seventh day, the tolerated group received a saline injection while the tolerated LD group received a 350 mg/kg dose of APAP. The naive and naïve LD groups received saline for six days. On the seventh day, the naive LD group received a lethal dose of APAP while the naïve group received saline. Six hours following the lethal dose, two animals from the naïve LD group had died. No animals died from the naïve, tolerated and tolerated LD groups and their livers were lighter in color and weight. Biochemical analysis revealed selective cytoplasmic glutathione depletion. Naïve animals showed a decrease in mitochondrial (29.4 +/- 5.4 to 11.9 +/- 9.2 μmole/gram total protein) but no change in cytoplasmic (34.4 +/- 10.5 to 15.2 +/- 13.6 μmole/gram total protein) total glutathione pools when challenged with a lethal dose of APAP. In contrast, tolerated animals showed increased mitochondrial (48.9 +/- 4.1 μmole/gram total protein) and cytoplasmic (56.2 +/- 14.3 μmole/gram total protein glutathione. When challenged with a lethal dose, tolerated animals showed a decrease in cytoplasmic (38.5 +/- 6.4 μmole/gram total protein) and increase mitochondrial (82.1 +/- 8.8 μmole/gram total protein) glutathione. Thus acquired tolerance to APAP involves mitochondrial resistant to glutathione depletion. This finding is significant in light of prior work demonstrating that depletion of mitochondrial glutathione sensitizes the hepatocyte to TNF dependent killing. In these same animals, we have conducted microarray gene analysis and comparing naïve to tolerated-lethal dose animals. Interestingly, major changes in mRNA expression in the tolerated animals are linked to TNF signaling. We hypothesize that selective depletion of cytosolic glutathione while favorable to TNF-induced apoptosis plays a part in the protective mechanism of adaptive tolerance to APAP. Future studies will involve the measurement of caspase 3 to assess apoptosis.

Disclosures:
The following people have nothing to disclose: Marjorie Bon Homme, Sreeleatha Channareddy, William W. Tucker, Roland Valdes, Mark W. Linder

1282
MALLORY BODY FORMATION IS AN EPIGENETIC PHENOMENON WHICH IS PREVENTED BY TREATMENT WITH SAME

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Background: Mice fed DDC (0.1%) for 10 weeks, and then withdrawn from the drug for 1 to 4 months, retain the ability to form Mallory Bodies (MBs) when the drug is refed for 7 days. The liver cells that form MBs increase and partially replace normal liver cells when the MBs form. Method: Microarray analysis was done with RNA from livers of mice treated with DDC and DDC+SAME. The hepatocytes forming MBs were identified immunohistochemically by over expressing ubiquitin in the cytoplasm. Results: Microarrays analysis of mice livers showed that MB formation was associated with changes in expression of a large number of genes. FAT10 was increased by 215 fold. FAT10, a new marker, identified these cells using an antibody to FAT10 (UbD or DiUbiquitin). FAT10 is over expressed in 90% of Human HCCs. This over expression was measured using Quantitative RT-PCR for the drug fed and drug primed mice which were refed the drug for 7 days. At this time MB formation was observed. When drug primed mice were refed DDC with S-adenosylmethionine (SAME) (7g/kg body weight), the over expression of FAT10 was prevented and MB formation was inhibited to the levels of the control mice. The inhibition of FAT10 over expression by SAME feeding was documented both by immunohistochemically (number of positive staining hepatocytes) and Quantitative RTPCR. Drug primed mice withdrawn from the drug for 8 to 9 months retained FAT10 positive hepatocytes. Hepatocellular tumors developed and stained positive for FAT10. A similar pattern of gene expression was found when KLF6, Alpha feto-protein and GSTmu2 were measured by Quantitative RTPCR. Conclusion: FAT10 is a marker for MB formation. FAT10 positive cells persist 9 months after drug withdrawal and form hepatocellular tumors. FAT10 over expression in drug primed livers is prevented by SAME, which induces hypermethylation of DNA and histones. This suggests that
1283
THE SPECTRUM OF LIVER DISEASE IN HIV-INFECTED INDIVIDUALS IN EAST AFRICA

Ponsiano Ocama, Jordan J. Feld, Michael Katwere, Theresa Piloya, Kenneth Opio, Andrew Kambugu, Elly Katabira, David L. Thomas, Robert Colebunders, Allan Ronald; Infectious Disease Institute, Mulago Hospital, Makerere University, Kampala, Uganda

Background: As patients are surviving longer, liver disease has emerged as an important cause of morbidity and mortality in patients with HIV infection. Despite the high burden of HIV in East Africa, little is known about the causes and consequences of liver disease in the HIV-infected population, partially because scarce resources often limit the extent of investigation and available treatment options. Aims: To define the spectrum of symptomatic liver disease in a large cohort of HIV-infected individuals in Kampala, Uganda. Methods: Patients presenting with jaundice, right upper quadrant (RUQ) pain, ascites and/or tender hepatomegaly were recruited for study participation. All study subjects underwent investigations to determine the cause of liver disease including HBV/HCV serology, liver enzyme evaluations, abdominal ultrasound and liver biopsy when indicated. Results: From May 2004 to March 2005, 8,715 HIV-positive patients (5,585 64% female) were seen at the Infectious Disease Clinic of Mulago hospital, 1,560 (18%) of whom were on antiretroviral therapy (ART). 77 (0.8%) patients met study inclusion criteria: 33 jaundice, 47 RUQ pain, 16 ascites and 5 tender hepatomegaly. The mean age was 37 years and 62 (81%) had a CD4 count below 200. Drug-induced liver injury (DILI) was the most common etiology (INH 10, nevirapine 7, both 5), presenting with jaundice in 14 and RUQ pain in the others and resolving in all patients with drug discontinuation. A cholestatic pattern predominated in patients taking INH (mean ALP 379 IU/L, ALT 92 IU/L) while a mixed pattern was seen in those taking nevirapine (mean ALP 733 IU/L, ALT 443 IU/L). 11 patients were HBsAg positive, 8 of whom had no other cause for liver disease identified. 7 patients presenting with RUQ pain, fever, hepatomegaly and high ALP (mean 2147 IU/L) (plus jaundice in 3), were treated for presumed tuberculous hepatitis and all responded well. Other diagnoses included: alcoholic liver disease (4), liver abscess (2), AIDS cholangiopathy (1), schistosomiasis (1), adenoma (1) and hemangiomia (1). Notably 12 (16%) patients died during follow-up, including 4 with hepato cellular carcinoma (HCC) and 3 with end-stage liver disease. Conclusions: Symptomatic liver disease is relatively common in HIV-infected individuals in Uganda and may portend a poor prognosis. Drug-induced liver injury is the most common cause and responds well to drug discontinuation. TB hepatitis should be considered in patients with a cholestatic presentation.

Disclosures:
The following people have nothing to disclose: Ponsiano Ocama, Jordan J. Feld, Michael Katwere, Theresa Piloya, Kenneth Opio, Andrew Kambugu, Elly Katabira, David L. Thomas, Robert Colebunders, Allan Ronald

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QUANTITATIVE PROTEOMIC ANALYSIS OF THE ETHANOL RESPONSE IN HEPATOCYTES USING THE ISOTOPE CODED AFFINITY TAG METHOD AND A SYSTEMS BIOLOGY APPROACH

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The mechanism of the ethanol–mediated liver injury involves many pathways not exclusive of one another that function in a coordinate manner. The aim of this work was to identify new proteins and pathways involved in the mechanism of ethanol toxicity in hepatocytes and examine the potential crosstalk between such pathways. We applied the isotope coded affinity tag (ICAT) proteomic method to compare protein abundance in hepatocytes from rats fed the Lieber-DeCarli diets. Hepatocytes were isolated and harvested immediately after perfusion. Proteins were labeled with the cleavable ICAT reagent followed by trypsin digestion. The ICAT labeled peptides which carry biotin tags were fractionated by cation exchange chromatography and purified by avidin affinity chromatography. Peptide mixtures were subjected to micro capillary HPLC and to mass spectrometry analysis using LTQ. Software tools available at the Seattle Proteome Center (Institute for Systems Biology) were used in the data analysis: 1) peptide tandem mass spectra matching to specific sequences in a database using SEQUEST, 2) assignment of a probability to the identified peptide sequence using PeptideProphet, 3) assignment of an overall probability to the protein identification using ProteinProphet, 4) calculation of ICAT ratio using the ASAP Ratio and EXPRESS programs, and 5) use of the ISB Experiment Analysis Management System. The ICAT method and the global proteomic analysis led to identification of more than 450 proteins from control and ethanol–treated hepatocytes with probability scores >0.5 (<5% error rate). Among the identified proteins were several well known alcohol regulated proteins such as those involved in fatty acid metabolism, oxidative phosphorylation, PPAR signaling pathway, and cytochrome P450 metabolism. Novel mitochondrial and ribosomal proteins were up- or down-regulated by alcohol treatment. Proteins identified by ICAT were explored for function, interactions, and pathway membership using a variety of annotation data sources integrated via the Gaggle http://gaggle.systemsbiology.org. Protein identifiers were mapped to Entrez GenIDs, which were then ‘broadcast’ to KEGG (for metabolic pathways), HPRD (for protein interactions), Bioconductor GOstats (for enriched molecular function and biological process) and EMBL STRING (for gene and protein associations). The resultant network of associations and interactions was broadcast back to cytoscape for further study, leading to the identification of putative novel and liver-specific regulatory components of the ethanol response. We are currently blocking critical pathways to assess the potential prevention of alcoholic liver injury.

Disclosures:
The following people have nothing to disclose: Wei Yan, Paul Shannon, Jose Moron, Laura Conde de la Rosa, Natalia Nieto
B-LYMPHOCYTES AS AN IN-VITRO MODEL TO STUDY INTERINDIVIDUAL VARIATION IN SENSITIVITY TO ACETAMINOPHEN TOXICITY

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Background: The dose of acetaminophen (APAP) required to cause liver injury varies among people. Indeed, some develop elevations in serum alanine aminotransferase with recurrent therapeutic dosing (JAMA 5:296(1):87-93, 2006). To define mechanisms underlying this variation in sensitivity, liver tissue is most relevant but is rarely available. Peripheral blood mononuclear cells (PBMCs) may represent a suitable alternate tissue for study because they express relevant metabolic pathways, including CYP enzymes and glutathione, plus they are easily accessible. Furthermore, loss of PBMCs accompanies APAP hepatotoxicity in mice. Goals: To determine if there exist interindividual differences in PBMC sensitivity to APAP toxicity and whether sensitivity varies by PBMC type. Methods: Human PBMCs were isolated by centrifugation on Ficoll-PaqueTM Plus and cultured for 48hrs with varying concentrations of APAP (1-10mM). The Cell Titer Blue® Assay and FACSScan analysis with propidium iodide were used to quantify cytotoxicity after APAP exposure. CD-specific antibodies were used to distinguish effects on subpopulations of PBMCs. Dynal Bead technology was used to isolate B lymphocytes from PBMCs. Statistical estimates of variance and confidence intervals were computed by fitting a single-group repeated-measures analysis of variance model in which "sensitivity" was the dependent variable. Results: Among 10 healthy adult volunteers, the APAP concentration required to kill 40% of the PBMCs at 48hrs ranged from 3.3mM to 16.6mM and the percent cell death resulting from 48hr exposure to 10mM ranged from 28% to 69%. Relative susceptibility "phenotype" was not changed with 3 repeat measurements separated by one week [n=5; intra-subject variance of 9.32% viability squared vs. inter-subject variance of 92.5% viability squared]. FACSScan analysis identified B lymphocytes as the subpopulation of PBMCs most susceptible to APAP toxicity. In contrast, T lymphocyte viability was relatively unchanged even at the maximal APAP concentration (10mM). Isolated B lymphocytes confirmed similar susceptibility to APAP toxicity as observed within PBMCs (>60% killed by 10mM APAP at 48hrs). Conclusion: There exists interindividual variation in the susceptibility of PBMCs to APAP-induced cytotoxicity and this phenotype appears to be reproducible within an individual. B lymphocytes are most sensitive to APAP toxicity, and may be the preferred cell population for mechanistic studies. These observations will have implications for the study of hepatotoxicity since B lymphocytes are being immortalized from all patients enrolled in the Drug Induced Liver Injury Network study.

Disclosures: The following people have nothing to disclose: Sara K. Peterson, Ray L. Hawke, Paul B. Watkins

ALCOHOL INDUCES GLOBAL HEPATIC PROTEIN HYPERACETYLYATION

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Chronic alcohol consumption can lead to serious liver disease. Although the disease progression is clinically well-described, the molecular basis for alcohol-induced hepatotoxicity is not well understood. Previously, we determined that microtubules were more highly acetylated and more stable in ethanol-treated WIF-B cells, an emerging model system for studies on liver injury. We further determined that microtubules were hyperacetylated to the same extent in livers from ethanol-fed rats indicating this result has physiologic importance. Besides tubulin, there is a growing number of proteins known to be acetylated on lysine residues including histones, transcription factors, nuclear import factors and p53. Large families of acetyltransferases and deacetylases have also been identified whose substrate specificities are not yet well defined. With this growing number of acetylated proteins and large number of modifying enzymes, it is likely that many other hepatic proteins are hyperacetylated in ethanol-treated cells. In support of this conclusion, we found in this study that nuclear histone deacetylase activity was impaired 35% in ethanol-treated WIF-B cells. In order to identify other hyperacetylated proteins, we have been immunoblotting samples prepared from livers of control and ethanol-fed rats with antibodies specific for acetylated lysine residues. Our analysis started globally where liver whole homogenates were compared. In general, the same 10-16 proteins ranging in molecular weight from 12-175 kDa were more highly acetylated in ethanol-treated homogenates. For most proteins, acetylation was enhanced 2-3 fold, but in some cases, acetylation was increased >20-fold. Among all samples examined, a cluster of 5 bands ranging from 30-45 kDa and a protein of 25 kDa were consistently hyperacetylated 2-3 fold and proteins at 175 and 12 kDa were hyperacetylated 2-10 fold. To further characterize the hyperacetylated proteins, we prepared nuclear, cytosolic and total membrane fractions by differential centrifugation. The 12 kDa protein was exclusively localized to the nuclear fraction as well as an additional cluster of hyperacetylated proteins ranging from 15-22 kDa that may likely be histones. The 175 kDa protein was recovered mainly in the membrane fraction whereas the 30-45 kDa cluster and the 25 kDa protein were mainly cytosolic. These results confirm our hypothesis that ethanol induces hyperacylation of a range of hepatic proteins. Such changes in acetylation likely have profound effects on hepatocyte function. Studies are underway to identify these proteins and determine how hyperacetylation alters proper hepatic function.

Disclosures: The following people have nothing to disclose: Blythe D. Shepard, Dean J. Tuma, Pamela L. Tuma

USEFULNESS OF FIBROSCAN FOR THE FOLLOW UP OF PATIENTS TREATED WITH METHOTREXATE

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Introduction: Liver stiffness measurement using FibroScan allows evaluating liver fibrosis. Recently, its has been shown that FibroScan could be useful for the follow-up of patients with Crohn’s disease treated with Methotrexate (MTX) and that significant fibrosis was rare in these patients (Laharie et al, 2006).
The aim of this prospective study was to evaluate significant fibrosis using FibroScan in a large cohort of patients with various diseases treated with MTX compared to patients without MTX. Methods: From January 2005 to April 2007, all consecutive patients treated with more than 1500 mg of MTX (group I, n=108) were compared to controls who never received MTX or received MTX ≤ 1500 mg (group II, n=199). In all patients, liver fibrosis was evaluated using FibroScan. For the diagnosis of significant fibrosis, published cut-off (> 8.7 kPa) was used (Ziol et al. Hepatology 2005). Results: A total of 307 patients (117 males, mean age 50.4 years, mean BMI 24.6 kg/m2, Crohn’s disease (n=85), psoriasis (n=73), rheumatoid arthritis (n=69), spondylarthritides (n=19), other inflammatory diseases (n=61), mean total dose of MTX in group I 3683 mg for 275 weeks) were included. Liver stiffness values ranged from 1.8 to 25.7 kPa (median: 4.4 kPa). For all patients, no correlation was observed between the total dose of MTX and FibroScan (Kendall Tau-b=0.034). Between group I and II, no difference was observed for liver fibrosis assessment. In group I, patients (n=6) with liver stiffness measurement > 8.7 kPa had a total dose of MTX between 2220 and 3240 mg. One patient had cirrhosis on liver biopsy. For others, 4/5 had BMI > 25 kg/m2 and 4/5 had no or mild fibrosis using Fibrotest. In group II, 4/10 patients with liver stiffness measurement > 8.7 kPa had never received MTX. For others (mean dose: 648 mg), one had cirrhosis on liver biopsy. For others, 4/5 had BMI > 25 kg/m2 and 5/5 had no or mild fibrosis using Fibrotest. Conclusion: In patients treated with MTX, whatever the indication of MTX treatment, there is no increased risk of significant fibrosis. According to these results, liver biopsy could be avoided during the follow-up of patients treated with MTX. A liver biopsy should be performed only in patients with persistent liver enzyme abnormalities or elevated liver stiffness measurement.

<table>
<thead>
<tr>
<th></th>
<th>Group I (n=108)</th>
<th>Group II (n=199)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FibroScan (kPa)</td>
<td>5.3 ± 3.0</td>
<td>4.9 ± 2.5</td>
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<tr>
<td>FibroScan &gt; 8.7 kPa (%)</td>
<td>6 (5.5)</td>
<td>10 (5.0)</td>
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Disclosures:
The following people have nothing to disclose: David Laharie, Edouard Chabrun, Thierry Schaeverbeke, Thomas Hubiche, Marie-Sylvie Doutré, Maîté Longy, Jean-Luc Pellegrin, Juliette Foucher, Laurent Castera, Florence Salau, Sandrine Villars, Frank Zerbib, Victor de Ledinghen

1288 RELATIONSHIP BETWEEN MEDICATION DOSE AND IDIOSYNCRATIC DRUG INDUCED LIVER INJURY (DILI)
Craig Lammert1, Einar Bjornsson2, Chandan Saha3, Naga P. Chalasani1; 1Indiana University School of Medicine, Indianapolis, IN; 2Sahlgrenska University Hospital, Gothenburg, Sweden

Introduction: Idiosyncratic DILI by definition is not dose-related but there are anecdotes suggesting that daily medication dosage may play a role in the pathogenesis of idiosyncratic DILI. Aim: To examine the relationship between the daily dose of oral prescription medications and the reports of hepatic adverse events. Methods: From a well known public database, we extracted the names of top 200 brand name and 200 generic drugs by prescription volume in the United States. After excluding the duplicate entries and non-oral medications, 234 medications were available for further consideration. These medications were categorized into <10 mg, 10-50 mg, >50mg according to recommended daily dosage. We subsequently reviewed the “DRUGDEX” to extract the details of reported hepatic adverse events for each medication belonging to these 3 groups. DRUGDEX, a comprehensive pharmaceutical database, consists of package insert data and published medical literature. Cochran-Armitage trend test and logistic regression analyses were done to test the association between outcome events and dosage groups. Results: Among 3 groups, there was no difference in the number of prescriptions filled in 2005 (p=0.35). The relationship between 3 dosage groups and reports of hepatic adverse events are shown in the table below. Logistic regression analyses showed that, compared to < 10 mg/daily group, more medications in the > 50 mg/day group had reports of increased ALT (OR: 3.28, 95% CI 1.62-6.63, p=0.001), liver failure (OR: 2.35, 95% CI 1.02-5.40, P=0.045), death” (OR: 3.09, 95% CI 1.18-8.04), and liver transplant (OR: 10.45, 2.3-47.44, P=0.002. There was no statistically significant difference between < 10 mg/d and 10-50 mg/d groups. Summary: (1) Significantly more number of medications given at daily doses > 50 mg have been associated with drug-induced liver failure, necessity for liver transplantation, and liver-related deaths, (2) daily dose had inconsistent relationship with elevated ALT, and (3) daily dose was not associated with cholestatic jaundice. Conclusion: We found a significant relationship between daily dose of oral medications and reports of hepatic adverse events. More studies are needed to validate and explore the significance of our observations.

<table>
<thead>
<tr>
<th>Reported Hepatic Adverse Events</th>
<th>&lt;10 mg/d (n=54)</th>
<th>10-50 mg/d (n=83)</th>
<th>50 mg/day (n=57)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. medications causing any ALT elevation (%)</td>
<td>48</td>
<td>60</td>
<td>75</td>
<td>&lt;0.001</td>
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<tr>
<td>No. medications causing ALT &gt; 3 ULN (%)</td>
<td>19</td>
<td>27</td>
<td>30</td>
<td>ns</td>
</tr>
<tr>
<td>No. medications causing cholestatic jaundice (%)</td>
<td>33</td>
<td>40</td>
<td>44</td>
<td>ns</td>
</tr>
<tr>
<td>No. medications causing liver failure (%)</td>
<td>17</td>
<td>12</td>
<td>32</td>
<td>0.009</td>
</tr>
<tr>
<td>No. medications leading to liver transplant (%)</td>
<td>0</td>
<td>2</td>
<td>13</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>No. medications causing liver-related death (%)</td>
<td>11</td>
<td>11</td>
<td>28</td>
<td>&lt;0.004</td>
</tr>
<tr>
<td>Median # of prescriptions in 2005</td>
<td>4,746,590</td>
<td>4,938,000</td>
<td>3,733,000</td>
<td>0.35</td>
</tr>
</tbody>
</table>

Disclosures:
The following people have nothing to disclose: Craig Lammert, Einar Bjornsson, Chandan Saha, Naga P. Chalasani

1289 THE CHOICE OF CLINICAL LABORATORY MAY CHANGE STUDY OUTCOME: AN INTER-LABORATORY EVALUATION OF AMINOTRANSFERASE MEASURES
Jody L. Green, Elizabeth Campagna, Gregory M. Bogdan, Kennon Heard, Richard C. Dart; Denver Health Rocky Mountain Poison and Drug Center, Denver, CO

Throughout the United States, clinical laboratories establish reference ranges, commonly referred to as the “normal” range for aminotransferase assays. Guidelines for determining reference ranges have been published but it is unknown how many or which laboratories follow the guidelines. Aminotransferase reference ranges vary between laboratories and may lead to difficulties in the interpretation of patient data in both clinical management and research. Drug-induced hepatotoxicity, as judged by alanine aminotransferase (ALT) serum activity in comparison to the reference range, is the single most common adverse effect leading to withdrawal of FDA-approved drugs. The purpose of this study was to determine if the classification of samples as “abnormal” would differ between two laboratories. We used 65 serum samples collected in a randomized clinical trial. Split samples were sent to two clinical laboratories for ALT testing, each used different instruments and reagents and
had different reference ranges. One laboratory had a higher ALT upper limit of reference range (ULRR) and the other a lower ULRR. The samples were stratified into three groups by the original ALT value: 1) ALT ≤ lowest ULRR (n=15), 2) ALT > lowest ULRR and ≤ highest ULRR (n=34) and 3) ALT > highest ULRR (n=16). Fifty-five of the specimens were from male subjects. Subject age ranged from 23 to 61 years (median 42 years). The overall ALT mean between laboratories was significantly different (p<0.001). The proportion of specimens judged abnormal (ALT greater than the ULRR) was 9% and 71% for Laboratory A and B, respectively (p<0.001). The proportion of samples with an ALT greater than three times the ULRR was zero at Laboratory A and 9% at Laboratory B (p=0.026).

Results for serum aspartate aminotransferase activity were similar. Our results indicate the classification of a sample as normal or abnormal differs between laboratories and that a patient’s serum ALT may be misclassified as normal or abnormal depending on the specific laboratory performing the assay. Interpretation would particularly be uncertain in large databases of spontaneous reports such as the FDA Adverse Event Reporting System (AERS). Adoption of standardized reference ranges and methodology are needed to allow consistent interpretation of results for patients or studies that are performed by different laboratories.

**Laboratory Reference Ranges and Results**

<table>
<thead>
<tr>
<th>Laboratory A</th>
<th>Laboratory B</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALT range (U/L)</td>
<td>17-63 (male), 14-54 (female)</td>
<td>5-31</td>
</tr>
<tr>
<td>ALT mean±SD</td>
<td>33.6 ± 22.9</td>
<td>47.9 ± 32.9</td>
</tr>
<tr>
<td>ALT &lt;ULRR, No. (%)</td>
<td>6 (9.2)</td>
<td>46 (70.8)</td>
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<tr>
<td>ALT &gt;3xULRR, No. (%)</td>
<td>0 (0)</td>
<td>6 (9)</td>
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</tbody>
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Disclosures:
The following people have nothing to disclose: Jody L. Green, Elizabeth Campagna, Gregory M. Bogdan, Kennon Heard, Richard C. Dart

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**1290 INHIBITION OF ATM ACTIVITY AMELIORATES THE ETHANOL METABOLISM-MEDIATED CELL CYCLE ARREST**

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Background: Chronic ethanol abuse results in hepatocyte injury and impairs hepatocyte replication. Impaired hepatocyte replication inhibits the replacement of cells lost to the toxic effects of ethanol. It has been shown that ethanol metabolism is responsible for the impairment of hepatocyte replication. We have shown that ethanol metabolism results in cell cycle arrest at the G2/M transition of the cell cycle and that this arrest is, in part, mediated by inhibitory phosphorylation of the mitotic cyclin-dependent kinase Cdc2 and its regulatory phosphatase Cdc25C. The checkpoint kinases, Chk1 and Chk2, phosphorylate Cdc25C, effectively rendering it inactive. Chk1 and Chk2 are in turn regulated by ataxia telangiectasia mutated (ATM) and ATM and Rad3-related (ATR) kinases. The purpose of these studies was to investigate the role of ATM in the ethanol-metabolism-mediated G2/M cell cycle arrest. Methods: Hep G2 cells that express either alcohol dehydrogenase (ADH) or both ADH and cytochrome P450 2E1, VA-13 and VL-17A cells respectively, were cultured for 4 days in the presence of 25 mM ethanol and/or 1 mM caffeine, an inhibitor of ATM. Immunoblots were performed to determine the effects of ethanol metabolism and inhibition of ATM. The effects of inhibition of ATM on the cell cycle and on Cdc2 was determined by monitoring the cell cycle progression of cultures and the phosphorylation state of Cdc2. Results: Culturing VA-13 and VL-17A cells in the presence of ethanol resulted in a 50-65% increase in the activity of ATM, and a corresponding increase in both Chk1 and Chk2. Additionally, ethanol metabolism resulted in a 3 to 4-fold increase in the inhibitory phosphorylation of Cdc2, and approximately 25-35% of the cells being arrested at the G2/M transition of the cell cycle. Inhibition of ATM resulted in approximately 50% of the cells being rescued from the G2/M cell cycle arrest, and almost completely ameliorated the inhibitory phosphorylation of Cdc2. Conclusions: Ethanol metabolism results in the activation of ATM. ATM can phosphorylate and activate the checkpoint kinases Chk1 and Chk2, ultimately resulting in the accumulation of the mitotic cyclin-dependent kinase, Cdc2, in the inactive phosphorylated form. This may, in part, explain the ethanol metabolism-mediated impairment in replication. The inhibition of hepatocyte replication may be important in the initiation and progression of alcoholic liver injury. Additionally, because ATM is activated in both VA-13 and VL-17A cells these data indicate that acetaldehyde has a significant role in this mechanism of ethanol metabolism-mediated cellular replication impairment.

Disclosures:
The following people have nothing to disclose: Dahn L. Clemens, Katrina J. Mahan, Robert F. Nuss, Michael F. Sorrell, Dean J. Tuma

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**1291 GENETIC POLYMORPHISMS OF IL-10, IL-4 AND TNF-α AND SUSCEPTIBILITY TO DEVELOP DRUG-INDUCED IDIOSYNCRATIC LIVER INJURY (DILI)**

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The aetiology of DILI is suggested to be multifactorial, determined by a combination of genetic, environmental and immunological factors. Emerging data indicate that immunoregulatory cytokines such as interleukin (IL) 10, IL-4 and tumour necrosis factor (TNF)α may be important candidates in determining susceptibility to develop DILI. Interleukin 10 (IL-10) is a potent anti-inflammatory Th2 cytokine that down-regulates the expression of major histocompatibility complex class I and class II molecules as well as the production of Th1 cytokines. Polymorphism of IL-10, IL-4 and TNFα is known to affect cytokine production. However, limited data exist on their effects in DILI appearance Aims: The aim of this study was to assess the relationship between polymorphisms involving three cytokine genes IL-10 (-1082 G/A), -819 C/T, and -592 C/A), IL-4 (-308 G/A) and TNFα (-308 G/A) and DILI susceptibility. Methods: Cytokine genotyping was analysed in genomic DNA by means of PCR-FRET in a total of 140 DILI patients and 175 healthy controls. An analysis in a subset of 29 patients with amoxicillin-clavulanate hepatotoxicity was also performed. Results: No
differences between allele frequencies, genotypes and haplotypes were found between DILI and healthy controls. Patients with cytokine gene polymorphisms did not differ in regard to clinical presentation of DILI, type of injury and outcome. When analysed by IL-10 haplotype carrier state, we identified the possible association between the low IL-10 producing haplotype (ACC, ATA) and absence of eosinophilia (p= 0.0004; OR 5.3, 95% CI 2.05-13.67). This association remained significant for cases of amoxicillin-clavulanate hepatotoxicity. Comparison of low IL-10 producing haplotype carriers and absence of eosinophils with high IL-10 producing haplotypes showed no difference in age, gender and duration of treatment between groups. Interestingly, cases of fulminant liver failure and transplant cases (n=3) as well as severe DILI cases (high bilirubin, altered prothrombin time) (n=5) were classified as low IL-10 producing haplotype. Searching in the general Registry database of Hepatotoxicity, out of 591 DILI patients 36 (6%) had a fatal outcome (death, transplant or fulminant liver failure) and none of them presented eosinophilia. While 113 patients out of the rest 555 (20%) had eosinophilia and a more favourable outcome. Conclusions: Our results show that IL-10, IL-4 and TNFα polymorphisms might not be predictable potential risk factors for DILI. Absence of eosinophilia in low IL-10 producing haplotype, which is compatible with an accelerated Th1-type response, might favour a worst DILI outcome. FIS 04/1759

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1292 GILBERT’S SYNDROME ASSOCIATED VARIABILITY: COMBINATION OF UDP GLUCURONOSYLTRANSFERASE (UGT) 1A1 AND UGT1A7 GENE POLYMORPHISMS INCREASE THE RISK OF IRINOTECAN TOXICITY

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Background: Gilbert’s syndrome is characterized by the absence of severe liver disease and is associated with a functional promoter polymorphism (SNP) of the UDP glucuronosyltransferase (UGT) 1A1 gene leading to a reduced glucuronidation which potentially impact a broad array of therapeutic range. This is potentially relevant for hepatological therapeutic range. Instead, enhanced glucuronidation and physiologically plausible risk factor for irinotecan toxicity. Based on the role of UGT1A7 the UGT1A1/UGT1A7 SNP combination haplotype is demonstrated to be a superior risk predictor than UGT1A1*28 alone, which contributes to the therapeutic individualization of this drug with a narrow therapeutic range. This is potentially relevant for hepatological patients undergoing chemotherapy.

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1293 ACETAMINOPHEN BIOTRANSFORMATION AT SINGLE AND REPEAT MAXIMUM DOSES IS SIMILAR BETWEEN ADULTS WITH AND WITHOUT CHRONIC LIVER DISEASE

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Background & Aim: Acetaminophen toxicity is understood to be dose dependent. Hepatotoxicity is not imparted directly by acetaminophen concentrations, but rather by the activated metabolite, NAPQI, if not adequately detoxified by glutathione after excessive overdoses. The question of whether the diseased liver can adequately detoxify therapeutic doses of acetaminophen was addressed in this study by measuring the amounts, fractions of dose, and formation clearances of metabolites produced after single and repeat dosing of acetaminophen at the maximum-labeled daily dose (4 g/d) for four days. Methods: Data for 12 subjects diagnosed with hepatocellular cirrhosis secondary to hepatitis C and/or alcohol abuse, and a Child-Pugh score of 7 to 9, indicating moderate hepatic impairment, were compared with 13 healthy matched-control subjects. After the single and final repeat 1 g doses, serial blood and pooled urine samples were collected for 36- and 24-h, respectively. They were assayed for acetaminophen and metabolites. Amino- transferases and clinical adverse events were monitored during the study. Results: Analysis of variance detected no differences in mean fractions of metabolites excreted, including thiols, between groups after single or repeat doses. Only the amount of unchanged acetaminophen excreted was statistically higher (p=0.0035) in carriers of the UGT1A risk alleles, who also had significantly higher rate of dose reductions. Conclusion: The association of irinotecan toxicity with a combination of reduced function UGT1A1 and UGT1A7 SNPs represents a physiologically plausible risk factor for irinotecan toxicity. Based on the role of UGT1A7 the UGT1A1/UGT1A7 SNP combination haplotype is demonstrated to be a superior risk predictor than UGT1A1*28 alone, which contributes to the therapeutic individualization of this drug with a narrow therapeutic range. This is potentially relevant for hepatological patients undergoing chemotherapy.

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hepatic-impaired adults show biotransformation that supports an expectation of diminished risk of hepatotoxicity with repeat therapeutic dosing at the maximum-labeled dose of 4 g/d. Aminotransferases did not change from baseline for any hepatic-impaired subject, and the doses were well tolerated.

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Brenda A. Zimmerman - Employee: Other
John T. Slattery - Consultant/Adviser: Other

1294
METABOLOMIC ANALYSIS OF URINE AND LIVER AFTER CHRONIC ALCOHOL TREATMENT IN C57BL/6J MICE
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Rodent models of alcohol-induced liver injury have been invaluable for understanding the mechanisms of this disease in humans. While the intragastric enteral alcohol feeding model (Tsukamoto-French) mimics liver injury in alcoholics, one criticism of this approach has been that it is not a normal feeding pattern for rodents. In this experiment we aimed to determine (i) whether a modified Lieber-DeCarli liquid alcohol-containing diet regimen results in a comparable degree of chronic liver injury; and (ii) what are the biochemical events evoked by this treatment. Male, C57BL/6J mice were fed an isocaloric control or alcohol-containing liquid diet with 35% of calories from corn oil, 18% protein and 47% carbohydrate/alcohol for up to 36 days ad libitum. Alcohol treatment was initiated at 7 g/kg/day and gradually reached a final dose of 21 g/kg/day. Urine samples were collected at 22, 30 and 36 days and in additional treatment groups, liver and serum samples were harvested at 28 days. In the alcohol group, a 5-fold increase in ALT and a 6-fold increase in liver injury score (necrosis, inflammation and steatosis) were observed. Metabolomic profiling was performed on liver and urine using Nuclear Magnetic Resonance (NMR) Spectroscopy and Electrospray infusion/ Fourier transform ion cyclotron resonance-mass spectrometry (ESI/FTICR-MS). In liver, alcohol had profound effects on metabolites. Glycogen levels were decreased which is consistent with stimulated glycolysis, a decrease in glucose and elevated lactate levels. Stimulated glycolysis can also account for an increase in amino acid metabolism giving rise to elevations in alanine, glutamate and glutathione. A secondary effect of stimulated glycolysis likely caused increases in valine, leucine and alanine. Taurine, which is a metabolite of glutathione, was also increased likely due to high levels of choline detected. The effect of increased glutamate could explain the increases in mitochondrial acetone production from ketogenesis. Alternatively, alcohol dehydrogenase-dependent alcohol metabolism could explain high levels of acetone due to elevated rates of ketogenesis. In urine, tyrosine, a marker of oxidative stress, was elevated and is a precursor for DOPA, epinephrine and thyroid hormones, all known to be altered by alcohol. Ethyl glucuronide was also markedly increased and is a known biomarker of alcohol exposure in urine. In summary, assessment of the biochemical networks affected by alcohol will enable interindividual comparisons of the metabolic differences and may provide insight into factors influencing susceptibility to alcohol-induced liver injury. (AA016258 and ES10126)

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1295
A PROSPECTIVE STUDY OF THE RELIABILITY OF THE RUCAM FOR ASSESSING CAUSALITY IN DRUG-INDUCED LIVER INJURY
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PURPOSE: We describe an empirical study to evaluate the reliability of the Roussel Uclaf Causality Assessment Method (RUCAM) in pedigree cases of drug-induced liver injury (DILI).

METHODS: The DILIN Retrospective study is enrolling cases of hepatotoxicity caused by isoniazid, phenytoin, clavulanate / amoxicillin and valproate occurring since 1994. These drugs are known to cause DILI, and have an identifiable clinical pattern of injury. Using the RUCAM, each case was adjudicated by three independent reviewers; after an interval of at least 4 months, they were re-adjudicated by the same reviewers. Simple descriptive statistics were used to summarize RUCAM differences between the two occasions and across the 3 reviewers. A random-effects regression model was applied for a formal reliability analysis; the upper 95% confidence limit (U95CL) was derived using bootstrapping methods.

RESULTS: Forty DILI cases were included (isoniazid=9, phenytoin=5, clavulanate / amoxicillin=15 and valproate=11). Mean age at onset was 44.8 [range=3.5 to 78.5] years; 68% female; 78% Caucasian and 15% African-American. mean BMI at onset was 28.5 [range=16.0 to 51.1] kg/m²; 28% had a prior history of a liver condition. All patients had at least one symptom including jaundice (73%), nausea (55%), and fever (18%); 18% had extrahepatic manifestations. Mean (s.d.) peak serum tests during the injury were: AST=1026 (1257), ALT=821 (799), AP=424 (456) /U/L and total bilirubin=14.2 (13.6) mg/dL. Cases were classified as cholestatic (28%), mixed (28%) or hepatocellular (44%). Using expert opinion achieved by consensus among DILIN hepatologists, causality was judged as: definite (26%), very likely (49%), probable (21%) and possible (5%). Mean (s.e.) RUCAM on the first review was 6.2 (0.2), and 6.4 (0.2) on the second review (p=0.99). On re-review by the same reviewer, however, there was complete agreement on only 26% of cases; changes greater than 2 points occurred in 19% of cases. On average, the maximum absolute value of the pairwise differences among the three reviewers was 3.1 on the first review and 2.7 on the second review. There was significant variation among the reviewers (p=0.03). The test-retest reliability was 0.52 (U95CL=0.69); the inter-rater reliability was 0.50 (U95CL=0.67).

CONCLUSIONS: A research instrument should achieve a test-retest reliability of at least 0.8 and an inter-rater reliability of 0.6. In well-recognized hepatotoxic drugs, however, the RUCAM met only the latter criterion. This raises questions about its suitability for assessing causality, and suggests that additional approaches should be considered. Supported by cooperative agreement, 5U01DK065176-04.

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DETECTION OF ACETAMINOPHEN PROTEIN ADDUCTS IN SERUM DURING THERAPEUTIC DOSING OF ACETAMINOPHEN IN HEALTHY VOLUNTEERS

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Background: Acetaminophen (APAP) protein adducts in serum have been proposed to be pathognomonic for APAP liver injury. The goal of this study was to determine whether adducts could be detected in healthy volunteers treated with the maximum recommended daily dose of APAP. Methods: 52 healthy men and women were housed as inpatients for 14 days and received APAP 4 grams daily for 7 days (days 4 - 11). Blood was drawn for adduct and ALT measurements each morning. Patients were stratified by ALT responder status: responder (change in ALT > 2 fold baseline; n=14); non-responder (change in ALT within 1.5 fold baseline; n=15); intermediate (> 1.5 and < 2 fold baseline; n=13). 10 subjects received placebo. A pharmacokinetic analysis was performed, presented as mean (+/- SD). Results: For all subjects receiving APAP, adducts were elevated compared to placebo (p<0.05) by 18 hours of dosing (day 5) and remained elevated until discontinuation of APAP (range: 0.067 nmol/mL serum). The elimination half-life (t1/2) was 2.9 (+/- 2.1) days; the maximum adduct concentration (Cmax) was 0.29 (+/- 0.11 nmol/mL); the change in adduct between days 4 and 5 (delta 4-5) was 0.14 (+/- 0.07 nmol/mL adducts). The t1/2, Cmax, and delta day 4-5 were higher in responders, but this was not statistically significant. The area under the curve for adducts was higher for responders/intermediate-responders combined (p<0.05), compared to non-responders. Adducts were lower than values in adults with APAP related acute liver failure (1.0-40 nmol/mL), adduct elevation preceded ALT elevation (Figure). Conclusions: Adducts are a sensitive marker of APAP exposure in healthy adult volunteers that receive therapeutic doses of APAP. The pharmacokinetics of adducts suggest that factors other than the extent of adduct formation underlie ALT response. Further studies in “at risk” populations are warranted to determine the biological significance of adducts and increases in ALT subsequent to therapeutic exposure to APAP. Supported by DK067999.

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ACETAMINOPHEN-PROTEIN ADDUCTS IN ALCOHOLIC PATIENTS RECEIVING ACETAMINOPHEN 4 G/DAY FOR 5 DAYS

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Background: Acetaminophen-protein adducts (APAP-ADD) are formed when the toxic metabolite of acetaminophen (APAP) binds to intracellular proteins. APAP-ADD are detectable in the serum of patients with acetaminophen poisoning. Objective: To determine whether APAP-ADD are detectable in alcoholic patients taking therapeutic doses of APAP. Methods: This was a sub study in a randomized, placebo controlled trial where 47 adult patients admitted to a controlled access facility received 1 gram APAP (40 patients) or placebo (7 patients) 4 times per day for 5 days. All patients were considered alcoholics (defined by CAGE questionnaire or Brief Michigan Alcoholism Screening Test), and were dosed at an alcohol detoxification center within 12 hours of their estimated sober time. Medications were dispensed by the nursing staff. Serum samples were collected at baseline and at days 2, 4, 6 and 7 and analyzed for APAP-ADD and ALT. APAP-ADD were quantified using a HPLC-EC assay. Each subject was monitored for clinical signs of hepatic injury as well as a standardized laboratory evaluation. Results: All five serial samples were available for all subjects. At day 0, APAP-ADD were detected in four (10%) APAP and one (14%) placebo subjects. Three of these subjects (2 APAP, 1 placebo) had a detectable serum APAP level at baseline and another reported ingestion of an APAP product prior to study enrollment. By day 6 APAP-ADD were detected in all (100%) APAP subjects and 2 (28%) placebo subjects. Mean APAP-ADD levels in APAP subjects increased during APAP dosing, reaching a peak of 0.310 ± 0.118 nmol/mL serum at day 6. Mean APAP-ADD level in APAP subjects decreased to 0.242 ± 0.100 nmol/mL serum on day 7. APAP subjects had a slight increase in mean ALT values during the study, from 42.55 ± 37.74 IU/L at day 0 to 57.30 ± 53.80 IU/L at day 7. No consistent trend was seen in ALT values in placebo subjects. Discussion: Alcoholic subjects taking the maximal therapeutic dose of APAP for 5 days had increasingly detectable APAP-ADD. The adducts detected in the placebo subjects likely represent unreported acetaminophen consumption. Our findings suggest that APAP-ADD occur during metabolism of therapeutic amounts of acetaminophen and that a low level of adducts characteristic of repeat therapeutic exposure to APAP can be detected in the absence of clinically relevant changes in ALT. As overdose patients have serum adducts 3- to 20-fold higher than the levels reported here, future studies will be needed to differentiate adduct levels consistent with therapeutic exposure and adduct levels indicative of acetaminophen toxicity.

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A HIGH BARRIER TO RESISTANCE MAY CONTRIBUTE TO THE ROBUST ANTIVIRAL EFFECT DEMONSTRATED BY R1626 IN HCV GENOTYPE 1-INFECTED TREATMENT-NAIVE PATIENTS

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R1626, a prodrug of R1479, is a potent inhibitor of HCV replication that has shown maximum mean (median) HCV RNA reductions of up to 3.7 (4.1) log10 following 2 weeks of monotherapy study, and 5.2 log10 following 4 weeks in combination with peginterferon alfa-2a (PEG-IFNα-2a) ± ribavirin (RBV) (phase 2A) in patients infected with HCV genotype 1. In vitro studies have identified NS5B polymerase amino acid substitutions, S96T or S96T/N142T, that result in 4-5-fold reduced sensitivity to R1479 as determined in the replicase assay. These mutations also resulted in a ~95% reduction in replication capacity compared to the wild type replicon. To study resistance development in vivo, phenotypic and genotypic analyses were performed on multiple serum samples from a total of 11 HCV patients treated with R1626 (3 from the monotherapy study and 8 from the combination study), who showed viral rebound before the end of treatment (defined as a sustained ≥ 0.5 log10 increase of viral load above nadir [lowest point], where nadir is a ≥ 0.5 log10 decrease from baseline). All samples tested in the HCV NS5B phenotypic assay were sensitive to inhibition by R1479 to a similar extent as the baseline samples and the 2 reference strains, Con1 and H77. Sequence analysis of the entire NS5B coding region revealed no known R1479 resistance mutations (S96T or S96T/N142T) or any other common amino acid substitutions. Three patients who failed to respond to R1626 (defined as <0.5 log10 decrease of viral load from baseline) had all received 500 mg of monotherapy. NS5B population sequence analysis and clonal sequence analysis of between 40–106 NS5B polymerase molecular clones for baseline samples of these 3 patients did not reveal pre-existing amino acid substitutions among the quasispecies of these patients that could be responsible for resistance to R1479. These findings are consistent with the sensitivity of these samples to inhibition by R1479 as observed in the phenotypic assay. In addition, sequence analysis demonstrated the absence of known R1479-resistance mutations in baseline samples of all patients who participated in both studies. In conclusion, there was no evidence for resistance selection to R1626 after 2 weeks of monotherapy or after 4 weeks in combination with PEG-IFNα-2a ± RBV. These findings suggest that there is a high barrier to the development of resistance to R1626 in vivo, that may contribute to the robust antiviral effect of this drug that has been observed in randomized clinical trials.

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Alan Kosaka - Employee: Roche
Steven Hu - Employee: Roche
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WHAT IS THE IMPACT OF HIGHER SENSITIVITY ASSAY ON RESPONSE-GUIDED THERAPY IN HEPATITIS C VIRUS (HCV)? COMPARATIVE ANALYSIS BETWEEN TAQMAN™ AND AMPLICOR™ TESTS FROM TWO LARGE RANDOMIZED INTERNATIONAL TRIALS OF PEGASYS® PLUS COPEGUS®

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Background: HCV treatment is rapidly evolving from a fixed duration of peginterferon alfa-2a (40KD) plus ribavirin (RBV) (48wks for genotype [G] 1; 24wks for G2/3) to response-guided therapy (RGT), adjusting duration according to on-treatment virologic response at wk4 and wk12; rapid virologic response (RVR) and complete early virologic response (cEVR), respectively. RGT originally used sensitive PCR assays (e.g. COBAS Amplicor HCV, v2.0; detection limit 50 IU/mL). It is unclear how the more sensitive TaqMan assay (COBAS AmpliPrep/COBAS TaqMan HCV Test: detection limit 15 IU/mL) will influence RGT. We therefore reanalysed serum samples stored at −70°C from two large international studies, ACCELERATE (G2/3) and NV15942 (G1,2&3), and compared clinical outcomes when using Amplicor or TaqMan.

Methods: In ACCELERATE, TaqMan data were available from patients who had sufficient sample volume at baseline (BL; n=122) and wk4 (n=663). In NV15942, TaqMan data were available from patients who had BL, wk4, wk12, end of treatment (EOT) and end of follow-up samples (n=629). RVR rates and sustained virologic response (SVR) rates in patients with RVR were compared by Amplicor or TaqMan (HCV RNA <15 IU/mL [undetectable or below the limit of detection] or Undetectable [true negative]) in G2/3 (ACCELERATE) and G1 (NV15942) patients treated with peginterferon alfa-2a (40KD) plus RBV standard dose. cEVR (HCV RNA-negative at wk12) and relapse rates were compared by these two tests in G1 and G2/3 patients in NV15942. BL viral loads were compared by Amplicor Monitor v2.0 and TaqMan in G2/3 patients in ACCELERATE.

Results: RVR and SVR rates were similar when RVR was defined as <50 IU/mL or <15 IU/mL regardless of genotype (Table). RVR rates were slightly lower when defined as HCV RNA undetectable by TaqMan. cEVR rates were similar when cEVR was defined as <50 IU/mL or <15 IU/mL. Relapse rates were lowest when TaqMan undetectable cut-off was utilized to determine EOT response. BL viral loads were well correlated between the two tests. Conclusions: A detection limit of <15 IU/mL by TaqMan serves as a reasonable definition of RVR and cEVR, along with <50 IU/mL, for RGT, regardless of genotype. The significance of HCV RNA "undetectable" by TaqMan should be investigated in prospective trials.
1300

**PATIENTS COINFECTED WITH HCV AND HIV WHO ACHIEVE AN RVR (HCV RNA < 50 IU/ML AT WEEK 4) OR CEVR (HCV RNA < 50 IU/ML AT WEEK 12) HAVE SIMILAR RATES OF SVR TO MONOINFECTED PATIENTS TREATED WITH PEGINTERFERON ALFA-2A (40KD) (PEGASYS®) AND RIBAVIRIN (COPEGUS®)**

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**Background:** Patients coinfected with HIV-HCV represent a challenging population to treat. In the APRICOT study coinfect ed patients treated with 180 µg/wk peginterferon alfa-2a (40KD) and 800 mg/d ribavirin achieved an SVR rate of 40% (29% genotype G1; 62% G2/3). These rates compare poorly to those in monoinfected patients. In monoinfected patients serum samples collected at wks 4 and 12 are increasingly being used to guide therapy decisions (response-guided therapy). There are limited data in coinfected patients on whether early treatment responses are also useful to predict rates of SVR and how rates of SVR in early responders compare between monoinfected and coinfected patients.

**Methods:** Patients included in this analysis were all patients from the APRICOT study randomised to 180 µg/wk peginterferon alfa-2a (40KD) and 800 mg/d ribavirin with G1, 2 or 3 HCV. Rates of SVR were determined as a function of response to therapy at wk 4 and 12. RVR was defined as undetectable HCV RNA (<50 IU/ML) at wk 4; complete EVR (cEVR) was defined as non-RVR but undetectable HCV RNA (<50 IU/ML) at wk 12; partial EVR (pEVR) was defined as non-RVR but ≥2 log reduction from baseline in HCV RNA but remaining detectable HCV RNA (>50 IU/ML) at wk 12; SVR was defined as undetectable HCV RNA (<50 IU/ML) 24 wks after the end of therapy.

**Results:** Data from 271 patients were included (Table). Rates of SVR for G1 and G2/3 were greatest in patients achieving an RVR (G1 = 81.8%, G2/3 94.3%), followed by cEVR (G1 = 63.2%; G2/3 69.7%) and then pEVR. Patients not achieving an RVR or cEVR/pEVR had minimal chance of achieving an SVR. Considering only patients with pEVR, rates of achieving SVR were influenced by several baseline and treatment factors indicating that response in this category of patients is heterogeneous.

**Conclusions:** Coinfected patients who achieve an RVR have a similarly high chance as monoinfected patients of achieving an SVR irrespective of genotype. Patients that achieve cEVR also have similarly high rates of SVR as in monoinfected patients. As in monoinfected patients RVR is the strongest predictor for SVR, but in addition achieving a cEVR is also highly predictive of achieving an SVR.

**Early response category**

<table>
<thead>
<tr>
<th>SVR rate n/N (%)</th>
<th>Genotype 1 (n=176)</th>
<th>Genotype 2/3 (n=95)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RVR</td>
<td>18/22 (81.8)</td>
<td>33/35 (94.3)</td>
</tr>
<tr>
<td>cEVR</td>
<td>24/38 (63.2)</td>
<td>23/35 (66.7)</td>
</tr>
<tr>
<td>pEVR</td>
<td>8/46 (17.4)</td>
<td>2/11 (18.2)</td>
</tr>
</tbody>
</table>

**Background:** The rate and extent of virological response to therapy in patients infected with HCV genotypes (G) 1 or 4 is highly variable. Retrospective analyses show that patients treated with peginterferon alfa-2a (40KD) plus ribavirin (RBV) who achieve an RVR (HCV RNA < 50 IU/ML at wk 4) may achieve an SVR after only 24 wks, while patients with a slower response may benefit from prolonged therapy. This prospective study investigated response-guided therapy of Peg-IFN alfa-2a (40KD) plus RBV in G1 and 4 patients based on RNA level at wk 4 & 12. We aimed to evaluate whether patients with RVR, EVR (HCV RNA < 600 IU/ML) have a higher chance of achieving an SVR.

**Methods:** Treatment-naive G1 and 4 chronic hepatitis C patients were treated with Peg-IFN alfa-2a (40KD) 180 µg/wk plus RBV 1000/1200 mg/day prior to allocation to one of four arms based on RNA tests at wk 4 & 12. At wk 4, patients with an RVR were assigned to a further 20 wks of therapy (Arm D). All other patients continued to receive treatment until wk 12 when RNA was retested. Patients with an EVR were randomized to a total of 48 (Arm A) or 72 wks (Arm B) of treatment. Wk 12 NRs
were offered to continue treatment for a total of 72 wks (Arm C). Treatment was stopped if RNA remained detectable at wk 24. Results: Of the 580 patients screened 510 patients (G1=443; G4=67) received treatment and were evaluable. Of these 121/443 (27.3%) G1 patients and 29/67 (43.3%) G4 patients had an RVR and were allocated to Arm D. RVR was associated with younger age, lower body weight, lower BL viral load, and G4. By multiple logistic regression analysis viral load (high vs. low OR: 0.26 [95% CI:0.16–0.41]) and genotype (1 vs. 4: OR: 0.29 [95% CI:0.16–0.56]), body weight and ALT but not fibrosis were significantly associated with not achieving an RVR. The outcome of patients with RVR is shown in the table. Conclusion: More patients with G4 than with G1 achieved an RVR. Both G1 and G4 patients with RVR achieved high rates of SVR with 24 wks of treatment. These rates are similar to rates of SVR in G1 patients achieving an RVR with 48 wks of therapy. We confirm that a substantial proportion of patients with G4 and G1 benefit from shortening therapy to 24 wks. Here we show that RVR is predictive of SVR in G4 patients and that G4 patients with an RVR have an even greater chance of achieving an SVR compared to G1 patients.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Pts with RVR (n,%</th>
<th>Per protocol SVR rate in pts with an RVR (%)</th>
<th>Per protocol relapse rate in pts with an RVR (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>12/104 (27.9)</td>
<td>90/103 (87.4%)</td>
<td>13/103 (12.6%)</td>
</tr>
<tr>
<td>4</td>
<td>28/67 (41.8)</td>
<td>25/26 (96.2%)</td>
<td>1/26 (3.8%)</td>
</tr>
</tbody>
</table>

Disclosures: Peter Ferenci - Grant/Research Support: Roche; Consultant/Adviser: Roche; Speaker's Bureau: Roche; Harald Brunner - Speaker's Bureau: Roche; Michael Ochswein: Speaker's Bureau: Roche; Karin Loschenberger - Employee: Roche; The following people have nothing to disclose: Hermann Laferl, Thomas-Matthias Scherzer, Andreas Maierson, Rudolf E. Stauber, Rainer Hubmann, Katharina Stauffer, Christian Datz, Martin Bischof, Petra E. Steinild-Munda, the Austrian Hepatitis Study Group On behalf of

1302 PHASE 1 EVALUATION OF ANTIVIRAL ACTIVITY OF THE NON-NUCLEOSIDE POLYMERASE INHIBITOR, HCV-796, IN COMBINATION WITH DIFFERENT PEGYLATED INTERFERONS IN TREATMENT-NAIVE PATIENTS WITH CHRONIC HCV

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Background: HCV-796 is an inhibitor of hepatitis C virus (HCV) RNA-dependent RNA polymerase that has demonstrated clinical antiviral activity across multiple HCV genotypes when administered as monotherapy or in combination with pegylated interferon alfa-2b (PEG2b). We further evaluated HCV-796 when administered with pegylated interferon alfa-2a (PEG2a). Methods: Evaluations were performed within a randomized, double-blind, Phase 1 study in adult patients with chronic HCV infection who were naive to therapy. In one group, patients were randomized to receive oral HCV-796 or placebo Q12h for 14 days, and all were to receive PEG2b (1.5 mcg/kg) on day -1 (one day before start of HCV-796/placebo) and day 7. In another group, the design was the same except the PEG therapy was PEG 2a (180 mcg) on day -1 and day 7. In each group 12-16 patients were to receive the active HCV-796 (500 mg Q12h) with one of the PEG therapies. Results: The median baseline HCV RNA level was 6.46±.3 log10 in each group and 71% of patients were infected with HCV genotype 1. For both PEG therapies, combination with HCV-796 reduced plasma HCV RNA levels to a greater extent than either PEG alone. At day 14, the mean reduction in HCV RNA for HCV-796+PEG2b was 3.4 log10 vs. 1.6 log10 for PEG2b alone. The mean reduction for HCV-796+PEG2a was 3.7 log10 vs. 1.1 log10 for PEG2a alone. For both groups, activity differed by HCV genotype. Mean HCV RNA reductions at day 14 for genotype 1 was 2.9 log10 for HCV-796+PEG2b and 3.2 log10 for HCV-796+PEG2a. For genotype non-1 the respective reductions were 4.4 vs. 4.7 log10. Combination of HCV-796 with either PEG2b or PEG2a provides similar antiviral activity across multiple HCV genotypes over 14 days of therapy. Results support clinical studies of more long-term administration of HCV-796 with either PEG therapy.

Disclosures: Stephen Villano - Employee: Other; Donald Raible - Employee: Wyeth; Dawn Harper - Employee: Wyeth; Priyamvada Chandra - Employee: Wyeth

The following people have nothing to disclose: Linda Bazisotto, Geraldine Bichier

1303 PEGINTERFERON ALFA-2A AND RIBAVIRIN FOR 12 OR 24 WEEKS IN PATIENTS WITH HCV GENOTYPE 2/3: THE NORDYNAMIC TRIAL

Martin Lagging1, Court Pedersen2, Mads Rauning Buhl3, Martti Färkkilä4, Nina Langeland1, Kristine March2, Johan Westin1, Åsa Alsiö1, Gunnar Norkrans1, Daniel Färkkilä1, Nina Langeland1, Kristine March2, Johan Westin1, Åsa Alsiö1, Gunnar Norkrans1

Background and Aims: Prior trials investigating the efficacy of treatment for less than 24 weeks in HCV genotype 2/3 infected patients have yielded discordant results. The aim of this study was to compare the efficacy of 12 or 24 weeks of combination therapy, as well as to identify patients achieving SVR using liver biopsy evaluation. IP-10 levels, α-interferon concentrations, ribavirin concentrations, and HCV RNA measurement using Roche COBAS TaqMan at baseline, day 3, day 7, day 8, week 4, and week 8. Methods: Three hundred and eighty-two genotype 2/3 infected patients at 31 centers in Denmark, Finland, Norway, and Sweden were randomized at baseline to 12 or 24 weeks of treatment with peginterferon α-2a 180 μg/week plus ribavirin 800 mg/day from February 2004 to November 2005. Results: Twelve weeks of combination therapy was inferior to 24 weeks for patients infected with genotype 2 (SVR rates 56% vs. 82%, p=0.007) and genotype 3 (58% vs. 78%, p=0.001). Likewise, 12 weeks of treatment was inferior for patients with non-significant fibrosis (p=0.024) and bridging fibrosis (p=0.003), and a similar trend was noted for patients with cirrhosis (p=0.078). Multivariate analysis demonstrated that age as well as HCV-RNA levels on day 7 and 29 were independent predictors of SVR following 12 weeks of therapy. For patients <40 years, no significant difference was noted between 12 and 24 weeks of therapy regardless of HCV-RNA level day 29. Similarly, for patients ≥40 years, no significant difference was noted between the treatment arms if both HCV-RNA day 7 was below 1,000 IU/mL and HCV-RNA was undetectable day 29. If both of these two criteria were not met for
patients ≥40 years, 24 weeks of therapy was superior (p<0.0001). Conclusion: Our findings indicate that 12 weeks of combination therapy may be acceptable for subgroups of, not just for all patients infected with HCV genotypes 2 or 3.

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**Outcome Grouped by Genotype**

![Graph showing outcome grouped by genotype](image)

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Disclosures:
The following people have nothing to disclose: Martin Lagging, Court Pedersen, Mads Rauing Buhl, Martti Farkkila, Nina Langeland, Kristine Merch, Johan Westin, Åsa Alsiö, Gunnar Norkrans

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**1304**

**HCV-SPECIFIC CELLULAR IMMUNITY, RNA REDUCTIONS, AND NORMALIZATION OF ALT IN CHRONIC HCV SUBJECTS AFTER TREATMENT WITH GI-5005, A YEAST-BASED IMMUNOTHERAPY TARGETING NS3 AND CORE: A RANDOMIZED, DOUBLE-BLIND, PLACEBO CONTROLLED PHASE 1B STUDY**

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**PURPOSE**: Evaluation of the efficacy, immunogenicity, and safety of GI-5005 in subjects with chronic HCV infection.

**METHODS**: GI-5005 is a whole heat-inactivated S. cerevisiae immunotherapy expressing HCV NS3 and Core. Subjects with chronic HCV infection who were interferon (IFN) partial responders, relapsers, or treatment naive were eligible. Five weekly subcutaneous (SC) doses of GI-5005 monotherapy over 29 days were followed by two monthly SC GI-5005 doses and 9 months of post-treatment follow-up. Dose groups of 0.05, 0.5, 2.5, 10, and 40 YU (1 YU = 10,000,000 yeast cells) were randomized 3:1 treated:placebo. RESULTS: Dose escalation was completed without a product-related dose limiting toxicity, serious adverse event, or exacerbation of hepatitis (66 subjects total; 6 in 0.05YU, 6 in 0.5YU, 7 in 2.5YU, 12 in 10.0YU, 11 in 20YU, 7 in 40YU, and 17 in placebo). HCV-specific cellular immune responses with broad epitope coverage were observed by ELISPOT assay in 33% of treated subjects (8/24) to date with a dose response for GI-5005, compared to no responses in the placebo group. Statistically significant improvements in ALT levels were observed in treated subjects with a mean maximum decrease from baseline of 29.3% versus 16.9% for placebo (47 treated versus 17 placebo respectively, p=0.02). Three of the 7 (43%) 20YU patients with abnormal ALT at baseline had normalized their ALT after treatment (> 2 consecutive visits WNL) compared to 0 of 29 treated subjects (14%) from the first 4 dose groups combined, and none of the placebo patients. Six subjects of 47 treated overall (13%) achieved a viral load nadir ranging from -0.75 to -1.04 log10 change from baseline with the 2 greatest reductions (2/11 subjects, 18%) observed in the 20YU group (-0.93 log10 and -1.04 log10). In several cases these viral load reductions persisted for months and in one case for the full 9 month post-treatment follow-up period. No HCV RNA reductions in -0.75 log10 to -1 log10 range were observed in the placebo group. CONCLUSIONS: GI-5005 was well tolerated and generated HCV-specific cellular immune responses with associated HCV RNA reductions and ALT normalizations in a subset of patients, which were not observed in the placebo group. These data support further development of GI-5005 in combination with IFN-based standard of care, or as a salvage treatment in patients who are intolerant or who fail IFN-based regimens. Future evaluation of combination of GI-5005 with small molecule inhibitors of HCV replication is also warranted based on its complementary immune mechanism of action.

Disclosures:
Eugene R. Schiff - Consultant/Adviser: Abbott; Consultant/Adviser: Achillion; Consultant/Adviser: Bayer; Consultant/Adviser: Bristol-Myers Squibb; Consultant/Adviser: Gilead; Consultant/Adviser: Indenix; Consultant/Adviser: Novartis; Consultant/Adviser: Ortho; Consultant/Adviser: Pfizer; Consultant/Adviser: Roche
Gregory T. Everson - Grant/Research Support: Roche; Consultant/Adviser: Roche; Speakers Bureau: Roche; Speakers Bureau: Ortho; Grant/Research Support: Roche; Consultant/Adviser: Gilead; Speakers Bureau: Gilead; Speakers Bureau: Ortho
Naoky Tsai - Grant/Research Support: Gilead; Grant/Research Support: Roche; Speakers Bureau: Roche; Speakers Bureau: Gilead; Speakers Bureau: Gilead; Speakers Bureau: Gilead; Grant/Research Support: Bristol-Myers Squibb; Grant/Research Support: Roche; Consultant/Adviser: GlaxoSmithKline; Consultant/Adviser: Valeant
Nathalie H. Bzowy - Grant/Research Support: Roche; Consultant/Adviser: Gilead; Grant/Research Support: Roche; Consultant/Adviser: Gilead; Consultant/Adviser: GlaxoSmithKline; Consultant/Adviser: Roche; Consultant/Adviser: Human Genome Sciences; Consultant/Adviser: Novartis; Consultant/Adviser: Ortho; Consultant/Adviser: Schering-Plough; Consultant/Adviser: SciClone; Consultant/Adviser: Valeant
John G. McHutchison - Grant/Research Support: Other
Ira M. Jacobson - Grant/Research Support: Other
Myron J. Tong - Grant/Research Support: Other
Donald M. Jensen - Grant/Research Support: Other
Georg M. Lauer - Grant/Research Support: Other
Scott Cruickshank - Consultant/Adviser: Other
John Ferraro - Employee: Other
Aurelia Haller - Employee: Other
Richard Duke - Major Stockholder: Other; Employee: Other
Timothy Rodell - Employee: Other; Major Stockholder: Other
David Apelian - Employee: Other; Major Stockholder: Other
ASSOCIATION OF PRE-TREATMENT AND ON-TREATMENT FACTORS WITH RAPID VIROLOGIC RESPONSE IN HCV GENOTYPE 1 INFECTED PATIENTS TREATED WITH PEGIFNα-2A/RBV

Maribel Rodriguez-Torres1, Mark Sulikowski2, Raymond T. Chung3, Fayezy Hamzeh4, Donald M. Jensen5; 1Fundacion Gastroenterologia de Diego, Santurce, PR; 2Johns Hopkins University, Baltimore, MD; 3Massachusetts General Hospital, Boston, MA; 4Roche Laboratories, Nutley, NJ; 5University of Chicago, Chicago, IL

Introduction Rapid virologic response (RVR), defined as undetectable serum HCV RNA after 4 weeks of treatment, is associated with likelihood of sustained virologic response. This retrospective analysis assessed the effect of baseline and demographic factors and drug dose/modification during the first 4 weeks of treatment on RVR. Methods Patients in 5 clinical trials who were infected with HCV genotype 1 and randomized to 180 µg/wk pegIFNα-2a/1000-1200 mg/d RBV were included. Baseline factors utilized for multiple logistic regression analyses included age (<40 vs >40 years), gender, race/ethnicity (non-Latino white vs other), BMI (<27 vs >27 kg/m²), baseline ALT quotient (<3 vs >3X ULN), baseline serum HCV RNA (<400,000 vs >400,000 IU/mL) and cirrhotic classification (cirrhotic vs non-cirrhotic). On-treatment factors included average daily exposure to RBV (<13 vs >13 mg/kg/day) and pegIFNα-2a dose reductions (yes vs no). Any factor with p≤0.2 was considered significant. Results Of 1550 treated patients, 234 (15.1%) attained RVR and 1316 (84.9%) did not; 1 (0.4%) and 16 (1.2%), respectively, withdrew for safety reasons, and 0 and 1 (0.1%), respectively, for non-safety reasons. Of these 1550 patients, 1050 (67.7%) were non-Latino whites, 295 (19.0%) were Latino whites, 154 (9.9%) were black, and 51 (3.3%) were Other; 1031 (66.5%) were men; 1112 (71.7%) were >40 years old; 797 (51.4%) had BMI >27 kg/m²; 1184 (76.4%) had ALT quotient >3X ULN; 1346 (86.8%) had serum HCV RNA >400,000 U/mL; and 239 (16.4%) were cirrhotic. Multiple logistic regression analysis showed that white non-Latino race (OR 1.48; 95% CI 1.04-2.12, p=.031), age ≤40 years (OR 1.63; 95% CI 1.27-2.26, p<.0035), baseline ALT quotient >3X ULN (OR 1.96; 95% CI 1.40-2.76, p<.0001), baseline serum HCV RNA >400,000 IU/mL (OR 8.67; 95% CI 6.13-12.37, p<.0001), cirrhotic status at baseline (OR 1.63; 95% CI 1.01-2.64, p=.0444), male sex (OR 1.37; 95% CI 0.98-1.91, p=.0687) and BMI ≤27 kg/m² (OR 1.37; 95% CI 0.99-1.91, p=.0545) were predictive of RVR. After adjusting for significant risk factors, multiple logistic regression analysis showed that average daily RBV >13 mg/kg/day (OR 2.15; 95% CI 1.41-3.37, p<.0004) was a significant predictor of RVR, whereas pegIFNα-2a dose reduction was not. Conclusion RVR in patients infected with HCV genotype 1 was associated with younger age, white race, higher ALT quotient, lower serum HCV RNA, absence of cirrhosis, male sex and lower BMI, and with greater exposure to RBV over the initial 4 weeks. Patients exposed to ≤13 mg/kg/day RBV over the first 4 weeks were less likely to achieve RVR.

Disclosures:
Maribel Rodriguez-Torres - Consultant/Adviser: Indenix; Consultant/Adviser: Roche; Consultant/Adviser: Valeant; Grant/Research Support: Other; Grant/Research Support: Bristol-Myers Squibb; Grant/Research Support: GlaxoSmithKline; Grant/Research Support: Novartis; Grant/Research Support: Schering-Plough
Mark Sulikowski - Grant/Research Support: Roche; Speakers Bureau: Roche; Consultant/Adviser: Roche
Raymond T. Chung - Employee: Roche
Fayezy Hamzeh - Employee: Roche

TREATMENT OF PEGINTERFERON/RIBAVIRIN NONRESPONDERS WITH DAILY DOSING OF CONSENSUS INTERFERON AND RIBAVIRIN - PRELIMINARY RESULTS OF THE GERMAN CONSENSUS INTERFERON MULTI-CENTER STUDY

Stephan Kaiser1, Wulf Boecker2, Joerg F. Schlaak3, Bettina Lutze1, Birgit Sauter1, Lennart Bissinger1, Christoph Werner4, Holger Hass2, Michael Gregor1; 1Dept. of Medicine I, University Hospital of Tuebingen, Tuebingen, Germany; 2Medicine, Marienhospital, Stuttgart, Germany; 3Medicine, University Hospital of Essen, Essen, Germany; 4Medicine, University Hospital of Mainz, Mainz, Germany

Objective: Current standard treatment with pegylated interferon (PEG IFN) and ribavirin (RBV) in genotype 1 patients shows sustained response rates of 31 – 47%, thus leaving more than half of the patients with a relapse or nonresponse to . Recently improved response rates have been observed in pilot trials using consensus interferon (CIFN) in combination therapy in PEG IFN / RBV nonresponders. Methods: The efficacy of CIFN daily dosing therapy compared to CIFN / RBV in PEG IFN combination treatment nonresponders was evaluated. 400 patients have been included, with 92% having genotype 1. Average weight of patients was 79.3 kg. Patients were either treated with CIFN at 9 ug QD for 16 weeks or with CIFN 27 ug QD for 4 weeks, followed by 12 weeks of CIFN 18 ug QD. Thereafter, treatment was continued in all treatment groups with CIFN at 9 ug QD with weight-based RBV for 32 - 56 weeks, depending when a patient became first PCR negative, ensuring a treatment period for 48 weeks with a negative PCR. Results: Preliminary data show that after the initial 12 weeks of CIFN monotherapy, a primary response with undetectable serum HCV-RNA was observed in 37 % of patients with a prior nonresponse to PEG IFN α2b and in 48% in prior PEG IFN α2a nonresponders (n=189). At the end of treatment, a negative PCR was observed in 34% in PEG IFN α2b nonresponders, and in 48% of PEG IFN α2a nonresponders. The sustained viral response rates [SVR] were 16% and 23% for PEG IFN α2b and PEG α2a nonresponders, respectively (n=143). When response rates were calculated according to the treatment arm used, the SVR for PEG IFN α2b nonresponders were 13% in the CIFN 9 ug arm and 19% in the CIFN high dose arm. For PEG IFN α2a nonresponders the SVR were 19% and 27% for the CIFN 9 ug dose and high dose arms, respectively. The overall tolerability of the CIFN 9 ug regimen was comparable to a standard therapy with pegylated IFN and RBV, while the CIFN 27/18/9 ug regimen was less tolerable during the high dose induction period. However, drop out rates were not different between the two dosing regimens. Conclusions: CIFN daily dosing / induction therapy together with subsequent RBV combination therapy thus shows promising response rates in previous PEG IFN combination therapy non-responders. Especially PEG IFN α2a nonresponders appear to have a benefit from CIFN QD retreatment. It is concluded that CIFN may be an effective treatment modality for this difficult-to-treat patient group.

Disclosures:
Stephan Kaiser - Speakers Bureau: Valeant; Grant/Research Support: Bristol-Myers Squibb; Grant/Research Support: Gilead; Grant/Research Support: GlaxoSmithKline; Grant/Research Support: Novartis; Grant/Research Support: Roche; Consultant/Adviser: Roche; Consultant/Adviser: Schering-Plough
The following people have nothing to disclose: Wulf Boecker, Joerg F. Schlaak, Bettina Lutze, Birgit Sauter, Lennart Bissinger, Christoph Werner, Holger Hass, Michael Gregor
PF-03491390 inhibits liver fibrosis in patients with chronic hepatitis C virus infection via suppression of pro-apoptotic caspase-activation.

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Background: In patients with chronic HCV infection, the pan-caspase inhibitor, PF-03491390 minimises hepatocellular damage, as reflected by reductions in elevated ALT and AST levels. As caspase activation plays a pivotal role in inflammatory and fibrotic liver injury in HCV-infection, we investigated caspase activation and a range of inflammatory and fibrotic serum biomarkers during therapy with this agent. Methods: 204 patients with chronic HCV infection and liver fibrosis were randomized to receive placebo or PF-03491390 5 mg, 25 mg, or 50 mg orally twice daily for 12 weeks in a placebo-controlled, double-blind, parallel-group study. If ALT and AST levels remained elevated at Week 10, the dose of study drug was doubled to Week 12. Changes in serum markers of inflammation, fibrosis and apoptosis are reported. Results: At Week 12, compared with placebo, PF-03491390 therapy was associated with decreases in serum levels of transforming growth factor (TGF) β1, α-2 macroglobulin, caspase-mediated cytokeratin-18 fragments (M30-Antigen), active caspases 3/7 and α-fetoprotein. PF-03491390 therapy was also associated with increases in serum levels of haptoglobin and Fas ligand, at Week 12, compared with placebo. PF-03491390 therapy had no apparent impact on serum levels of the other measured biomarkers (Table 1). Conclusions: In patients with chronic HCV infection, 12 weeks of PF-03491390 therapy appears to inhibit liver inflammation and fibrosis via suppression of pro-apoptotic caspase-activation. Long-term studies are required to assess the mechanism of action and effects of PF-03491390 in patients with liver fibrosis.

Table 1: Median absolute change of serum markers of inflammation, fibrosis and apoptosis from baseline at Week 12

<table>
<thead>
<tr>
<th>Serum marker</th>
<th>Placebo</th>
<th>PF-03491390</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5 mg bid</td>
<td>25 mg bid</td>
</tr>
<tr>
<td>Fibrosis</td>
<td>N=47.50</td>
<td>N=49.53</td>
</tr>
<tr>
<td>M30-Antigen (U/L)</td>
<td>-17.0</td>
<td>-11.45</td>
</tr>
<tr>
<td>Active caspases 3/7 (RU)</td>
<td>-46.0</td>
<td>-39.20</td>
</tr>
<tr>
<td>TGFβ1 (ng/ml)</td>
<td>0.22</td>
<td>-0.95</td>
</tr>
<tr>
<td>α-2-macroglobulin (mg/dl)</td>
<td>2.00</td>
<td>-4.00</td>
</tr>
<tr>
<td>Haptoglobin (mg/dl)</td>
<td>-0.50</td>
<td>9.00</td>
</tr>
<tr>
<td>Apolipoprotein A1 (mg/dl)</td>
<td>0.50</td>
<td>-3.00</td>
</tr>
<tr>
<td>Inflammation</td>
<td>N=48.51</td>
<td>N=49.55</td>
</tr>
<tr>
<td>TNFα receptor II (pg/ml)</td>
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<td>0.10</td>
</tr>
<tr>
<td>C-reactive protein (mg/dl)</td>
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<td>0.00</td>
</tr>
<tr>
<td>α-fetoprotein (ng/ml)</td>
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<td>-0.60</td>
</tr>
<tr>
<td>Interleukin-6 (pg/ml)</td>
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<td>0.20</td>
</tr>
<tr>
<td>Interleukin-8 (pg/ml)</td>
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<td>0.00</td>
</tr>
<tr>
<td>Fas ligand (pg/ml)</td>
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<td>2.10</td>
</tr>
<tr>
<td>Mechanism of Action</td>
<td>N=25</td>
<td>N=28</td>
</tr>
<tr>
<td>Interferon 1β (pg/ml)</td>
<td>0.00</td>
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</table>

Disclosures:
Gary Burgess - Employee: Pfizer
Peter Colman - Employee: Pfizer
Emanuel Engmann - Employee: Pfizer

PF-03491390 differentiates of early virologic response (EVR) into RVR, complete EVR (CEVR) and partial EVR (PEVR) allows for a more precise prediction of SVR in HCV genotype 1 patients treated with peginterferon alfa-2A (40KD) (Pegasys®) and ribavirin (Copegus®).

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Background: Early on-treatment responses in HCV RNA at wks 4 & 12 post initiation of therapy are increasingly being used to predict those pts likely to achieve an SVR. Pts achieving an RVR (HCV RNA <50 IU/mL at wk 4 and maintained at wk 12) have a high rate of SVR irrespective of genotype. The standard definition of an early virologic response (EVR) had been defined as pts at wk 12 achieving either an undetectable HCV RNA (<50 IU/mL) or a ≥2 log drop in HCV RNA but still detectable. However, rates of SVR in pts achieving an EVR by this definition are heterogeneous. By further subdividing pts achieving early responses into RVR, complete EVR (cEVR or non-RVR but HCV RNA <50 IU/mL at wk 12) and partial EVR (pEVR or non-RVR but HCV RNA ≥2 log drop in HCV RNA at wk 12 but still detectable) it may be possible to improve the prediction of pts likely to achieve an SVR and may allow for tailoring of treatment duration. Here we performed a retrospective analysis of 2 large, multinational phase III studies of genotype 1 pts treated with peginterferon alfa2a (40KD) in combination with ribavirin (RBV) (Fried et al. NEJM 2002 & Hadziyannis et al. Ann Intern Med 2004). Methods: 569 pts treated for 48 wks with 180 µg/wk peginterferon alfa2a (40KD) and 1000/1200 mg/d RBV were included in the present analysis (ITT). Early responses were divided into 4 mutually exclusive categories as defined above: RVR, cEVR, pEVR and non-EVR (<2 log drop at wk 12). Rates of SVR were then calculated for each category. Results: 16% (90/569) pts were classified as achieving an RVR, 42% (240/569) pts a cEVR, 22% (128/569) pts a pEVR and 20% (111/569) non-EVR. Rates of achieving an SVR in these groups were 87% (78/90) for RVR, complete cEVR, 22% (128/569) pts a pEVR and 20% (111/569) non-EVR pts. Conclusions: Pts achieving an RVR have high rates of SVR and may benefit from shortened treatment duration (24 wks; also see Jensen et al. Hepatol 2006). Pts with a cEVR also have high rates of SVR but should be encouraged to remain on therapy for the standard duration of therapy (48 wks). Pts with a pEVR have lower rates of SVR with the standard 48 wks of therapy and may benefit from intensified treatment (72 wks; also see Sánchez-Tapias et al. Gastroenterol 2006) and pts that are non-EVR at wk 12 have a low chance of achieving an SVR and consideration should be given to change treatment strategy. Early on-treatment virologic responses in HCV RNA are highly predictive of achieving an SVR and subdividing early responses into RVR, cEVR and pEVR allows for a more precise prediction of achieving an SVR.

Disclosures:
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Donald M. Jensen - Speakers Bureau: Roche; Consultant/Adviser: Roche
Stephanos J. Hadziyannis - Consultant/Adviser: Roche; Speakers Bureau: Roche
1309 FINAL RESULTS OF PATIENTS RECEIVING PEG-INTERFERON-ALFA-2A (PEG-IFN) AND RIBAVIRIN (RBV) AFTER A 14-DAY STUDY OF THE HEPATITIS C PROTEASE INHIBITOR TELAPREVIR (VX-950), WITH PEG-IFN
Christine J. Weegink1, Nicole Forestier2,3, Peter L. Jansen1, Stefan Zeuzem2,3, Henk W. Reesink1; 1Academic Medical Center, Amsterdam, Netherlands; 2J.W.Goethe University Hospital, Frankfurt a.M., Germany; 3Saarland University Hospital, Homburg/Saar, Germany

Purpose: Telaprevir (TVR, VX-950) is a highly-selective peptidomimetic inhibitor of the hepatitis C virus (HCV) NS3/4A protease that is designed to block HCV replication. This 14-day study was designed to explore the viral kinetics and safety during dosing with TVR in combination with peginterferon-alfa-2a (Peg-IFN). Here we report the final results of patient status after stopping follow-on standard therapy with Peg-IFN and ribavirin (RBV). Methods: The VX04-950-103 clinical study randomized twenty treatment-naïve patients with chronic genotype 1 hepatitis C infection to three dosing arms. Eight patients received TVR (750 mg as tablets q8h) with Peg-IFN on Days 1 and 8, and eight patients received TVR alone. Four patients received Peg-IFN alone on Days 1 and 8. At the completion of the 14-day study, off-study therapy with Peg-IFN and RBV was offered to all patients. Nineteen of 20 patients began therapy within 5 days of completing the 14-day dosing period. The patient who refused post-study Peg-IFN/RBV was in the TVR-alone group. Results: At week 12 of therapy, all 8 patients in the TVR/Peg-IFN group and 5 of 7 patients in the TVR alone group had undetectable HCV RNA. At week 24, all 15 patients who received TVR had undetectable HCV RNA (<10 IU/mL). Ten patients (6/8 TVR/Peg-IFN and 4/7 TVR alone) chose to stop Peg-IFN/RBV treatment at week 24 and 5 patients chose to continue Peg-IFN/RBV for a total of 48 weeks. All groups were followed for the subsequent 24 weeks. In patients who had received TVR-based therapy for 14 days before starting off-study Peg-IFN/RBV therapy, 7/10 patients treated for a total of 24 weeks and 2/5 patients treated for a total of 48 weeks achieved a sustained viral response (SVR) One patient, treated for 48 weeks, was lost to follow-up. From the group who received Peg-IFN alone before 48 weeks of Peg-IFN/RBV therapy, 1/4 patients achieved SVR. The side effect profile observed during the post-study dosing was consistent with the expected profile of Peg-IFN/RBV therapy. Sequence analysis of the 5 patients who relapsed after TVR-based therapy is in progress. Conclusions: SVR was achieved in 9 of 15 patients treated for 14 days with TVR or TVR/Peg IFN followed by Peg-IFN/RBV therapy for a total of 24 or 48 weeks. These results suggest that TVR-based regimens may increase SVR rates compared to current therapies. Large Phase 2 clinical studies of TVR-based regimens are now ongoing to evaluate this hypothesis and the possibility of shortening the duration of therapy.

Disclosures: The following people have nothing to disclose: Christine J. Weegink, Nicole Forestier, Peter L. Jansen, Stefan Zeuzem, Henk W. Reesink

1310 RETREATMENT OF HCV GENOTYPE 1 RELAPSE PATIENTS TO PEGINTERFERON/RIBAVIRIN THERAPY WITH AN EXTENDED TREATMENT REGIMEN OF 72 WEEKS WITH CONSENSUS INTERFERON/RIBAVIRIN VERSUS PEGINTERFERON ALFA/RIBAVIRIN
Stephan Kaiser1, Bettina Lutze1, Birgit Sauter1, Lennart Bissinger1, Christoph Werner1, Holger Hass2, Michael Gregor1; 1Dept. of Medicine I, University Hospital of Tuebingen, Tuebingen, Germany; 2Medicine, Marien hospital, Stuttgart, Germany

Objective: Treatment with pegylated interferon and RBV for 48 weeks in naive chronic Hepatitis C patients results in relapse rates of about 20–30%. Recently improved response rates have been observed in treatment-naive patients with a slow viral response as well as in retreatment trials using an extended treatment duration of 72 weeks. However, the optimal retreatment regimen and treatment time remains unclear at present. Methods: The efficacy of CIFN daily dosing + RBV versus PEG IFN a2a + RBV for 72 weeks in patients with a prior relapse to 48 weeks of treatment with PEG IFN + RBV was evaluated. 120 patients with genotype 1. Average weight of patients was 79 kg. Patients were either treated with CIFN at 9 ug QD for 72 weeks or with PEG IFN a2a at 180 ug QW for 72 weeks, both in combination with weight-based RBV. Results: Data show that after the initial 12 weeks a primary response with undetectable serum HCV RNA was observed in 85% of patients in the CIFN QD group and in 81% in the PEG IFN 180 ug group (diff. = n.s.). At the end of treatment at week 72, a negative PCR was observed in 86% in the CIFN group, and in 78% of the PEG IFN 180 ug group (diff. = n.s.). The sustained viral response rates (SVR) were 69% for the CIFN arm and 42% for the PEG IFN a2a arm, respectively (diff. = 0.5), indicating a significantly higher relapse rate in patients being retreated with PEG IFN a2a. No growth factors were used in this study. 5 patients experienced grade III thrombocytopenias, while no grade IV neutropenias or thrombocytopenias were observed. The overall tolerability of the CIFN QD regimen was comparable to the PEG IFN a2a therapy, while the CIFN QD regimen lead to a higher rate of injection site reactions and a slightly higher drop out rate of 19% versus 11% for the PEG IFN a2a group. In contrast, hematologic grade III alterations were higher in the PEG IFN a2a group. Conclusions: Both extended CIFN daily dosing combination therapy and PEG IFN a2a combination therapy for 72 weeks show promising response and SVR rates in previous relapse patients to standard PEG IFN / RBV therapy, while relapse rates are significantly lower in the CIFN retreated patients leading finally to higher SVR rates. Although a significant proportion of patients experienced a second relapse in both treatment regimens after cessation of therapy, the overall sustained response rates are nevertheless promising showing a SVR in up to 70% of patients. It is concluded that extended treatment especially with CIFN in combination with RBV may be an effective treatment modality for this difficult-to-treat patient group.

Disclosures: The following people have nothing to disclose: Bettina Lutze, Birgit Sauter, Lennart Bissinger, Christoph Werner, Holger Hass, Michael Gregor
1311
LONG-TERM LOW DOSE TREATMENT WITH PEGYLATED INTERFERON ALPHA 2B LEADS TO A SIGNIFICANT REDUCTION IN FIBROSIS AND INFLAMMATORY SCORE IN CHRONIC HEPATITIS C NONRESPONDER PATIENTS WITH FIBROSIS OR CIRRHOSIS
Stephan Kaiser1, Bettina Lutze1, Birgit Sauter1, Lennart Bissinger1, Christoph Werner1, Holger Hass2, Michael Gregory1; 1Dept. of Medicine I, University Hospital of Tuebingen, Tuebingen, Germany; 2Medicine, Marienhospital, Stuttgart, Germany

Objective: Treatment with current standard antiviral therapy leaves about 50% of patients without viral clearance with the risk of progression of their liver disease. Recent studies have suggested an antifibrotic effect of low dose interferon treatment. Methods: The efficacy of low dose pegylated interferon alfa 2b with 0.5 ug/kg weekly given for 36 months as monotherapy was evaluated based on histological examination and liver function in 182 patients with chronic HCV, nonresponse to antiviral combination therapy and significant fibrosis / cirrhosis (Ishak staging 3-6) and compared to an observational control group (n=83). Histology was evaluated at baseline, at 18 months of treatment and 6 months after end of treatment. Results: At 18 months therapy an increase in fibrosis score from 3.71 to 4.17 and at 6 months post observation to 4.79 was detectable in the control group (n=78). In the treatment group a decrease from 3.83 at baseline to 2.51 at 18 months and 2.05 at 6 months post therapy was noted (n=167). The necroinflammatory score showed constant levels with 7.89 at baseline, 7.56 at month 18 and 7.73 at 6 months post observation in the control group. In the treatment group the score decreased from 8.61 at baseline to 5.89 at month 18 and then relapsed again to 7.54 post therapy. 61% of patients in the treatment group showed an HCV viral load decline of > 1 log, and 6% had a negative PCR, which however was not maintained for any patient upon cessation of therapy. The drop out rate was 3% and the dose reduction rate was 13%. 22 SAEs were observed, of which 17 were complications of cirrhosis (6 hydropic decompensations, 6 variceal bleeds and 5 HCC development). There was no significant difference of these complications between treatment and observational groups. Conclusions: Low dose therapy with pegylated interferon alfa 2b in patients with HCV and advanced fibrosis or cirrhosis shows a significant and persistent decrease in fibrosis in comparison to a control group. In contrast the also observed significant decrease in the necroinflammatory score is only temporary as long as treatment lasts. As treatment was well tolerated even for patients with cirrhosis, this treatment could evolve as a salvage therapy for patients with advanced liver disease with HCV where standard antiviral therapy has failed.

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1312
CORRELATION BETWEEN POLYMORPHISMS OF NS5B AND EARLY CLEARANCE OF HEPATITIS C VIRUS BY PEG-INTERFERON PLUS RIBAVIRIN TREATMENT
Mitsuyasu Nakamura1,2, Hidetsugu Saito2, Naoki Kumagai4, Shinichiro Tada2, Masanori Ikeda2, Nobuyuki Kato3, Toshifumi Hibi2, Soichiro Murai1; 1Internal Medicine, National Defence Medical College, Tokorozawa, Japan; 2Internal medicine, Keio University Scool of Medicine, Tokyo, Japan; 3Molecular Biology, Okayama University School of Medicine and Dentistry, Okayama, Japan; 4Research Center for Liver Diseases, Kitasato, Tokyo, Japan

[Purpose] We previously reported the pilot study indicating relationship between the viral RNA polymerase polymorphisms and the initial decline in viral load induced by IFN-a and ribavirin (RBV) therapy in genotype 1b-related chronic hepatitis C patients. (Kumagai, et al. J Viral hepatit 2004) Substitution of glutamic acid to lysine at the 124th position (E124K) and of isoleucine to valine at the 85th position (I85V) of NS5B was closely associated with viral clearance at 8 weeks of treatment. We have made a prospective study with 69 patients, and analyzed relationship between the NS5B polymorphism and early viral clearance (EVC) at 12 weeks of peg-IFN-a 2b and RBV combination treatment. And we also investigated the mechanism how this polymorphism of NS5B protein affect EVC in vitro using the replicon system. [Methods] The patients received peg-IFN-a 2b and RBV combination therapy for 48 weeks (peg-IFN α2b 1.5 ug/kg a week plus RBV 400-1000 mg daily). HCV RNA was analyzed in pretreatment sera of patients, and correlation between clinical aspects including EVC and HCV polymorphisms in the NS5B region was analyzed. According to the previous report, we analyzed the significance of amino acid substitution at the 85th and 124th of NS5B (V85I, K124M) and its clinical aspect was confirmed by in vitro replicon systems. Identified polymorphisms of NS5B might affect viral replication and finally EVC in peg-IFN + RBV therapy.

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The following people have nothing to disclose: Mitsuyasu Nakamura, Hidetsugu Saito, Naoki Kumagai, Shinichiro Tada, Masanori Ikeda, Nobuyuki Kato, Toshifumi Hibi, Soichiro Murai
1313
EFFECT OF INFlixIMAB ON THE EFFICACY OF PEGINTERFERON ALFA-2B (PEG-2B) PLUS RIBAVIRIN (RBV) THERAPY IN TREATMENT-NAIVE PATIENTS WITH HEPATITIS C GENOTYPE 1 AND HIGH TUMOR NECROSIS FACTOR $\alpha$ (TNF-$\alpha$) LEVELS

Curtis Cooper1, Stephen Shafran2, Susan Greenbloom3, Robert Enns4, John Farley5, Manuela Neuman6, Nabil Abadir7; 1The Ottawa Hospital, Ottawa, ON, Canada; 2University of Alberta, Edmonton, AB, Canada; 3Toronto Digestive Disease Associates, Toronto, ON, Canada; 4St Paul's Hospital, University of British Columbia, Vancouver, BC, Canada; 5Private Practice, Vancouver, BC, Canada; 6University of Toronto, Toronto, ON, Canada; 7Scher- ing-Plough Canada Inc, Pointe Claire, QC, Canada

Background: High levels of TNF-$\alpha$ may contribute to the pathogenesis of hepatitis C virus (HCV) infection. This study evaluated the safety of infliximab in HCV-infected patients and assessed the effect of infliximab induction therapy on early virologic response and sustained virologic response (SVR). This interim analysis reports viral kinetics during the first 12 weeks of treatment. Methods: This was a randomized, prospective, open-label trial conducted at 8 academic and community sites in Canada. Treatment-naive patients with HCV G1 infection and high serum TNF-$\alpha$ levels (>300pg/mL) were randomly assigned to receive either a single pretreatment induction infusion of infliximab (5mg/kg) 7 days before antiviral therapy (Arm A) or no pretreatment (Arm B). All patients received PEG-2b (1.5µg/kg/wk) plus weight-based RBV (800–1400mg/d) for up to 48 weeks. Rapid virologic response (RVR) was defined as undetectable HCV RNA at week 12 between study arms (11/16 Arm A, 6/13 Arm B). All patients received PEG-2b (1.5µg/kg/wk) plus weight-based RBV (800–1400mg/d) for up to 48 weeks. Rapid virologic response (RVR) was defined as undetectable HCV RNA at week 12 <50IU/mL HCV RNA; Amplicor HCV Monitor V2. Results: This analysis is based on the first 29 randomly assigned patients (16 Arm A, 13 Arm B) who received 12 weeks of PEG-2b + RBV; 70% of participants were male. Infliximab was well tolerated, without excessive side effects. More infliximab recipients had advanced (F3) fibrosis (38% vs 15%). At initiation of PEG-2b + RBV, lower mean serum TNF-$\alpha$ levels were observed in patients in Arm A than in Arm B (P<.013). In Arm A, 7/16 (43.8%) patients attained RVR, compared with 4/13 (30.8%) patients in Arm B. By week 8, significantly more patients in Arm A (11/16, 69%) than in Arm B had undetectable HCV RNA than in Arm B (6/13, 46%; P=.024). The number of patients who attained undetectable HCV RNA at week 12 was similar between study arms (11/16 patients in Arm A vs 13 patients in Arm B; P=.183), suggesting that the effect of infliximab may not be sustained past week 8. Conclusion: The anti-TNF-$\alpha$ effect of infliximab on HCV may provide viral decline during the first 8 weeks of HCV therapy. It is unknown whether infliximab treatment before combination PEG-2b + RBV therapy will translate into greater SVR rates.

<table>
<thead>
<tr>
<th>HCV RNA Log$_{10}$</th>
<th>Arm A</th>
<th>Arm B</th>
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<tbody>
<tr>
<td>Visit, wk</td>
<td>Mean</td>
<td>SD</td>
</tr>
<tr>
<td>1(-3)</td>
<td>5.9</td>
<td>0.78</td>
</tr>
<tr>
<td>2(-1)</td>
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<td>4(1)</td>
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<tr>
<td>5(2)</td>
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</tr>
<tr>
<td>8(12)</td>
<td>1.23</td>
<td>2.44</td>
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Disclosures:
Curtis Cooper - Consultant/Adviser: Schering-Plough; Grant/Research Support: Schering-Plough
Stephen Shafran - Grant/Research Support: Schering-Plough
Susan Greenbloom - Grant/Research Support: Schering-Plough
Robert Enns - Grant/Research Support: Schering-Plough
John Farley - Grant/Research Support: Schering-Plough
Manuela Neuman - Consultant/Adviser: Schering-Plough
Nabil Abadir - Employee: Schering-Plough

1314
TREATMENT OF ALCOHOLIC HCV PATIENTS WITH ACETAMINOPHEN 4 G/DAY FOR 5 DAYS DOES NOT AFFECT HEPATIC TESTS COMPARED TO PLACEBO

Jody L. Green1, Kennon Heard1, Gregory M. Bagdan1, Bruna Brands2, Richard C. Dart1; 1Denver Health Rocky Mountain Poison and Drug Center, Denver, CO; 2Centre for Addiction and Mental Health, Toronto, ON, Canada

Retrospective accounts question the safety of maximum labeled daily dose of APAP in alcoholics and in patients with hepatitis C. As part of a larger trial of APAP use in alcoholic patients, we evaluated the effect of APAP on hepatic tests in alcoholic patients who also had hepatitis C virus (HCV) antibody. This was a randomized, double-blind, placebo-controlled trial of recently abstinent alcoholics. Patients were randomized 1:1 to APAP (1g every 4 hr for 4 doses x 5 days) or placebo. Exclusion criteria included a baseline serum APAP > 20 mcg/mL, aspartate aminotransferase (AST) or alanine aminotransferase (ALT) > 200 IU/L, or international normalized ratio (INR) > 1.5. Laboratory measures were obtained at baseline and days 2, 4, 6, and 7. Of 142 patients (74 APAP group, 68 placebo group), 50 subjects were positive for HCV antibody (24 APAP, 26 placebo). Demographics, nutritional status and baseline measures were similar between groups (p>0.05). Baseline ALT was significantly higher in the HCV reactive group (59 ± 38 IU/L) than in the HCV non-reactive group (42 ± 32 IU/L, p<0.05). The ALT in HCV reactive patients was significantly higher throughout the study than HCV non-reactive patients (p<0.05), regardless of treatment group assignment. Of the HCV reactive subjects, the ALT increased during the trial in both groups. However, the ALT was not different between APAP and control groups. The peak ALT mean of HCV reactive subjects was on Day 7 (71 ± 54 IU/L in APAP group, 68 ± 58 in placebo group). Maximum reported ALT was 216 IU/L in APAP group and 246 IU/L in placebo group. Total bilirubin decreased significantly after Day 2, regardless of treatment group assignment. The INR was unaffected in either group. Short-term treatment of alcoholic HCV patients with APAP at the maximum labeled daily dose, 4 g/d, appears safe.

ALT (Mean, Standard Deviation and Range) in Alco- holics with HCV During and Following Maximum Dos- ing of Acetaminophen for 5 Days

<table>
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<th>Group</th>
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<th>ALT Day 4</th>
<th>ALT Day 6</th>
<th>ALT Day 7</th>
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<tr>
<td>APAP (n=24)</td>
<td>56.3 ± 37.3</td>
<td>51.1 ± 38.6</td>
<td>55.5 ± 44.5</td>
<td>63.6 ± 44.9</td>
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<td>Placebo (n=33)</td>
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Gregory M. Bagdan - Grant/Research Support: Other
Richard C. Dart - Grant/Research Support: Other
The following people have nothing to disclose: Bruna Brands
FAVORABLE QUALITY OF LIFE (QoL) WITH ALBINTERFERON ALFA-2B PLUS RIBAVIRIN IN GENOTYPE 1, IFN-NAIVE, CHRONIC HEPATITIS C (CHC) PATIENTS

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Background/Aim This Phase 2b, active controlled study evaluated the efficacy and safety of albinterferon alfa-2b (alb-IFN) in IFN-naïve, CHC patients. The effects of alb-IFN on subject quality of life (QoL) and disability days were compared with those of PEG-IFNα-2a (PEG-IFN). Methods 458 patients were randomized and treated in 4 groups: PEG-IFN 180mcg Q1w or one of 3 alb-IFN arms (900mcg Q2w, 1200mcg Q2w or 1200mcg Q4w). The primary efficacy end-point was SVR. SF-36v.2 and the Hospital Anxiety and Depression Scale (HADS) were used to assess subject QoL and anxiety/depression. Subject disability days were evaluated by a number of missed work days or days with impaired activity. Missing data for patient reported outcomes were handled using LOCF (last observation carried forward). Results Based on ITT analysis, SVR rates were comparable (p=NS) across treatment groups. SVR in the 900Q2w was 58.5% and for PEG-IFN was 57.9%. Overall, QoL was similar or favorable for all alb-IFN arms compared to PEG-IFN at all timepoints assessed. At w12 and w24 on treatment, the 900Q2w cohort performed better in all 10 SF-36 domains relative to PEG-IFN. Statistically significant and clinically meaningful differences were observed in mental health (figure), bodily pain, vitality, and social functioning domains. Changes in mental health correlated strongly (r=0.7) with changes in HADS and by w12 post-treatment had recovered to baseline in 67% of 900Q2w subjects and 57% of PEG-IFN subjects. Subjects receiving alb-IFN 900Q2w experienced significantly fewer days of missed work: at w12 and 24, ~5% of 900Q2w subjects missed at least 7 days of work the previous month compared to ~20% for PEG-IFN. Conclusions The alb-IFN 900mcg Q2w dose was associated with the most favorable QoL and fewest missed work days while maintaining efficacy at least comparable to PEG-IFN.

HIGH CHANCE OF CURE IN HCV GENOTYPE 1 PATIENTS WITH A LOW VIRAL LOAD ACHIEVING AN RVR TREATED FOR 24 WKs WITH PEGIFN ALFA-2A (PEGASYS®) PLUS RIBAVIRIN (COPEGUS®): PROSPECTIVE, RANDOMIZED, CONTROLLED STUDY COMPARING 24 AND 48 WEEKS OF TREATMENT

Ming-Lung Yu1,2, Chia-Yen Dai1,3, Jee-Fu Huang4, Li-Po Lee5, Ming-Yen Hsieh1, Chang-Fu Chiu5, Nai-Jen Hou4, Zu-Yau Lin1,2, Shinn-Cheng Chen1,2, Ming-Yuh Hsieh1,2, Liang-Yen Wang1,2, Wen-Yu Chang1,2, Wan-Long Chuang1,2; 1Department of Internal Medicine, Kaohsiung Medical University, Kaohsiung, Taiwan; 2Department of Internal Medicine, Kaohsiung Medical University Hospital, Kaohsiung, Taiwan; 3Department of Internal Medicine, Kaohsiung Municipal Hsiao-Kang Hospital, Kaohsiung, Taiwan; 4Department of Internal Medicine, Foo Yin Hospital, Pintung, Taiwan; 5Department of Internal Medicine, Paochien Hospital, Pintung, Taiwan

Background: Recommended treatment for patients with HCV genotype 1 (G1) infection is Peg-IFN plus ribavirin (RBV) for 48 wks and 24 wks for HCV G2/3. A rapid virological response (RVR; <50 IU/mL HCV RNA at wk 4) is a strong predictor of sustained virological response (SVR; <50 IU/mL HCV RNA 24 wks after untreated follow-up). SVR rates of >80% with a shorter treatment duration of 12-16 wks peginterferon alfa-2a (40KD) plus RBV in HCV G2/3 pts with an RVR have questioned whether shorter treatment duration can yield high SVR rates for G1 patients with an RVR. Therefore, we determined the efficacy of 24 wks therapy to standard 48 wks treatment in HCV G1 patients with an RVR. Methods: In a controlled, multicenter, open-label study in Taiwan, 200 treatment-naïve G1 patients were randomized (1:1) to 24 wks or 48 wks peginterferon alfa-2a (40KD) 180 µg/wk plus RBV in HCV G2/3 pts with an RVR have questioned whether shorter treatment duration can yield high SVR rates for G1 patients with an RVR. Therefore, we determined the efficacy of 24 wks therapy to standard 48 wks treatment in HCV G1 patients with an RVR. Methods: In a controlled, multicenter, open-label study in Taiwan, 200 treatment-naïve G1 patients were randomized (1:1) to 24 wks or 48 wks peginterferon alfa-2a (40KD) 180 µg/wk plus RBV 1000/1200 mg/d, with a follow-up period of 24 wks. The primary endpoint was an SVR. Results: Baseline (BL) characteristics were similar in the 24 and 48 wk arms. At BL, respectively for the 24 and 48 wk arms, male patients accounted for 57/58% of all patients, with a mean age of 49.7/49.1 years, a mean weight of 65.5/67.5 kg and with 25/19% having a BL diagnosis of advanced hepatic fibrosis (F3/4). BL log HCV RNA (IU/mL) was 5.43 (24 wk) and 5.66 (48 wk). Overall, the 48 wk arm had a significantly lower relapse rate and a higher SVR rate than the 24 wk arm (ITT). Patients with an RVR had a significantly higher SVR rate than patients without an RVR in both treatment arms. For patients with a lower BL viral load (LVL, <400,000 IU/mL) and an RVR at wk 4, the rates of relapse and SVR in the 24 wk arm was comparable to those in the 48 wk arm. MLR analysis in all patients showed that an RVR was the strongest independent factor associated with an SVR, followed by treatment duration, adherence and BL viral load. The 48 wk arm had a significantly higher rate of discontinuation than the 24 wk arm. Conclusion: In this study, high SVR rates (>95%) were seen with both 24 and 48 wks of peginterferon alfa-2a (40KD) plus RBV 1000/1200 mg/d in HCV G1 patients with a LVL and an RVR. BL viral load and an RVR at wk 4 could provide decision-making information for a shorter treatment duration for HCV G1 patients.
1317 TREATMENT OF HEPATITIS C PATIENTS WITH CHILD A AND B CIRRHOSIS WITH CONSENSUS INTERFERON AND RIBAVIRIN IN A LOW ASCENDING DOSING REGIMEN LEADS TO SIGNIFICANT VIRAL ELIMINATION RATES

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Objective: Antiviral treatment response in patients with chronic hepatitis C and liver cirrhosis is considerably lower than in non-cirrhotic patients and therapy is complicated by high dropout rates, less tolerability of side effects and high rates of hematological complications. Pegylated interferons have shown higher response rates than standard interferons, however, also higher dose-reduction and drop-out rates due to a lower tolerability. Consensus interferon (CIFN) is an interferon with a relatively low half-life, but stronger antiviral potency as shown by high efficacy in nonresponders. Methods: The efficacy of CIFN together with ribavirin (RBV) was evaluated in 120 patients with chronic hepatitis C and cirrhosis Child A and B (average Child score 7.9, maximum MELD score 20) . All patients had histologically proven cirrhosis, elevated ALT values and were viremic, with 79% having genotype 1. Child A patients were treated with CIFN 9 ug TIW for 4 weeks, followed by 9 ug QD for another 4 weeks. Continuing treatment consisted of CIFN 9 ug QD with RBV with a stepwise increase from 400 mg by 200 mg increments at 4 week intervals for a total of another 52 weeks. For Child B patients the two lead-in phases with CIFN monotherapy were extended to 6 weeks each, and the starting dose of RBV was 200mg with an otherwise identical therapy as with Child A patients. Based on tolerability the dosing of RBV was increased to a weight-based dosing for all patients. Results: At 60 weeks therapy an undetectable HCV-RNA was observed in 76% and 47% of Child A and B patients, respectively, with drop out rates of 13% and 27%. Sustained response rates showed a 57% and 27% response for Child A and B, respectively. Due to side effects CIFN had to be dose reduced in 17% and 34%, mainly due to low platelet counts. As growth factors erythropoetin as well as G-CSF was used. 11 patients experienced grade III and 7 patients grade IV thrombocytopenia. Overall tolerability of the CIFN QD regimen was comparable to a standard therapy with pegylated IFN and RBV, while CIFN even as QD treatment resulted in a lower rate of thrombocytopenias. Conclusions: CIFN as a low ascending and finally daily dosing regimen with subsequent escalating RBV shows significant response rates in Child A and B cirrhotic patients. Therapy is also safe, however, a significant portion of patients was unable to even tolerate lower doses of CIFN or RBV. These data suggest that for a subgroup of cirrhotic patients even in stage Child B a combination therapy of CIFN and RBV may lead to viral eradication.

Disclosures: Stephan Kaiser - Speakers Bureau: Valeant; Grant/Research Support: Bristol-Myers Squibb; Grant/Research Support: Gilead; Grant/Research Support: GlaxoSmithKline; Grant/Research Support: Novartis; Grant/Research Support: Roche; Grant/Research Support: Schering-Plough

The following people have nothing to disclose: Bettina Lutze, Birgit Sauter, Lennart Bissinger, Christoph Werner, Holger Hass, Michael Gregor

1318 RESULTS OF A PHASE I PLACEBO-CONTROLLED TRIAL IN HEALTHY VOLUNTEERS TO EXAMINE THE SAFETY, TOLERABILITY AND PHARMACOKINETICS OF THE HCV PROTEASE INHIBITOR TMC435350 AFTER SINGLE AND REPEATED DOSING

Rene Verloes1, Khalid Abou Farha2, Andre van Vliet2, Gerben van ‘t Klooster1, Fatima Aharchi1, Kris Marien1, Herman de Kock1, Kenneth Simmen1; 1Tibotec Pharmaceuticals Ltd., Eastgate Village, Little Island, Cork, Ireland; 2PRA International EDS, Stationsweg 163, 9471 GP Zuidlaren, Netherlands

Background: This trial studied the safety, tolerability and plasma pharmacokinetics (PK) of a novel HCV NS3/4A protease inhibitor, TMC435350, after single oral dosing and, in a second step, after 5 days of oral dosing in HCV-negative volunteers. Methods: The single ascending dose (SAD) part was studied under fed conditions using two panels of 9 males or females followed by an investigation on the effects of fasting. In the multiple ascending dose (MAD) part, 4 panels of 9 volunteers were included. Each panel was designed to have 6 subjects receiving TMC435350 solution and 3 subjects receiving placebo. Safety monitoring included physical examination, vital signs, laboratory parameters (haematology, biochemistry, urinalysis), extensive cardiovascular safety (ECGs, and additional biomarkers and echocardiography during the MAD phase), and adverse events. In the SAD study a full PK profile was evaluated up to 72 h post-dose. In the MAD study, a full PK profile was studied on Days 1 and 5, with samples taken up to 72 h post-dose. Results: In the SAD study, oral doses up to 600 mg were well tolerated without attaining any dose-limiting toxicity. The plasma exposure increased in a more than dose proportional fashion. A single dose of 200 mg studied under fasted conditions was well tolerated and yielded comparable exposure to the fed condition. TMC435350 displayed good plasma exposure, with a Tmax of 4.6 hours, which together with a half-life of ~12 hours, supports once daily dosing (qd) in the MAD phase. In that phase, a starting dose of 100 mg was given qd for 5 days. Subsequent doses were 200 mg qd, 200 mg bid and 400 mg qd. All doses of TMC435350 or placebo were well tolerated. There were no grade 3 or 4 adverse events and no clinically relevant changes from baseline on laboratory parameters, vital signs, ECG recordings and echocardiographic evaluations. Minor effects observed were mainly gastrointestinal tract related. Mild, short-lasting erythema was also noted after sun exposure in a few subjects receiving the 200 mg bid dose or placebo. The plasma levels of TMC435350 detected 24 hours after the Day 5 dosing were substantially in excess of the replicon EC50 value for all doses. Conclusions: The data suggest that TMC435350 is safe when given as single doses up to 600 mg and 5 days of dosing up to 400 mg qd. The compound will be further investigated following once-daily administration in HCV patients.
Mean PK parameters after single doses of TMC435350.

<table>
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<tr>
<th>Dose</th>
<th>50 mg</th>
<th>100 mg</th>
<th>200 mg</th>
<th>300 mg</th>
<th>450 mg</th>
<th>600 mg</th>
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<tr>
<td>t_{1/2, a}</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>6</td>
<td>6</td>
<td>6</td>
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<tr>
<td>C_{max, ng/mL}</td>
<td>293.5</td>
<td>582.0</td>
<td>2957</td>
<td>4960</td>
<td>10460</td>
<td>13550</td>
</tr>
<tr>
<td>AUC_{24h, ng/mL}</td>
<td>3355</td>
<td>6269</td>
<td>30050</td>
<td>46380</td>
<td>125000</td>
<td>166700</td>
</tr>
</tbody>
</table>

Disclosures:

The following people have nothing to disclose: Rene Verloes, Khalid Abou Farha, Andre van Vliet, Geberen van 't Klooster, Fatima Aharchi, Kris Marien, Herman de Kock, Kenneth Simmen

1319 ANALYSIS OF RELAPSE RATES IN PEGYLATED INTERFERON AND RIBAVIRIN NON-RESPONDERS TREATED WITH DAILY CONSENSUS INTERFERON AND RIBAVIRIN

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Background: Relapse rates in HCV treatment-naive patients treated with pegIFN/RBV occurs in over 30% of patients. A recent controlled trial of pegIFN/RBV non-responders retreated with a subsequent course of pegIFN/RBV showed a relapse rate of more than 80% and a sustained response in only 3% of patients. While several factors associated with relapse after therapy of naïve patients with pegIFN/RBV have been identified (genotype 1, high viral load, advanced histology, and non-adherence to treatment regimen), predictors of relapse after re-treatment of pegIFN/RBV nonresponders with consensus interferon (CIFN) and RBV remain unknown. Therefore, viral and host factors associated with relapse following 48 weeks of therapy with CIFN/RBV in patients who failed previous pegIFN/RBV treatment were analyzed. Methods: The DIRECT clinical trial is Phase 3, multi-center, and open-label US-based study. 27 genotype 1 patients who had an end-of-treatment response and received daily CIFN (15µg/d) and RBV (1.0-1.2 g/day) were identified for this retrospective analysis. 54% of these patients had a high baseline viral load (VL; ≥850,000 IU/mL), 41% had advanced liver disease/cirrhosis, 44% had evidence of steatosis, and a mean weight of 89kg. 59% of patients had a <2log10 drop in VL during their previous course of pegIFN/RBV therapy. Relapse was defined as VL negative at end of treatment and virus detectable at anytime within the 24-week follow-up period. No adjunctive growth factors were used. Patients had a negative VL if virus was undetectable by both bDNA and TMA assays. Results: The relapse rate in the 27 patients was 59%. The relapse rate in patients with steatosis was 83% compared to 38% in patients who did not have steatosis. Patients that achieved viral negativity by week 12 had the lowest relapse rate (33%), followed by patients that were negative by week 24 (45%). All patients that became viral negative after week 24 relapsed. There was no apparent difference in relapse rates between patients that had a null response (<2log10 drop in VL) versus a partial response (>2log10 drop in VL but detectable HCV RNA) to previous pegIFN/RBV therapy (63% vs. 57%, respectively). Conclusions: Previous pegIFN/RBV non-responders treated with daily CIFN/RBV that achieved viral negativity earlier in the course of treatment were less likely to relapse. The presence of steatosis also appeared to worsen the relapse rates. Previous response to pegIFN/RBV did not appear to have an impact on relapse with retreatment with daily CIFN 15µg/d and RBV. To reduce relapse rates in this population, additional studies evaluating longer duration of CIFN and higher doses of RBV are warranted.

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Janet Hammond - Employee: Valeant

The following people have nothing to disclose: Tarek Hassanein, Reem H. Ghalib, Nizar N. Zein, Kenneth D. Rothstein, Shobha N. Joshi, Paul Yien Kwo

1320 THE EFFECT OF COMPLETE AND PARTIAL RESPONSE AT WEEK 12 ON SUSTAINED Virologic RESPONSE: RESULTS FROM CONTROLLED TRIALS IN NAIVE HCV GENOTYPE 1 PATIENTS TREATED WITH PEGYLATED INTERFERON AND RIBAVIRIN

Mitchell L. Shiffman1, Hank Mansbach2, Janet Hammond2, Mark O’Neill2, 1Hepatology Section, Virginia Commonwealth University Medical Center, Richmond, VA; 2Valeant Pharmaceuticals North America, Aliso Viejo, CA.

Background: Early virologic response (EVR) has been defined as a >2log10 decline in HCV RNA from baseline or undetectable HCV RNA at treatment week 12. However, within the context of EVR are patients who are HCV RNA undetectable at treatment week 12 (complete responders) and a second group that remains HCV RNA positive at week 12 (partial responders). Previous studies have suggested that this latter group has a significantly lower chance of achieving a sustained virologic response (SVR). We, therefore, analyzed a large database of HCV patients who received either pegIFN alfa-2a or -2b along with ribavirin (RBV) and determined the impact of EVR, complete, and partial response on SVR. Methods: A retrospective review of the active control arms (pegIFN/RBV) of two global phase 3, multi-center, randomized, parallel group, double-blind studies in treatment-naive patients was performed. This analysis pooled 392 genotype 1 patients, 204 of whom were treated with pegIFN alfa-2b (1.5mg/kg/wk) and 188 treated with pegIFN alfa-2a (180µg/wk). Both studies used standard weight-based doses of RBV (1.0-1.2 g/day). Week 12 response was categorized as HCV RNA negative (complete responder), >2log10 decline but HCV RNA positive (partial responder), and <2log10 decline in HCV RNA (null responder). Each category of response was evaluated with respect to its ability to achieve SVR. HCV RNA was assessed using the NGI SuperQuant assay (sensitivity to 39 IU/mL). Results: The patient population was 82% Caucasian; mean body weight 81kg; 71% high viral load (>2 million copies), and 34% with advanced fibrosis or cirrhosis (F3-4). At week 12, 56% of patients were complete responders (HCV RNA undetectable), 29% partial responders, and 15% null responders. 335 patients (85%) achieved an EVR and 54% of these patients went on to achieve an SVR. 220 of the EVR patients (66%) were complete responders, and 115 patients (34%) were partial responders. 162 (74%) of complete responders went on to achieve SVR. In contrast, SVR was achieved by only 18 (16%) of partial responders. Only 1 of 57 (2%) null responders achieved an SVR. Conclusions: Approximately 56% of genotype 1 treatment-naive patients treated with pegIFN/RBV became HCV RNA undetectable at week 12, and 74% of these patients achieved SVR. In contrast, only 16% of patients with partial response at week 12 achieved SVR. A study to determine if SVR can be increased in partial responders (those with EVR but HCV RNA positive at week 12) by switching to a more aggressive IFN regimen at week 12 is, therefore, warranted.
1321
COMPARISON OF STANDARD VS EXTENDED PEGYLATED INTERFERON PLUS RIBAVIRIN TREATMENT ACCORDING TO THE TIME OF HCV RNA NEGATIVE IN PATIENTS WITH GENOTYPE 1 AND HIGH VIRAL LOAD
Tatsuya Ide, Teruko Arinaga, Ichiro Miyajima, Kei Ogata, Reiichiro Kuwahara, Yuriko Koga, Kouichirou Kuhara, Ryukichi Kumashiro, Michio Sata; Division of Gastroenterology, Kurume University School of Medicine, Kurume, Japan

Background and aims: Standard duration of pegylated interferon and ribavirin therapy for HCV genotype 1 and high viral load is 48 weeks in Japan. Prior trials investigating the efficacy of longer treatment duration than 48 weeks for HCV genotype 1 demonstrated high sustained virologic response (SVR) rates. Many studies extended the duration of therapy from 48 weeks to 72 weeks. However, in some patients, 72 weeks therapy might be excessive and we should set up the optimal duration of therapy. On the other hand, in patients whose HCV RNA became negative at 4 weeks, SVR can be obtained in almost 100%. These patients maintained HCV RNA negative for 44 weeks. Therefore, we designed extended the therapy which was set up to be HCV RNA negative for 44 weeks and conducted a prospective, randomized, controlled trial investigating whether this extended treatment have higher SVR rate than standard 48 weeks treatment. Methods: 83 genotype 1b and high viral load (>100 KIU/ml, Roche amplicore) patients were randomized at baseline for standard (n=41) or extended (n=42) treatment group. Standard group patients received 48 weeks of treatment. Duration of extended treatment was determined by the time of HCV RNA negative to be HCV RNA negative for 44 weeks (ex. If HCV RNA became negative at week 16, total treatment duration was 60 weeks.). If HCV RNA is positive at week 24, the patient was dropped out of the trial. Results: Two patients in standard group were lost to follow-up. 9 in standard group and 8 in extended group were dropped out because of HCV RNA positive at 24 weeks. 6 in each group discontinued treatment because of the side effects and other reasons. SVR rates were 56.0% (14/25) in standard group versus 81.1% (23/28). Especially, in patients who obtained HCV RNA negative at from week 12 to week 24, SVR rate was significantly higher in extended group (standard vs extended: 35.3% vs 78.9%, P<0.05). Conclusion: Extension of treatment group and 8 in extended group were dropped out because of HCV RNA positive at 24 weeks. 6 in each group discontinued treatment because of the side effects and other reasons. SVR rates were 56.0% (14/25) in standard group versus 81.1% (23/28). Especially, in patients who obtained HCV RNA negative at from week 12 to week 24, SVR rate was significantly higher in extended group (standard vs extended: 35.3% vs 78.9%, P<0.05). Conclusion: Extension of treatment with pegylated interferon plus ribavirin therapy significantly increased the SVR rate in patients with HCV RNA undetectable at 12-24 of treatment.

Disclosures:
The following people have nothing to disclose: Tatsuya Ide, Teruko Arinaga, Ichiro Miyajima, Kei Ogata, Reiichiro Kuwahara, Yuriko Koga, Kouichirou Kuhara, Ryukichi Kumashiro, Michio Sata

1322
TOLERABILITY OF LOW-DOSE PEGINTERFERON ALFA-2b IN HEPATITIS C PATIENTS WITH CIRRHOSIS: RESULTS FROM THE COPILOT TRIAL
Manan B. Shah1, Robert S. Brown2, Tera Barski3, Bradley Freilich4, Robert A. Levine5, Nezam H. Afdhal1; 1Gastroenterology and Hepatology, Beth Israel Deaconess Medical Center, Boston, MA; 2Division of Digestive and Liver Diseases, Columbia-Presbyterian Medical Center, New York, NY; 3SUNY Upstate Medical University, Syracuse, NY; 4Kansas City Gastroenterology, Kansas City, MO

INTRODUCTION: Quality of life (QOL) is critical for the utilization of long term maintenance therapies. The aims of this study were to evaluate baseline QOL in patients with advanced fibrosis and to evaluate changes with Peginterferon alfa-2b (PEG-IFN) vs. Colchicine (COLC). RATIONALE: Given side effects of standard doses of PEG-IFN, it is unclear whether lower-dose maintenance therapy would improve or impair QOL. METHODS: 475 patients with advanced Hepatitis C refractory to conventional treatment with Interferon and/or Ribavirin were randomized to receive 0.5µg/kg PEG-IFN alfa 2b weekly vs. COLC 0.6mg PO BID. QOL was assessed by administering the Medical Outcome Trust’s SF-36© survey annually throughout the study. Responses were scored using the norm-based scoring approach, where 50 is the mean for the general population and 10 is the standard deviation. RESULTS: Mental Component Summary (MCS) scores and Physical Component Summary (PCS) scores were calculated for each of the two groups at baseline, 48-weeks, and 96-weeks. Mean values are reported in Table 1. All demographics including age, gender, duration of disease, histology, viral load, biochemical tests, and Child Pugh classification were comparable between the 2 groups. Mean MCS and PCS scores were not significantly different between the study groups at baseline. QOL was relatively stable in the groups with no statistically significant change between baseline and week 96. Mixed procedure analysis revealed a statistically significant effect of treatment assignment only on MCS score (p = 0.02) at week 48 in the COLC group, and values returned to baseline by week 96. The actual difference in score was a modest 4-point reduction in MCS. There was no significant effect of treatment assignment on QOL over time (p = 0.16) in either group. CONCLUSION: Surprisingly, cirrhotic patients were not more than 1 SD below the normal population in either MCS or PCS at baseline. Treatment with low-dose PEG-IFN did not adversely affect QOL over a 2-year period in patients staying on therapy, suggesting that it can be tolerated as a maintenance therapy.

Mean MCS and PCS Scores

<table>
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<tr>
<th>Randomization</th>
<th>Week</th>
<th>N</th>
<th>MCS Score (Mean ± SE)</th>
<th>PCS Score (Mean ± SE)</th>
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<tr>
<td>Colchicine</td>
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<td>161</td>
<td>46.4 ± 0.9</td>
<td>42.6 ± 0.8</td>
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<td></td>
<td>48</td>
<td>88</td>
<td>42.6 ± 1.3</td>
<td>40.6 ± 1.2</td>
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<tr>
<td></td>
<td>96</td>
<td>69</td>
<td>46.2 ± 1.2</td>
<td>43.9 ± 1.2</td>
</tr>
<tr>
<td>PEG-IFN</td>
<td>0</td>
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<td>42.1 ± 0.8</td>
</tr>
<tr>
<td></td>
<td>48</td>
<td>106</td>
<td>45.3 ± 1.1</td>
<td>41.6 ± 1.1</td>
</tr>
<tr>
<td></td>
<td>96</td>
<td>76</td>
<td>43.7 ± 1.2</td>
<td>41.0 ± 1.3</td>
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</table>

Disclosures:
The following people have nothing to disclose: Manan B. Shah, Robert S. Brown, Tera Barski, Bradley Freilich, Robert A. Levine, Nezam H. Afdhal
Introduction: Consensus interferon (CIFN) is a synthetic type 1 interferon with enhanced in vitro activity compared to conventional IFN-alfa (IFNa). In the prospective, randomized multicenter Peglntron-Against-Consensus-Trial (PACT), the efficacy and safety of daily CIFN-treatment and ribavirin is compared to Peglnterferon (PegIFN) alfa2b plus ribavirin in genotype 2 and 3 patients. Methods: 262 patients with chronic HCV infection and genotype 2 or 3 were randomly assigned to treatment with peg-IFNa2b (1.5 mcg/kg body weight once weekly, group A) or 9 mcg qd CIFN (group B) for 24 weeks. 194 patients received weight based ribavirin doses, however due to a later protocol amendment, 68 patients received a fixed ribavirin dose of 800 mg qd. Follow up was 24 weeks. Results: There were no significant differences in patient baseline characteristics between both treatment groups concerning age, gender, genotype and viral load. No significant differences were detected between both treatments at end of therapy (EoT) and for sustained virological response (SVR) rates in the intent to treat (ITT) and the per protocol (PP) analysis (ITT: 82% vs. 75% at EoT and 67% vs. 65% SVR; PP: 95% vs. 95% at EoT and 84 vs. 90% SVR). However, the CIFN group displayed a significantly lower relapse rate than group A (3% vs. 9%, p = 0.042) resulting in a slightly higher SVR rate in group B in the PP analysis. Furthermore, no difference in the treatment outcome was found between weight based or fixed ribavirin dose regimens. Treatment was well tolerated in both treatment groups, despite more dose modifications for leucopenia in group B (9% vs. 3%, p = 0.015) without an increased discontinuation rate. Conclusions: In treatment-naive patients with chronic hepatitis C and genotype 2 or 3, daily treatment with CIFN combined with ribavirin has similar antiviral efficacy and safety profile as weight adjusted PegIFNa2b.

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Correlation Between Liver Biopsy and FibroSURE during Screening for a Phase II Study to Assess the Antifibrotic Activity of Farglitazar in Chronic Hepatitis C Infection

Keyur Patel1, John G. McHutchison1, Zachary D. Goodman2, Dickens Theodore3, Alison Webster4, Margaret Schultz5, Britt Stancil6, Martin Gardlan7, Stephen Gardner8, 1Gastroenterology, Duke Clinical Research Institute and Duke University Medical Center, Durham, NC; 2Armed Forces Institute of Pathology, Washington, DC; 3GlaxoSmithKline, Research Triangle Park, NC

Background: Non-invasive biomarkers have been proposed as an alternative to liver biopsy for initial staging and assessing changes in fibrosis with therapy in chronic hepatitis C (CHC) patients. Our aim was to assess the utility of the FibroSURE panel in staging fibrosis during screening for enrollment into a multicenter phase II, placebo-controlled, antifibrotic study of a PPARγ agonist (Farglitazar) in CHC infection. Methods: 482 adult CHC non-responders with compensated liver disease had a screening biopsy, and FibroSURE (FS) assay obtained through a central laboratory. Liver biopsy assessment for Ishak staging was performed independently by a single central histopathologist; subjects with Ishak stages 2-4 were subsequently randomized into the study. Screening data were analyzed to determine the accuracy of FS for predicting Ishak fibrosis stage. Results: CHC patients were mostly Caucasians (363/482, 75%), male (297/482, 62%) and with a mean age 52 ± 6.7 yrs. Mean biopsy length was 22.6 ± 11.4 mm. Overall Spearman correlation for Ishak stage and FS, rs = 0.38 (CI, 0.31 – 0.47; p<0.0001); weighted Kappa = 0.15 (CI, 0.11 – 0.19; p<0.0001). FS demonstrated good sensitivity but relatively poor performance for the detection of moderate-to-severe stage (F3-F6) disease [AUC=0.69]. For FS detection of cirrhosis (F5-F6), AUC=0.73, rs=0.25 (95% CI, 0.16 – 0.34). For FS index scores 0.32–0.58 that predicted stage F2-F3 disease, biopsy indicated F0-F1=53/134 (40%), F2-F3=72/134 (54%), and F4-F6 = 9/134 (7%). Conclusions: Non-invasive serum biomarkers such as FibroSURE demonstrate good performance for exclusion of significant disease. However, their utility in differentiation of moderate stage disease is relatively poor, and this may limit their ability to accurately follow changes in fibrosis stage with treatment. This will be further evaluated based on follow-up biopsies at the end-of-treatment in this study.

Performance characteristics of FibroSURE for detection of moderate and advanced disease

<table>
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<tr>
<th>Ishak Fibrosis stage</th>
<th>Prevalence</th>
<th>FS Index Score</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
<th>NPV</th>
<th>AUC(CI)</th>
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</thead>
<tbody>
<tr>
<td>F3-F6</td>
<td>40%</td>
<td>≤0.32 (n=386)</td>
<td>0.90</td>
<td>0.27</td>
<td>0.45</td>
<td>0.80</td>
<td>0.69 (0.62)</td>
</tr>
<tr>
<td>F4-F6</td>
<td>15%</td>
<td>≤0.59 (n=252)</td>
<td>0.80</td>
<td>0.53</td>
<td>0.21</td>
<td>0.93</td>
<td>0.70 (0.63)</td>
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<td>8%</td>
<td>≤0.73 (n=151)</td>
<td>0.70</td>
<td>0.72</td>
<td>0.19</td>
<td>0.96</td>
<td>0.73 (0.63)</td>
</tr>
</tbody>
</table>

AUC, Area under ROC curve

Disclosures:
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A 52 Week Multi-Centre, Randomized, Double-Blind Placebo-Controlled Trial Evaluating the Efficacy and Safety of Glycyrrhizin in Patients with Chronic Hepatitis C Not Responding to IFNα or Peg-IFN Plus Ribavirin Therapy

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Background: A significant number of patients with chronic hepatitis C genotype 1 fail to respond to the standard therapy of Peg-IFN-α plus RBV, or cannot be treated for various reasons. There are no options for non-responders to previous standard therapies. Glycyrrhizin (GL) is used in Japan for more than 20 years to treat such cases (SNMC, Minophagen). Studies in non-Asian patients are missing. Objectives: Confirm efficacy (based on ALT and histology) and safety of GL in non-responders to combination therapy. Methods: The trial consists of 2 phases (12 plus 40 weeks). Phase 1 is a randomized, double-blind, placebo-controlled, parallel group comparison of i.v. injections of GL (200 mg glycyr rhizic acid), administered intravenously 5x/ week, or 3x/ week placebo, for 12 weeks. The following phase 2 is a randomized, open comparison of GL 200 mg administered iv 5x/ week versus 3x/ week, for 40 weeks (no placebo for ethical reasons). Adaptive design plan and 2 primary efficacy endpoints: 1 - proportion of patients with ≥ 50% ALT reduction after 12 weeks, and 2 - proportion of patients (≥60%) with improved necroinflammation score after 52 weeks. Inclusion criteria: pos. HCV-RNA, non-Asian, no active ALT, liver biopsy at entry or performed within 6 months. In this study were enrolled 374 patients from 2002 to 2004, [5x/week GL, 3x/week GL, and placebo with N= 123, 127, and 129, respectively], carrying genotype type 1 in 73 % of cases. Baselines (mean, SD): ALT 77 ±49 IU/L, necroinflammation score 7.6 ± 2.5, fibrosis score 3.1 (±1.8). Results: The rate of ALT reduction ≥50% after 12 weeks was significantly higher with 5x/week GL (30%, p = 0.000003) and 3x/week GL (32%, p = 0.000001) compared to placebo (6%). Significant ALT (mean, 95% CI) decrease under active treatment (-33 % -40 to -25) and (-27 % -33 to -21) respectively, but not under placebo (1 %, -8 to +10). Based on evaluable biopsies taken within 6 months before randomization and at the end of the 52 week treatment (N=249 patients) the rate of improvement, defined as at least 1 point scoring difference, in the necroinflammation reached 45% after 52 weeks of treatment, and remained stable in 17%, i.e. no deterioration in 62%. Despite these positive results the second formal primary endpoint (>60% of patients with improved necroinflammation) could not be reached. 4.2% of patients dropped-out for treatment related AEs. Favourable results were also seen in secondary endpoints, including quality of life. Conclusion: GL appears to be effective and well tolerated in the treatment of chronic hepatitis C not responding to IFNα or Peg-IFN plus RBV therapy.
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COMPARISON OF PEGINTERFERON ALFA-2A AND RIBAVIRIN FOR 12 OR 24 WEEKS IN PATIENTS WITH HCV GENOTYPE 2 OR 3: THE CLEO TRIAL

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Background: In chronic hepatitis C (CHC) patients with genotype 2 or 3, 24 weeks treatment with peg-interferon and ribavirin induces a sustained virological response (SVR) in about 80% of the cases. Recent trials have shown that a similar response rate may be obtained with a shorter treatment period (12 or 16 weeks), especially in patients with rapid virologic response (RVR). Endpoints Primary endpoint of the study was to assess whether 12 week treatment with peg-interferon and ribavirin is as efficacious as 24 week treatment in inducing a SVR in CHC patients with genotype 2 or 3. Secondary endpoint was the rate of relapsers among groups. Methods We performed a multicenter, prospective, randomized trial on 180 historically confirmed CHC patients with genotype 2 or 3 enrolled in 11 italian centers. All patients were treated with peg-interferon alpha2a (180 mcg/week) and ribavirine (800-1200mg/day). After 4 weeks of treatment, the patients with HCV-RNA <600 UI/ml (RVR) were randomized either to 12 weeks (Group A1; n=60) or to 24 weeks (Group A2; n=60) of combination therapy in a 1:1 ratio. The patients without RVR continued standard 24 weeks combination therapy (Group B n=60). In all groups of patients HCV-RNA was checked 12 and 24 weeks after the end of therapy. Results: At the end of the study period SVR was observed in 74% of patients of Group A1, in 84% of patients of Group A2 and in 54% of patients of Group B (P<0.05 vs Groups A1 and A2). Relapsers rate at the end of the study period was 3% in Group A1, 2% in Group A2 and 6% in Group B. Logistic regression analysis showed that baseline HCV-RNA< 1 million/UL (OR: 3.5; P<0.001), a baseline histological inflammation score <7 (OR:2.5; P<0.001), a fibrosis score <2 (OR: 3.3; P<0.001) and a RVR <4 week (OR: 3.5; P<0.001) were the strongest covariates predictive of SVR. Conclusions: In CHC patients with genotype 2 or 3, 12 week combination therapy is as efficacious as 24 week therapy. A RVR is an independent covariate predictive of SVR along with low baseline HCV levels and a low baseline histological inflammation and fibrosis score without significant differences in the rate of relapsers.

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INTERFERON THERAPY PREEMPTIVE PARTIAL SPLENIC EMBOLIZATION FOR HEPATITIS C PATIENTS WITH THROMBOCYTOPENIA

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INTRODUCTION: Interferon therapy for advanced chronic hepatitis C patients (CH-C) is often difficult because of accompanied thrombocytopenia with hypersplenism. Partial splenic embolization (PSE) is one of the treatments for portal hypertension and hypersplenism. We have introduced PSE to increase platelet count for IFN therapy in CH-C. AIMS & METHODS: The aim of this study was to reveal the efficacy and safeness of PSE before IFN therapy. Subjects were 30 patients (M/F: 13/17; mean age: 57.5) with chronic hepatitis who could not be allowed to have IFN therapy because of thrombocytopenia, platelet count less than 70 thousand /ml, with hypersplenism. We underwent PSE to them before IFN therapy between October 2003 and March 2007. Liver fibrosis grade F1/F2/F3/F4: unknown: 0/2/16/5/7, HCV RNA genotype 1b/2a/2b: 15/11/4, HCV viral load is 989.3±995.8(23-5000) KIU/ml. 25 cases were treated by PEG-IFNα2b+Ribavirin, 3 were IFNα2b+Ribavirin and 2 were PEG-IFNα2a, respectively. RESULTS: Mean rate of embolized-volume of the spleen is 71.7% on CT volumetry. At 2 weeks after PSE, platelet count increased from 7.0±1.9×10⁴/µl to 17.9±5.9×10⁴/µl(P<0.001). WBC count increased from 3526.2±1050.7/µl to 5326.4±1050.7/µl(P<0.001). Hb, T-Bil, Alb, ALT and AST did not change significantly. As for the adverse events by PSE, fever and abdominal pain appeared in all the patients, pleural effusion in 2 cases, ascites in 1 case. No severe complications such as splenic abscess, splenic rupture nor sepsis were observed and all patients could start IFN therapy after PSE. The waiting period after PSE to IFN therapy is 24.2 days. 10 cases are still on treatment. IFN therapy was discontinued because of neutropenia in 2 cases, and strong fatigue in 1 case, but thrombocytopenia was not the reason for IFN discontinuation. Up to now a sustained viral response was obtained for clinical efficacy in 5 of the 16 cases (31%). CONCLUSION: Based on this study, we conclude that PSE is effective to improve thrombocytopenia in patients with CH-C and enable them to have IFN therapy safely.

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1329 VIRAL KINETICS OF HCV GENOTYPE 4 DURING PEGYLATED INTERFERON ALPHA 2A – RIBAVIRIN THERAPY

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Background: Kinetics of Hepatitis C virus (HCV) during pegylated interferon (PEG-IFN) and early monitoring of viral decline were recently described to predict treatment outcomes and in turn reduce the course of treatment, adverse effects and cost. There is limited information on the viral dynamics of HCV-4.

Aim: To follow the HCV-RNA kinetics during Peg-IFN-α2a and Ribavirin therapy and the best time for predicting SVR in genotype 4 patients. Methods: Serum HCV-RNA levels before initial dosing, 24h later then at weeks 1, 4, 12, 24, 48 and 72 were assessed in 84 HCV genotype 4 patients treated weekly by PEG-IFN alpha2a 180microgram and daily Ribavirin(1000-1200mg). Results: At the end of treatment, out of the 84 treated patients, 19 (22.6%) were non-responders while 65 (77%) showed end of treatment response (ETR). However 9 patients relapsed (9.5%), thus the sustained viral response (SVR) was observed in 57 patients (67.9%). Younger patients were more likely to attain SVR, where the odds of SVR increased by a factor of 0.94 for each year increase in age (95% CI 0.90, 0.99, p=0.019). Although a significant negative correlation between stage of fibrosis and rate of viral decline at week 1 & 4 (p<0.005 & 0.001, respectively) was seen, neither fibrosis stage (X2 =3.4882, p>0.1) nor grade of inflammation (X2= 0.0057, p>0.1) significantly predicted response to treatment. Non-responders had no or only a limited decline at week 1 and week 4, whereas sustained virological responders had a significant decline both at week 1 and week 4.

Area under (ROC) curve revealed that week 12 is better than any other time point in predicting SVR (AUC 0.97; 95% CI 0.94, 1.01), (sensitivity 98.3%; 95% CI 90.7, 99.9), (specificity 88.5%; 95% CI 71.0, 96.0), positive predictive value (PPV) of 94.9% and negative predictive value (NPV) of 95.8%. A drop of more than 1.17 log viral load at week 1 and viral clearance or decline >3log at week 4 were considered as the earliest predictors of SVR. Conclusion: In genotype 4 patients, while failure to achieve an EVR at week 12 predicts non-response, and RVR at week 1 & week 4 98% guaranteed SVR. These findings further re-enforce the value of week 12 in the course of IFN treatment. Genotype 4 patients who show significant viral clearance (>1.17 log viral load) by the first week of treatment and viral clearance >3log by week 4 are expected to show SVR and would therefore be assigned to a shorter drug regimen lasting for 24 weeks. Those unfortunate cases who do not achieve viral clearance by week 1 or week 4 should not be deprived from treatment but rather given more time till week 12 before being classified as non-responders.

1330 EFFECTS OF CHRONIC HCV INFECTION ON HEALTH-RELATED QUALITY OF LIFE AND FATIGUE: THE ROLE OF LIVER FIBROSIS PROGRESSION

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Background and Aims: Health-related quality of life (HRQoL) is significantly impaired in patients with chronic hepatitis C. However, only few studies have been published investigating the relationship between the stage of liver disease and quality of life and revealed conflicting results. Therefore, we investigated HRQoL and fatigue in patients with chronic hepatitis C virus (HCV) infection in relation to the degree of fibrosis while controlling for the influence of relevant demographic and medical variables. Patients and Methods: HRQoL and fatigue were assessed in a cross-sectional multi-center study including a total of 215 consecutive outpatients (92 women and 123 men, mean age 46.7) with chronic HCV infection using the Medical Outcomes Study 36-item Short-Form Health Survey (SF-36) and the German version of the Fatigue Impact Scale (FIS-D). Applying multiple linear regression analyses, the contribution to the variability of these psychometric scores was evaluated for the degree of fibrosis (Ishak Score) as well as viremia, gender, age, mode of transmission, genotype, and ALT. Results: Concerning HRQoL, there was a strong negative association between the degree of liver fibrosis and the physical SF-36 summary score (p=0.016), mainly due to the subscale “general health” (p=0.046). No such relationship was found with respect to the mental domain of SF-36. These effects were independent of the covariate age, also significantly predicting physical HRQoL (p=0.001). Similarly, the absolute FIS score – including the subscale “physical functioning” (p=0.011) – was significantly increased in patients with advanced fibrosis (p=0.043), indicating a higher burden of fatigue symptoms. A further noticeable finding was the more pronounced impairment of the mental SF-36 summary score (p=0.007) and fatigue (p=0.017) in females. However, the psychometric scores of HRQoL and fatigue were found to be unrelated to viremia, mode of transmission, genotype and ALT levels. Conclusions: The present study suggests a significant association of the physical aspects of both HRQoL (SF-36) and fatigue (FIS-D) with the extent of fibrosis in patients with chronic hepatitis C while viral factors such as viremia and genotype exert no significant effect. Female HCV patients were particularly affected by impaired mental components of HRQoL and fatigue, possibly indicating gender-specific aspects of coping. Our findings stress the need for considering fibrosis stage for the identification and management of HCV patients at risk for reduced physical HRQoL.

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Michael R. Kraus - Consultant/Adviser: Schering-Plough

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1331 RETREATMENT OF TREATMENT EXPERIENCED HCV PATIENTS WITH PEGYLATED INTERFERON ALFA-2A AND THYMOSIN ALPHA-1: POOLED ANALYSIS OF TWO RANDOMIZED CONTROLLED TRIALS

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BACKGROUND: Management of HCV-infected treatment non-responders remains a significant clinical challenge. Early pilot studies suggested that an immunomodulatory peptide, thymosin alpha-1 (TA-1) may play a role in enhanced viral clearance, histologic improvement and sustained viral response (SVR). We performed two parallel randomized controlled trials to evaluate efficacy of TA-1 in this difficult to treat population. METHODS: Both trials had similar designs. Patients were randomized to receive either pegylated interferon alfa 2a 180 mcg (PEG) SQ qwek + TA-1 for 48 weeks, or PEG with placebo TA-1 for 48 weeks. One trial (803) permitted subjects with mild fibrosis through bridging fibrosis (Metavir F1-3) while the other (804) enrolled subjects with more advanced baseline fibrosis (F3 or F4). Each trial planned enrollment of at least 500 subjects with a 1:1 randomization between TA-1 and placebo TA-1. Baseline and post-treatment liver biopsies were obtained. RESULTS: Cumulative enrollment in the two trials was 1061 subjects. Nearly all were genotype 1 (93.5%), and 83% had previously failed treatment with interferon alfa + ribavirin and 17% with interferon alfa alone. HCV RNA viral load at baseline was similar between the two trials and averaged log 6.2 copies/ml. 71% of subjects were male and their mean age was approximately 50 years. By intention-to-treatment analysis (ITT), viral clearance at week 48 occurred in 74/533 (13.9%) of subjects treated with PEG + TA-1 vs. 76/528 (14.4%) in the control arm. SVR was observed in 13 subjects (2.4%) in the active treatment arm and 9 subjects (1.7%) in the placebo arm (not significant). The proportion of patients demonstrating an improvement of Knodell Histologic Activity Score (HAI) by 2 or more points was similar in treatment and control groups. Decreases in circulating CD4 lymphocytes were lower among subjects with more advanced fibrosis who received TA-1 vs those receiving placebo. Treatment emergent toxicities were similar between treatment groups. CONCLUSION: Among treatment experienced subjects with HCV infection, retreatment with PEG + TA-1 resulted in a similar sustained virologic response when compared to use of PEG monotherapy. Studies utilizing PEG, TA-1 and ribavirin are in progress.


1332 U.S. MULTICENTER PILOT STUDY OF DAILY CONSENSUS INTERFERON (INFERGEN) PLUS RIBAVIRIN FOR “DIFFICULT-TO-TREAT” HCV GENOTYPE 1 PATIENTS: TOLERANCE AND ON-THERAPY VIROLOGIC RESPONSE

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Patients in the U.S. with HCV genotype 1 respond poorly to treatment with pegylated interferon alfa and ribavirin (RBV). Consensus interferon (CIFN; interferon alfacon1) demonstrates enhanced activity in vitro compared with other interferons. Genotype 1 patients may benefit from an interferon with increased activity, more favorable viral kinetics from daily dosing, and a longer duration of therapy. Purpose: To determine the efficacy and safety of daily CIFN+RBV for initial treatment of patients with HCV genotype 1 and other “difficult-to-treat” characteristics. Methods: Patients with HCV genotype 1 were prospectively enrolled in 7 Veterans Affairs (VA) and 2 private medical centers. Patients were randomized to treatment with daily CIFN (15 mcg/d sq) and RBV (1-1.2 gm/d PO) given for (A) 52 weeks, vs (B) 52 to 72 weeks (from time of initial virologic response + 48 wks). Treatment is discontinued if HCV RNA is detectable at wk 24. Results: 64 patients initiated treatment (92% male, 33% African American, 78% VA, 67% high viral load; 59% stage III/IV fibrosis, mean body weight 92.5±2.1 kg). ITT analysis of 64 patients with virologic data through 24 wks indicated that 43 (67%) achieved undetectable viral levels; overall 21 (33%) demonstrated a rapid virologic response (RVR) at 4 wks, 16 (25%) were early virologic responders between 8-12 wks, and 6 (9%) were late virologic responders between 12-24 wks. During treatment 27 (42%) patients required CIFN dose reduction and 18 (28%) required RBV dose reduction. Five patients were discontinued at 24 weeks due to virologic non-response. Overall early discontinuation of treatment before 24 wks occurred in 22/64 (34%) patients (9 due to intolerance; 10 due to noncompliance; 1 due to chest pain; and 2 due to cellulitis). This is comparable with the 32% discontinuation rate reported in a large VA cohort treated with interferon alfa-2b+RBV (J Viral Hep 2006;13:242). Final ETR and SVR data are pending. Conclusions: Patients with hepatitis C genotype 1 and “difficult-to-treat” characteristics treated with daily CIFN+RBV demonstrated a high RVR rate, with 33% virus-negative at 4 wks and 67% virus negative by 24 wks. This compares favorably with RVR rates of 19-22% for genotype 1 patients treated with pegylated interferon alfa-2a and ribavirin (Gastroenterology 2006;130:1086 and 131:451). Although adherence and tolerability are limitations, these data indicate that daily CIFN+RBV for initial treatment of U.S. genotype 1 patients have promise primarily via an enhanced RVR rate. (Supported by Valeant Pharmaceuticals; Veterans Affairs Research Service)
Predicted outcomes after 48wks treatment with PegIFN alfa-2a/RBV in G1

<table>
<thead>
<tr>
<th>RBV dose (mg/d)</th>
<th>800</th>
<th>1000 (&lt;75kg) or 1200 (&gt;75kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Predicted median SVR (9, 95% CI)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HCV mono-infection</td>
<td>41 (36-45)</td>
<td>49 (45-53)</td>
</tr>
<tr>
<td>HIV-HCV co-infection</td>
<td>29 (23-35)</td>
<td>37 (29-45)</td>
</tr>
<tr>
<td>Predicted median incidence of anemia (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HCV mono-infection</td>
<td>7 (5-9)</td>
<td>13 (11-15)</td>
</tr>
<tr>
<td>HIV-HCV co-infection</td>
<td>14 (11-17)</td>
<td>22 (18-30)</td>
</tr>
</tbody>
</table>

Anemia=Hgb <10 g/dL; SVR=undetectable HCV RNA (<50 IU/mL) at wk72

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Marcos Pedrosa - Grant/Research Support: Roche; Speakers Bureau: Roche

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OUTCOMES IN HIV-HCV CO-INFECTED GENOTYPE 1 PATIENTS TREATED WITH PEGINTERFERON ALFA-2A (40KD) PLUS RIBAVIRIN (RBV) 1000/1200 MG/D: PREDICTIONS BASED ON A GENERALIZED ADDITIVE MODEL (GAM)
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Background: It is now well established that HCV genotype 1 (G1) mono-infected patients benefit from starting RBV at a dose of 1000/1200 mg/d instead of the lower 800 mg/d dose used in earlier studies. In the APRICOT trial, HIV-HCV co-infected patients were treated with peginterferon alpha-2a (40KD) plus RBV 800 mg/d for 48 weeks. The 800 mg/d dose of RBV was selected to minimize hematologic toxicity and the potential for drug–drug interactions with nucleoside reverse transcriptase inhibitors. In APRICOT, the rate of SVR was 40% overall and 29% in G1 patients. We modeled the probability of SVR and anemia in co-infected G1 patients and predicted the outcomes for treatment with RBV 1000/1200 mg/d. Methods: Binary data from 176 G1 patients in APRICOT were incorporated into an existing generalized additive model (GAM) established with data from 817 mono-infected G1 patients enrolled in two phase III clinical trials. The effect of prognostic factors on SVR and anemia (Hgb <10 g/dL) were analyzed and simulations run with the updated GAM to predict SVR and the incidence of anemia in co-infected G1 patients treated with peginterferon alfa-2a (40KD) plus RBV 1000/1200 mg/d. Uncertainty was quantified by 'bootstrap'ing. Results: After incorporating data from APRICOT, significant predictors of SVR retained in the model included baseline (BL) viral load, RBV dose (mg/kg), age, BL ALT quotient, histological diagnosis (cirrhosis vs no cirrhosis) and HIV status (yes vs no). BL factors predictive of anemia included BL Hgb level, RBV dose (mg/kg), age, sex, HIV status, BL ALT quotient and BL viral load. Use of RBV 1000/1200 mg/d in co-infected G1 patients is predicted to increase the SVR rate by 8% (from 29% to 37%) and the incidence of anemia from 14% to 23% (Table). Conclusions: Our simulations suggest that increasing RBV dose to 1000/1200 mg/d, would improve SVR rates in patients co-infected with HIV and HCV G1. However, higher doses of RBV would also be associated with a higher incidence of anemia. Therefore, Hgb levels should be monitored closely and the RBV doses should be decreased in small decrements to preserve chances for higher end of treatment responses and to prevent relapses.

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HEPATIC SOCS3 EXPRESSION IS STRONGLY ASSOCIATED WITH NON-RESPONSE TO IFN TREATMENT IN CHRONIC HCV
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Background/Aims: The combination of pegylated interferon (IFN) and ribavirin (RBV) is the most effective treatment for chronic hepatitis C (CHC), but response rates vary depending on viral and host factors. We hypothesized that key regulators of the IFN signaling pathway are predictive of treatment outcome. We measured the intrahepatic protein expression of STAT1, SOCS3 and ISG15, each of which play a key role in IFN signaling and are known to be altered in hepatitis C virus (HCV) infection, and correlated their expression with treatment outcome. Methods: 49 unselected patients treated with at least 12 weeks of PegIFN and RBV (36 genotype 1, 13 genotype 2/3) with CHC were analyzed. STAT1, SOCS3 and ISG15 were stained using the immunoperoxidase method on pretreatment liver biopsy samples and quantified by NIH Image J software. Results were expressed as percent area positive. Staining was correlated with either sustained virologic response (responder) or nonresponse/response-relapse (nonresponder). Results: Pretreatment hepatic SOCS3 protein expression was significantly higher in nonresponders than in responders for patients with CHC (21.6±± 2.7 vs 10±±1.3, p<0.001). When compared only among genotype 1 patients, SOCS3 expression remained significantly higher in nonresponders than responders in patients with CHC (23.0±± 2.9 vs 12.6±±1.8, p<0.05). Genotype 1 responders had similar levels of staining as genotype 2/3 responders. There were no clinical, virological and histological factors associated with hepatic SOCS3 expression other than IFN response/nonresponse. The hepatic expression of STAT1 and ISG15 did not differ between nonresponders and responders. Conclusions: Pretreatment intrahepatic SOCS3 levels are correlated with outcomes of interferon-based treatment in patients with chronic HCV. The
finding that SOCS3 levels were comparable among responders irrespective of genotype suggests that SOCS3 may be a more powerful pre-treatment predictor of response than genotype. These data strongly implicate SOCS3 as an important antagonist of the in vivo response to IFN. Prospective validation of SOCS3 as a clinical tool to predict response is warranted.

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1335 EUROPEAN LIVER FIBROSIS (ELF) PANEL OF SERUM MARKERS CAN PREDICT CLINICAL OUTCOME IN A COHORT OF PATIENTS FROM ENGLAND WITH MIXED AETIOLOGY CHRONIC LIVER DISEASE

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Introduction The performance of non-invasive tests has been evaluated against fibrosis on biopsy but use of clinical outcomes as the reference standard would be ideal. The ELF panel of markers (TIMP1, HA, PIIINP) has been shown to have excellent ability to identify significant fibrosis on biopsy. To evaluate ELF performance in predicting clinical outcomes, the original ELF cohort was followed-up at 8.9 years. Methods: Patients recruited to the ELF study at 6 centres were followed up for liver morbidity and mortality by examination of clinical data. Those lost to follow up were followed up for morbidity by questionnaire to the family practitioner. Primary outcome measure was liver related morbidity or death; secondary outcome was all-cause mortality. Results: 448 patients were followed up after a median of 7.7 years representing 99% of those recruited at 6 sites. Median age at infection (p<0.0001) were associated with accelerated fibrosis rate. Body mass index (BMI) (p= 0.018) and increased time interval to first biopsy (“long-term rate”). Linear regression was used to assess the relationship of demographic variables to fibrosis rate. Linearity of long-term fibrosis change over time was assessed using polynomial regression. Results: Of the 161 paired biopsy patients, 136 had historical information allowing estimation of a long term rate of fibrosis prior to therapy: mean time interval to first biopsy 23.6 years; stages: <1 (17.6%, mean 21.4 yrs), 1-2 (71.3%, mean 24.0 yrs), >2 (11.0%, mean 24.8 yrs); overall fibrosis rate .06545 stages/year with “best fit” statistical analysis revealing non-linearity of the stages (p<0.001). Body mass index (BMI) (p= 0.018) and increased age at infection (p<0.0001) were associated with accelerated rates of fibrosis. The mean interval between biopsy samples was 4.1 years; overall stage increased by just 0.168 stages (8.7% decreased, 67.1% stable, 10.6% increased by 0.5 stage and 13.7% by one stage or more), resulting in an overall short term fibrosis rate of 0.03995 stages/year; BMI

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1336 TRACKING THE COURSE OF HEPATITIS C WITH PAIRED BIOPSIES - A CRITICAL ANALYSIS

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Purpose: To determine if paired liver biopsies in following the course of patients with hepatitis C is of value. Methods: Blinded histologic re-examination of liver biopsy samples was undertaken from a cohort of 161 chronic hepatitis C patients who had undergone at least two (non-protocol) liver biopsies. Fibrosis was assessed using the Batts/Ludwig 0-4 staging system, with intermediate stages (e.g. 1.5) being used in an attempt to maximize the sensitivity. Grade and other co-morbid conditions were also recorded. The rate of fibrosis (stage/year) was determined both from the paired samples (“short-term rate”) and from the estimated time of infection by hepatitis C (per history) to the first liver biopsy (“long-term rate”). Linear regression was used to assess the relationship of demographic variables to fibrosis rate. Linearity of long-term fibrosis change over time was assessed via polynomial regression. Results: Of the 161 paired biopsy patients, 136 had historical information allowing estimation of a long term rate of fibrosis prior to therapy: mean time interval to first biopsy 23.6 years; stages: <1 (17.6%, mean 21.4 yrs), 1-2 (71.3%, mean 24.0 yrs), >2 (11.0%, mean 24.8 yrs); overall fibrosis rate .06545 stages/year with “best fit” statistical analysis revealing non-linearity of the stages (p<0.001). Body mass index (BMI) (p= 0.018) and increased age at infection (p<0.0001) were associated with accelerated rates of fibrosis. The mean interval between biopsy samples was 4.1 years; overall stage increased by just 0.168 stages (8.7% decreased, 67.1% stable, 10.6% increased by 0.5 stage and 13.7% by one stage or more), resulting in an overall short term fibrosis rate of 0.03995 stages/year; BMI
The mean rate of fibrosis was detected in 123 untreated patients (57.4%), and men and 0.08 ± 0.02 respectively. Progression of fibrosis scores was detected in all treated patients with sustained virologic response. The rate of progression was non-linear in HCV with accelerated disease over time in untreated patients resulting in complications of chronic liver disease, hepatocellular carcinoma and death. Cohort studies and clinical decisions based on single biopsy estimates of disease progression may underestimate the risk of fibrosis progression.

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1338
DIAGNOSTIC ACCURACY OF THE APRI FOR THE PREDICTION OF HEPATITIS C-RELATED FIBROSIS: A SYSTEMATIC REVIEW
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Background: The development of noninvasive markers of liver fibrosis is a clinical and research priority. The aspartate aminotransferase to platelet ratio index (APRI) is a promising tool with limited expense and widespread availability. Our objective was to systematically review the performance of the APRI in hepatitis C virus (HCV)-infected patients. Methods: An electronic search on Medline, EMBASE, and the Cochrane Library (01/1997-12/2006), supplemented with a manual search, identified studies comparing the APRI with liver biopsy for the assessment of HCV-related fibrosis. Random effects meta-analyses and areas under summary receiver operating characteristic curves (AUC) were examined to characterize APRI accuracy for significant fibrosis (stages 2-4) and cirrhosis. Random effects meta-regression was used to determine the impact of study and patient-related factors on APRI performance. Results: In 22 studies including 4,266 patients (median age, 44 years; 61% male), the prevalence of significant fibrosis and cirrhosis were 47% (range 9-72%) and 15% (range 7-33%), respectively. The summary AUCs of the APRI for these outcomes were 0.76 (95% CI 0.74-0.79) and 0.82 (95% CI 0.79-0.86), respectively. For significant fibrosis, an APRI threshold of 0.5 was 81% sensitive and 50% specific. At a 40% prevalence of significant fibrosis, this threshold had a negative predictive value (NPV) of 80%, but could reduce the necessity of liver biopsy by only 35%. For cirrhosis, a threshold of 1.0 was 76% sensitive and 71% specific. At a 15% cirrhosis prevalence, the NPV of this threshold was 91%. Higher APRI thresholds had sub-optimal positive predictive values except in settings with a high prevalence of cirrhosis. APRI accuracy for significant fibrosis was not affected by the prevalence of advanced fibrosis, or study and biopsy quality. However, the accuracy for cirrhosis was greater in studies including HIV/HCV-coinfected patients. Conclusions: The major strength of the APRI is the exclusion of significant HCV-related fibrosis. Future studies of novel markers should demonstrate improved accuracy and cost-effectiveness compared to this economical and widely available index.

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1337
LONG-TERM MORBIDITY AND MORTALITY OF HCV INFECTION: A 25 YEARS FOLLOW-UP
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Background/Aims: The natural history of HCV is highly variable and not well characterized. Most cohort studies have not been well controlled with serial liver biopsies to estimate true disease progression. The aim of this study was to investigate the long-term clinical and histological outcome of a cohort of untreated and treated subjects followed for 25 years with a known onset of acute HCV. Study design/methods: In this retrospective-prospective longitudinal cohort study, archived serial serum samples from 420 subjects with non A-non B acute hepatitis between 1981-1982 were re-screened for HCV (ELISA, PCR). Of the 385 subjects with proven acute HCV, 36 subjects refused enrolment, 45 had confections and were excluded, and 214 were enrolled and prospectively followed. Clinical, virological and HRQOL evaluations were performed. Liver biopsies were either available at entry (n=49) or were performed at enrollment (n=96). Liver biopsies were repeated6-17 years after the initial biopsies in patients considered for treatment. Ishak score and fibrosis progression rates were determined. The clinical end-points were end-stage disease, hepatocellular carcinoma (HCC), liver transplantation or death. Results: The disease duration ranged between 22-25 years. Of the 385 patients with acute hepatitis C, 127 subjects (33%) had spontaneous persistent resolution. At baseline, none of the 214 patients with chronic hepatitis had clinical decompensation, HCC or significant impairment of HRQOL. Eighty-two patients (38%) had history of interferon therapy or were treated during the study. The baseline mean necroinflammatory and fibrosis scores were 2.8±3.9 and 0.5±0.02 respectively. Progression of fibrosis was detected in 123 untreated patients (57.4%), and 18 patients with no response to treatment. The mean rate of fibrosis progression in untreated patients was 0.19±0.08 in men and 0.08±0.01 in women (p=0.05). Improvement in fibrosis scores was detected in all treated patients with sustained virologic response. The rate of progression was non-linear and higher in older patients. During follow-up significant impairment of HRQOL was reported in 63 subjects (29.4%), end-stage disease in 41 (19%), liver transplantation in 23 (10.7%), HCC in 23 (10.7%). Death due to liver-related causes was reported in 23 patients (11%). Conclusion: Disease progression is non-linear in HCV with accelerated disease over time in untreated patients resulting in complications of chronic liver disease, hepatocellular carcinoma and death.
COMPARISON OF REPRODUCIBILITY OF HISTOLOGY, BLOOD TESTS AND FIBROSCAN FOR LIVER FIBROSIS
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Our aim was to compare the reproducibility of histology, blood tests and Fibroscan for liver fibrosis by comparing the intra-method and the inter-method agreements by taking as histological reference the reading by a senior expert pathologist. Several studies were used. All patients had chronic liver disease. Agreements were evaluated with kappa index (κ) and intraclas correlation coefficient (Ric). Results. 1/ Intra method agreement. Blood tests. In study 1, 33 patients and 12 laboratories were included; Ric was: APRI: 0.897, FibroMeter: 0.942. In study 2, 20 patients and 10 laboratories were included; Ric was: APRI: 0.949, Fibrotest: 0.984, FibroMeter: 0.963. In study 3, 33 patients and 2 laboratories were included; Ric for FibroMeters was: virus: 0.991, alcohol: 0.976, NAFLD: 0.990. Liver biopsy. Intra center agreement. In study 4, the Metavir staging was evaluated in 44 patients by 4 pathologists from an academic hospital: κ=0.59. In study 5, agreement was evaluated between one senior and one junior expert in 157 patients: κ=0.48. Inter center agreement. In 1998 (study 6), agreement was evaluated in 26 patients between an academic senior expert and 10 non-expert out-pathologists: κ=0.13 and Ric=0.69. In 2003 (study 7), 33 patients were evaluated by 8 out-pathologists: κ=0.18 and Ric=0.69. In 2004, the agreement was between one expert from the Metavir group and 6 out-pathologists in 205 chronic hepatitis C (study 8): κ=0.336 and Ric=0.649. Fibroscan. 46 patients and 4 observers were enrolled into the study 9: Ric=0.93. 2/ Comparison between methods. Non invasive tests translated into Metavir F stages. The agreement of FibroMeters was: Ric=0.965, κ=0.874 (study 3). Agreement of Fibroscan was: Ric=0.84-0.89, κ=0.63-0.65 (study 9). Misclassification rates. The misclassification rates for significant fibrosis were: blood tests: FibroMeter: 23.3%, Fibrotest: 27.9%, Hepascore: 27.8% in 825 patients (study 10); liver biopsy: 22.9% against senior expert (study 8), 9.6% against consensus reading (study 5); Fibroscan: 16.5% in 194 patients (study 11), 20% in 65 patients (study 11) and 24.1% in 461 patients (study 13). Conclusions. Intra method reproducibility is as follows: blood test > Fibroscan > histology. Considering the liver interpretation by a senior expert or consensus reading as the reference, the agreement between measurements is as follows: blood test > Fibroscan > histology. In routine practice, the observed misclassification rate of non invasive tests is similar to that of liver biopsy. Finally, liver biopsy should be considered as a specialized tool read by experts whereas non invasive tests are more reproducible in clinical practice.

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HISTOLOGICAL OUTCOMES AFTER 30 YEARS IN UNTREATED IRISH WOMEN WITH CHRONIC HCV GENOTYPE 1B, DO GENETIC FACTORS INFLUENCE?
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Background and Aims: The cohort of Irish women infected with Hepatitis C virus (HCV) genotype 1b in 1977, represent a unique homogenous group to investigate the natural course of HCV infection. We have previously demonstrated strong genetic associations with viral clearance in this cohort. More recently, we have demonstrated minimally progressive disease and histological improvement (after 27 years) in serial liver biopsies. To date we have not investigated whether genetic factors are responsible for the favourable histological outcomes observed in serial liver biopsies. In the current study we aim to describe the natural history of infection after 30 years and to investigate whether genetic factors including HLA Class I and Class II (DRB1 and DQB1) alleles or polymorphisms of TNF-alpha, TGF-beta, IL-10, IL-6, and IFN-gamma genes could predict biochemical or histological outcomes. Methods: The study cohort comprised of 56 untreated women with persistent viremia who had paired or multiple liver biopsies. All were infected with HCV genotype 1b in 1977. Biochemical (alanine aminotransferase [ALT]) and histological assessments in serial liver biopsies were performed. Baseline and sequential biopsy specimens were assessed for grade (increase/decrease of 2 points) and stage change (increase/decrease of 1 point). Genotyping for the various genetic markers was carried out on genomic DNA using polymerase chain reaction sequence-specific primers. Results: The mean age of the 56 women at the time of baseline biopsy was 44.3 [range 23-57]. The mean baseline grade and stage scores were 3.89 [range 2-9] and 0.72 [range 0-3] respectively. Mean number of biopsies was 2.73±0.7 and mean interval to last biopsy was 7.5 years [range 1-13]. Over this time period, grade scores remained unchanged in 51.8%, increased in 28.6% and improved in 19.6% patients; stage scores remained unchanged in 55.4%, increased in 17.9% and improved in 17.9% patients. The mean baseline ALT was 45.5 [range 11-110]. There were positive correlations between baseline ALT, grade and stage and between sequential grade and stage scores. Only DQB1 0203 was associated with increased ALT values (p<0.024). There were no significant associations between genetic factors and histological outcomes. Conclusion: A benign course of HCV genotype 1b infection with 17.9% disease regression was observed in untreated women with paired or multiple liver biopsies 30 years after infection. Genetic factors including HLA Class I and Class II (DRB1 and DQB1) alleles or polymorphisms of TNF-alpha, TGF-beta, IL-10, IL-6, and IFN-gamma genes do not account for this favourable histological outcome.

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1341
HIGHER CAFFEINE CONSUMPTION IS ASSOCIATED WITH Milder FIBROSIS IN PATIENTS WITH CHRONIC LIVER DISEASES

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Introduction: Two recent population-based surveys (NHANES I and III) have reported that higher caffeine consumption was associated with lower risk of elevated ALT levels and lower risk of chronic liver disease (CLD). Aim: To develop an instrument to assess caffeine consumption and evaluate the association of caffeine intake with severity of fibrosis in pts with CLD. Methods: A questionnaire aimed at measuring caffeine consumption over the previous month was developed and administered to all pts undergoing liver biopsy. Modified from a Nurses Health study questionnaire, it asked the frequency of consumption of caffeine-containing foods and beverages, including soft drinks, coffee, tea, cocoa as well as caffeine-fortified drinks, chocolate bars, caffeine-containing medications and alcohol intake. Caffeine consumption was quantified as the average mg of caffeine per day in which one 8 oz cup of coffee = 136 mg. Routine liver tests were obtained at the time of questionnaire completion, and liver histology was scored using a modified Ishak scoring system for activity and fibrosis. Logistic regression was performed to evaluate the association of caffeine with advanced liver fibrosis (Ishak score≥3).

Results: Among the 182 pts (60% male, 58% Caucasian, mean age 48 years), 112 had HCV, 38 HBV, 3 HDV, 21 NASH, 4 PBC and 4 AIH. 137 pts underwent liver biopsy and 30% had advanced fibrosis. The average caffeine intake was 193 mg/day (~1.5 cups of coffee/day). No patient reported more than minimal alcohol intake. Repeat administration of the questionnaire (> 2 weeks after initial) demonstrated consistency in reporting of caffeine intake (Cronbach coefficient alpha = 0.97). Among the entire cohort, after adjusting for other factors known to be associated with fibrosis (age, sex and baseline ALT), caffeine intake ≥ 300 mg/day (~2.2 cups of coffee daily) was associated with reduced fibrosis compared to lesser amounts or no caffeine intake (OR=0.39, 95% CI 0.15-1.03, p=0.057). The effect of caffeine was even more pronounced in pts with HCV. HCV pts with caffeine intake ≥ 300 mg/d were 88% less likely to have advanced fibrosis than pts with lower consumption both by univariate and multivariate regression (adjusted OR=0.22, 95% CI 0.06-0.89, p= 0.03). Higher caffeine consumption was also independently associated with ALT values below the median (58 IU/l) for the HCV cohort (OR=0.37 95% CI 0.15-0.92, p=0.03). Conclusions: Higher caffeine consumption is associated with milder fibrosis in pts with CLD, particularly those with HCV infection.

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LARGE SCALE VALIDATION OF THE SAFE (SEQUENTIAL ALGORITHMS FOR FIBROSIS EVALUATION) BIOPSY IN CHRONIC HEPATITIS C

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Background: Noninvasive methods have been proposed in hepatitis C as surrogates of liver fibrosis but their diagnostic performance is not such to completely substitute liver biopsy. Moreover, large validation studies are still pending. We have recently proposed sequential algorithms that combine AST/Platelets Ratio Index (APRI) and Fibrotest (FT) with liver biopsy, with excellent performance and >50% reduction in liver biopsy needed (J Hepatol 2006;44:686-93). These algorithms (SAFE biopsy) are based on sequential use of APRI and FT, restricting liver biopsy to cases in which these noninvasive markers show insufficient accuracy. Aim: large-scale validation of the SAFE biopsy to identify significant fibrosis (≥F2) by METAVIR and cirrhosis (F4) in hepatitis C. Methods: International, multicenter study of 1978 HCV monoinfected cases (1112 males, 866 females; mean age: 46.9±11.9yrs). FT and APRI were measured at the time of liver biopsy, taken as gold standard. Subgroup analysis was conducted to determine whether age, gender, HCV genotype and BMI modify the performance of SAFE biopsy. Results: Performance of the SAFE biopsy using the cutoff for APRI and FT of the original studies is shown in table. Overall, SAFE biopsy for significant fibrosis saved 47.1% of liver biopsies with >95% accuracy. SAFE biopsy for cirrhosis also had excellent performance and saved 82% of liver biopsies. The performance of SAFE biopsy was excellent across all major HCV genotypes while it was somehow reduced for ≥F2 with age >50yrs (AUC=0.83, p=0.001 vs. overall AUC) and for F4 with BMI>30 (AUC=0.84, p=0.01). Therefore, specific cutoff for FT (0.57 for ≥F2; 0.84 for F4 with BMI>30) were identified, allowing to optimise the performance of SAFE biopsy (AUC=0.87 for ≥F2 with >50yrs; AUC=0.90 for F4 with BMI>30). SAFE biopsy applied to HCV patients with normal ALT showed excellent performance (AUC=0.94 for ≥F2; AUC=0.89 for F4). Conclusions: this large-scale, multicenter validation study confirms that SAFE biopsy is a rational way of combining noninvasive markers with marked reduction in liver biopsies needed to correctly classify liver fibrosis in hepatitis C. This approach can be useful for large scale screening of HCV patients.
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1343 ANTI-LIVER KIDNEY MICROSMO TYPE ONE (αLKM1) POSITIVE HCV-RELATED CHRONIC LIVER DISEASE: PRESENTATION AND FOLLOW UP

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Our study aims to explore presentation and follow up of anti-LKM1 positive HCV-related chronic liver disease (CLD). Methods: 63 patients with anti-LKM1 positive HCV-related CLD (LKM) were matched for age (median 51 years) and sex (M/F = 78/111) with 126 controls with HCV related CLD negative for non-organ specific autoantibodies (NOSA) (C); no differences in viral genotype distribution were observed between LKM and C. The evaluation was made at the time of presentation in our liver unit and after a median follow up of 72 (LKM) and 51 (C) months. NOSA presence was tested by indirect immunofluorescence (IFL) at a 1:40 serum dilution. On frozen hepatic liver sections, HCV-antigens and CD8 lymphocytes were evaluated by an immunoperoxidase technique and their number estimated. Conclusions: Our data indicate a more active disease in LKM patients (higher transaminase levels, more frequent advanced disease at presentation). Higher immunoglobulin and hepatic CD8 cell levels, in association with lower HCV-infected hepatocytes, suggest that at least in part the hepatic damage in LKM could be autoimmune-driven via molecular mimicry mechanisms between HCV polyprotein and CYP450IID6. This hypothesis is supported by the frequent disappearance of anti-LKM1 antibody after antiviral therapy in long term responders (84.6%) compared to 44.4% in non responders/relapsers (p < 0.0743). Although anti-LKM presence doesn’t seem to affect antiviral therapy results, hepatic flares occurring in LKM suggest the importance of a careful monitoring of these patients under treatment.

Results

Disclosures:
The following people have nothing to disclose: Silvia Ferri, Giorgio Ballardini, Valentina Cipriano, Alessandro Granito, Alberto Grassi, Luigi Muratori, Paolo Muratori, Georgios Pappas, Chiara Quarneti, Francesco B. Bianchi, Marco Lenzi
ently associated with the median inter-observer gap (P < 0.001). Four groups (Gr) were defined according to IQR/LSM. Gr1: Both operators found an IQR/LSM < 0.2 (n = 119): median inter-observer gap = 0.15; ICC = 0.69 and kappa index = 0.87; Gr2: At least one of the two operators found an IQR/LSM > 0.2 and < 0.3 (n = 124): median inter-observer gap = 0.21; ICC = 0.68 and kappa index = 0.86; Gr3: At least one of the two operators found an IQR/LSM > 0.3 and < 0.5 (n = 84): median inter-observer gap = 0.23; ICC = 0.64 and kappa index = 0.80; Gr4: At least one of the two operators found an IQR/LSM > 0.5 (n = 42): median inter-observer gap = 0.41; ICC = 0.41 and kappa index = 0.58. The ICC and kappa index of Gr1 were different from those of the 3 other groups (P < 0.05). Conclusion: The reproducibility of transient elastography is excellent. However, IQR/LSM is a key factor of inter-observer agreement. The reproducibility of transient elastography is significantly reduced in patients with IQR/LSM > 0.2. 1) Ganne-Carrié et al. Hepatology 2006;44:1511-7.

1345 THE USE OF NEW NORMAL ALT VALUES DIFFERENTIATE BETTER PATIENTS WITH HBeAg NEGATIVE CHRONIC HEPATITIS B FROM INACTIVE CHRONIC CARRIERS

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Background: Wild type HBeAg-positive CHB, inactive healthy carriers and HBeAg negative CHB are the three most common clinical forms of chronic hepatitis B infection (CHB). Aim: To determine whether the new normal ALT values and lower HBV DNA cut-off levels differentiate better patients with HBeAg negative CHB from inactive chronic carriers. Methods: Of 240 chronic HBV infected patients, 90 patients were tested for HBV DNA levels by PCR-based assay [Cobas Amplicor HBV Monitor, Sensitivity <200 copies/ml]. HBeAg-positive patients with normal ALT (immune tolerant) and patients with co-infection with HCV or HIV were excluded. ALT levels were tested twice during the last six months [new normal values: ULN 30 IU for male, 19 IU for female]. Results: 31 patients were HBeAg-positive, 19 inactive HBsAg carriers (HBeAg negative, ALT normal) and 40 HBeAg-negative CHB [ALT elevation]. There was no significant difference in inflammatory grade (4.6±1.9 Vs 4.4±1.2, P<0.5) and in fibrosis stage (1.3 ±1.4 Vs 1.1±1.3) between HBeAg-positive and HBeAg-negative patients respectively. Serum HBV DNA levels of the HBeAg-negative patients were significantly lower than those of the HBeAg-positive patients (Median 2.0×10E+5 Vs 1.9×10E+11 copies/ml, P < 0.0001) and higher than those of the inactive healthy carriers (Median 2.0×10E+5 Vs 4.1×10E+10 copies/ml, P<0.001). Using the normal new ALT definition, the lowest optimal HBV DNA levels that differentiate between HBeAg-negative CHB from inactive carriers was 50.000 cc/ml (91% accuracy, NPV 95%, PPV 78%, SS 78%, SP 93%) and not the recent suggested HBV DNA cut-off of 5000 cc/ml (70% accuracy, NPV 52%, PPV 76%, SS 75%, SP 57%). The diagnostic accuracy of HBV DNA levels was similar with the 50,000 and 100,000 cut-off level. The serum HBV DNA levels were lower than the cut-off value in 97% (18/19) of the inactive carriers, and in 27% (11/40) of the HBeAg negative CHB patients. If ALT values are less than <30 in men and less than <19 in women and HBV DNA levels <100,000, the risk of having HBeAg-negative CHB is 4%. On the other hand, if ALT values are more than >30 in men and more than >19 in women and HBV DNA levels is more than >100,000, the risk of HBeAg−negative CHB is 80%. Conclusion: using the new normal ALT values, HBV DNA level <100,000 proposed by NIH workshop to characterize inactive carriers is appropriate.

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1346 THE RESULT OF LIVER STIFFNESS MEASUREMENT IS INFLUENCED BY THE SERUM BILIRUBIN LEVEL

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Background/Aim: Although liver stiffness measurement (LSM) using FibroScan is usually well correlated with the fibrosis score on liver biopsy, discrepancies between the results of LSM and fibrosis score could appear in some patients. Until now, except fibrosis score, any factors which could influence the result of LSM were not identified. This study was performed to evaluate whether acute liver injury could affect the result of LSM. Methods: All consecutive patients with acute hepatitis who admitted to our hospital during 6 months were included. Patients with previous or family history of liver disease were excluded. These patients were subjected to FibroScan during admission and followed-up 1-6 months later. At the time of LSM, serum AST, ALT, ALP, GGT and bilirubin levels were examined. For validation, correlation between the LSM and fibrosis score were compared according to the results of biochemical tests in patients who underwent liver biopsy and LSM. Results: Seventy-five patients with acute hepatitis were enrolled [median age, 30 years; M:F; 40:35]. The causes of acute hepatitis were acute hepatitis A in 61 patients, acute hepatitis B in 3, toxic hepatitis in 8, and others in 3. Baseline LSM was 10.6±6.9 kPa (median, 8.8). There was no difference in the results of AST, ALT, ALP and GGT between patients with higher LSM and lower LSM, but bilirubin level was significantly higher in patients with high LSM (P=0.024). Bilirubin level was the only significant correlated factor with LSM in bivariate correlation analysis (Kendall's correlation coefficient, 0.331; P <0.001). LSM decreased to ≤6 kPa in 44 patients during follow-up. The duration for LSM ≤6 kPa was not different according to sex, age, cause of acute hepatitis, the levels of ALT, AST, ALP, and GGT, but it was significantly longer in patients with higher bilirubin level (80±11 days) than patients with lower bilirubin level (36±4 days; P <0.001). Similarly, bilirubin level was the only significant factor on multivariate analysis (OR, 0.265; 95% CI, 0.127-0.525). In 92 patients who performed liver biopsy, Kendall's correlation coefficient between the results of LSM and fibrosis stage was 0.553 and AUROC for ≥F2, ≥F3, and F4 was 0.808, 0.877, and 0.913, respectively. When 7 patients with jaundice were excluded; the improvement of correlation and increase of AUROC were noted (Kendall's correlation coefficient, 0.650; AUROC for ≥F2, ≥F3, and F4, 0.891, 0.915, and 0.923, respectively). Conclusion: Serum bilirubin level seems to affect the result of LSM. Caution is required when interpret the result of LSM in patients with jaundice.

Disclosures:
The following people have nothing to disclose: Yeon Seok Seo, Sang Jun Suh, Yong Dae Kwon, Sanghun Park, Bora Keum, Beom Jin Park, Yong Sik Kim, Yoon Tae Jeen, Hoon Jai Chun, Chang Duck Kim, Ho Sang Ryu, Soon Ho Uhm
Obesity is a contributing factor to the development of steatosis and in treatment failure in chronic HCV. Aims: Evaluate the interaction between obesity and fibrosis progression in a cohort of HCV patients with paired liver biopsies. Methods: We identified all HCV patients with at least two liver biopsies (1997-2006). Patients with insufficient tissue (<10 portal tracts) or lack of body mass index (BMI) were excluded. A hepatopathologist scored 215 biopsies from 102 patients for necroinflammation (modified histologic activity index [HAI]) and fibrosis (Ishak, stage 0-6). A change by at least one histologic stage was used to define progression or regression. Univariable and multivariable logistic regression analysis was performed to assess the association of baseline factors with advanced fibrosis on each of the paired biopsies separately. To assess factors associated with progression between the two biopsies, a Cox regression model was used. Backward elimination method was used to select factors included in the final model. Results: Time between 1st and 2nd biopsy was 1-13.5 years in which 43% of subjects had progression, 29% had no change and 27% had regression. Rate of change between 1st and 2nd biopsy was -0.16 to 0.49 units annually. Older age, higher HAI score and diabetes were independently associated with advanced fibrosis in 1st and 2nd biopsy. Obesity (BMI >30) was the only factor found to be significantly associated with fibrosis progression between 1st and 2nd biopsy by univariable (P=0.023) and multivariable (P=0.006) analysis, independent of antiviral therapy. A Kaplan-Meier plot was constructed (Figure) to outline the association between obesity and fibrosis progression. Conclusions: 1)Older age, higher HAI score and diabetes in chronic HCV patients are independently associated with advanced fibrosis in a cross-sectional single time point biopsy. 2)Using paired biopsy specimens, obesity is a strong predictor of fibrosis progression over a defined time interval between biopsies.

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The following people have nothing to disclose: Anjana A. Pillai, Lisa M. Yerian, Rocio Lopez, Ibrahim A. Hanouneh, Nizar N. Zein

Accurate identification of liver fibrosis using the point-of-care continuous 13C methacetin breath test: a decision making tool in the treatment of patients with chronic HCV infection

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Current treatment of chronic HCV infection is based on the degree of hepatic fibrosis. Liver biopsy has been the gold standard for this assessment. The point-of-care non-invasive BreathID® continuous online 13C methacetin breath test (MBT) reflects hepatic microsomal function (CYP1A2) and was shown to correlate accurately with the degree of hepatic fibrosis. Aim: To develop a treatment algorithm for patients with chronic HCV infection based on detection of the degree of liver fibrosis using MBT. Methods: 100 patients with chronic HCV infection and 100 healthy, age and sex-matched controls underwent 13C MBT following ingestion of 75 mg methacetin. All HCV patients had undergone a liver biopsy within 6 months of performing the MBT. Breath test parameters tested included PDR Peak (percentage dose recovered), PDR Peak Time, and both PDR and CPDR (cumulative PDR) 10, 20, 30 and 60 minutes after ingestion of methacetin, respectively. The correlation between breath test parameters and the Ishak modified histological activity index (HAI) fibrosis score, age, weight and BMI was determined. The ability of MBT to correctly identify liver fibrosis was assessed. Patients with a HAI fibrosis score ≤ 2 or > 2 were defined as non-significant or significant fibrosis, respectively. Results: MBT parameters were significantly correlated with the stage of fibrosis (p<0.0001). By using an algorithm that includes age, PDR Peak, PDR Peak Time, PDR30 and CPDR20-60, 67% of liver biopsies performed in the patient group could have been avoided. This algorithm achieved an area under the curve (AUC) of 0.92, with a sensitivity of 91% and a specificity of 88%, a PPV of 88% and NPV of 91%. Thirty three patients were identified as having significant fibrosis, including 4 false positives; two with a HAI fibrosis score of 2 and an additional two with a score of 1. Thirty four patients were identified as having non-significant fibrosis including 4 false negatives; two with a HAI fibrosis score of 3 and one with a score of 5. There was no correlation between age or BMI and MBT scores for patients with the same histological score. Conclusions: The BreathID® continuous online 13C MBT accurately detects liver fibrosis in patients with chronic HCV infection enabling a non-invasive alternative to liver biopsy. Using the above algorithm, liver biopsy could have been avoided in two thirds of this patient population. MBT is a practical non-invasive tool for decision-making in the evaluation of patients with chronic HCV infection.

Disclosures:

Yaron Ilan - Consultant/Adviser: Other

The following people have nothing to disclose: Gadi Lalazar, Orit Pappo
Iron overload does not affect the quantification of fibrosis by liver stiffness measurement

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Background and aims: Several studies report a close relation between liver stiffness measured by FibroScan (Echosens, France) and fibrosis evaluated by liver biopsy (LB), but the influence of iron overload on liver stiffness measurement (LSM) is unknown. Aim of our study was to evaluate the correlation between liver fibrosis, iron overload and LSM in patients with Homozygous β-Thalassemia (HbT) and in patients with chronic hepatitis C. Methods: 52 patients with HbT (mean age 27 years; 18 HCV RNA positive and 34 HCV RNA negative) and 104 non-thalassemic adults with chronic hepatitis C (mean age 55±10.6 years, mean BMI 27.3±4.7 Kg/m2) underwent LB (mean length of biopsy 17 mm) and simultaneous LSM. Liver inflammation and fibrosis were scored according to METAVIR, steatosis according to Brunt’s score and LIC was measured on fresh tissue cores by atomic absorption spectrometry and was expressed as mg/gr of liver dry weight (normal values < 1.6 mg/gr). Results: The degree of liver fibrosis, the LSM expressed as KPa, and the LIC in patients with HbT and in patients with chronic hepatitis C are reported in the table 1. LSM increased proportionally to the METAVIR stage, with a highly significant relation to fibrosis (rho= 0.70; p <0.001 by Spearman’s test) independently of LIC values (r= 0.16; p= 0.07 by Spearman’s test), both for thalassemics and non-thalassemics patients. At univariate analysis, LSM correlated with histological grading (95% CI: 3.0–37.5; p < 0.0001), presence of steatosis (p < 0.0001) and degree of fibrosis (95% CI: 2.8–37.5; p < 0.0001). At multivariate analysis, presence of steatosis (r= 0.362; p < 0.0007 and fibrosis (r= 0.684; p < 0.0001) remained factors associated with LSM. LSM had excellent value proportionally to the METAVIR stage, with a highly significant relation to fibrosis (rho= 0.70; p <0.001 by Spearman’s test) independently of LIC values (r= 0.16; p= 0.07 by Spearman’s test), both for thalassemics and non-thalassemics patients. At univariate analysis, LSM correlated with histological grading (95% CI: 3.0–37.5; p < 0.0001), presence of steatosis (p < 0.0001) and degree of fibrosis (95% CI: 2.8–37.5; p < 0.0001). At multivariate analysis, presence of steatosis (r= 0.362; p < 0.0007 and fibrosis (r= 0.684; p < 0.0001) remained factors associated with LSM. LSM had excellent value in discriminating patients with F4 fibrosis (i.e. cirrhosis), but was less performing at lower stages of fibrosis. Conclusion: LSM by FibroScan is an adequate and reliable non-invasive tool to diagnose advanced liver fibrosis even in subjects with severe liver iron overload.

Liver fibrosis, LSM (expressed as KPa) and the LIC (expressed as mg/gr of liver dry weight) in patients with HbT and in patients with chronic hepatitis C

<table>
<thead>
<tr>
<th>Liver Fibrosis (METAVIR)</th>
<th>HbT patients (52)</th>
<th>LIC (median, range)</th>
<th>Chronic Hepatitis C patients (66)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(median, range)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>F0</td>
<td>23</td>
<td>4.9 (3.2–10.1)</td>
<td>3.51 (0.50–10.7)</td>
</tr>
<tr>
<td>F1</td>
<td>14</td>
<td>5.1 (2.8–6.8)</td>
<td>2.86 (0.77–22.03)</td>
</tr>
<tr>
<td>F2</td>
<td>14</td>
<td>5.1 (2.8–6.8)</td>
<td>2.86 (0.77–22.03)</td>
</tr>
<tr>
<td>F3</td>
<td>8</td>
<td>7.1 (4.7–10.4)</td>
<td>2.73 (0.23–4.86)</td>
</tr>
<tr>
<td>F4</td>
<td>7</td>
<td>13.9 (13.1–21.3)</td>
<td>4.66 (0.66–11.7)</td>
</tr>
</tbody>
</table>

Disclosures:
The following people have nothing to disclose: Vito Di Marco, Fabrizio Bronte, Daniela Cabibi, Francesca Barbària, Zelia Borsettino, Giuseppe Alaimo, Vincenza Calvaruso, Stefania Ciminnisi, Marcello Capra, Aurelio Maggio, Piero Luigi Almasio, Antonio Craxi

HCV genotype determination in clinical practice: weaknesses of assays based on the 5'-noncoding region and improvement with the core-coding region

Stephane Chevaliez, Magali Bouvier-Alias, Claire Vandervenet, Jean-Michel Pawlotsky; Henri Mondor Hospital, University of Paris 12, Creteil, France.

HCV genotype determination is mandatory to tailor peginterferon-ribavirin treatment dose and duration. Subtype determination is likely to become useful to tailor specific HCV inhibitor therapy and interpret resistance data in the future. In clinical practice, genotype determination is most often based on assays targeting the 5’-noncoding region (5’NCR). However, this region does not discriminate well between the different subtypes of genotype 1 and between genotypes 1 and 6. Objectives: (i) To determine the frequency of erroneous HCV genotype 1 subtype determination with genotyping technologies targeting the 5’NCR. (ii) To assess whether targeting the core-coding region in addition to the 5’NCR improves HCV typing. METHODS: 505 clinical specimens from patients with chronic hepatitis C included in a multicenter national trial, locally considered as genotype 1, have been studied. HCV genotype-subtype determination has been performed by means of three different techniques: (i) the reference method, i.e. direct sequence analysis of a portion of the N558 coding region followed by phylogenetic analysis; (ii) direct sequence analysis of the 5’NCR (Trugene HCV 5’NCR Assay, Siemens); (iii) reverse hybrization with the second-generation line probe assay (INNO-LiPA 2.0, Innogenetics), that includes probes located in the core-coding region in addition to the 5’NCR probes. RESULTS: Among the 505 clinical specimens, the reference method showed that 496 patients were infected with HCV genotype 1, including 233 with subtype 1a, 253 with subtype 1b and 10 with another subtype, and 9 patients were infected with an HCV genotype other than 1. Among the 496 patients infected with HCV genotype 1, sequence analysis of the 5’NCR gave an erroneous subtype in 71 patients (14.3%), including 46 with subtype 1a, 15 with subtype 1b and 10 with other subtypes. In the second-generation line probe assay, the subtype was erroneous in only 19 patients (3.8%), including 4 with subtype 1a, 5 with subtype 1b and 10 with other subtypes. Among the patients infected with an HCV genotype other than 1, 6 were infected with HCV genotype 6 and all of them were correctly classified by the second-generation line probe assay. CONCLUSION: HCV genotyping assays targeting the 5’NCR misclassify HCV genotype 1 subtypes in nearly 15% of cases. Using the second-generation line probe assay, that contains probes targeting both the 5’NCR and the core-coding region, improves the accuracy of HCV genotype 1 subtyping and differentiates among genotypes 1 and 6. However, approximately 4% of the samples remain mistyped with this technique.

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The following people have nothing to disclose: Stephane Chevaliez, Magali Bouvier-Alias, Claire Vandervenet, Jean-Michel Pawlotsky.
1351
SERUM LEVELS OF SH2A, A SECRETED FORM OF THE ASIALOGLYCOPROTEIN RECEPTOR, AS A NON-INVASIVE SENSITIVE MARKER FOR LIVER FUNCTION
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Study’s purpose: We have evaluated the possible use of a soluble secreted form of the human asialoglycoprotein receptor (ASGPR sh2a) as a new non-invasive marker for the sensitive assessment of liver function. The current markers (prothrombin time, albumin and others) can only detect liver dysfunction in advanced disease. On the other hand, blood levels of enzymes such as alanine and aspartate aminotransferases (ALT and AST) indicate liver damage and not function and can appear normal in many cases of liver disease or abnormal in a wide range of non-hepatic diseases. sh2a is liver-specific as it is secreted to the serum exclusively by hepatocytes. Methods: We have measured sh2a levels in serum using a monoclonal antibody and an ELISA assay that we developed, comparing with routine liver function tests. Results: We determined in a series of double blind studies that sh2a was present at very constant levels in serum from 57 out of 63 healthy control individuals. The <10% of controls that showed abnormal levels of sh2a also revealed borderline levels for one or more of the established liver function markers. A study of 43 HCV patients showed abnormal sh2a values in 25% of those at fibrosis stage 0, 37% of those at stage 1, 57% at stages 2-3 and 100% at stage 4 (cirrhosis). The combined analysis of sh2a levels and those of ALT and AST allows prediction of fibrosis stage. Conclusions: sh2a appears to be a uniquely sensitive marker of liver function with potential widespread use. In combination with routine markers it could also be used for non-invasive determination of fibrosis stage.

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1352
COMPARISON OF A NEW ASSAY FOR HEPATITIS C VIRUS (HCV) GENOTYPING TARGETING BOTH 5’NC AND NS5B GENOMIC REGIONS, WITH REVERSE HYBRIDIZATION AND SEQUENCING METHODS
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Responsiveness to current combined therapy varies depending on genotype. Most genotyping methods used in clinical settings target the conserved 5’NC region, while sequencing and phylogenetic analysis of more variable genomic regions, such as NS5b, is considered the reference method. Our aim was to evaluate a new assay based on real-time PCR targeting the NS5b region for genotype 1 and the 5’NC for genotypes 2 to 6 (Abbott Diagnostic) in comparison with 5’NC reverse hybridization (InnoLiPA HCV II, Innogenetics) and 5’NC sequencing (Trugene HCV 5’NC, Bayer Healthcare). After RNA extraction from serum, a total of 295 specimens were tested by real-time PCR (method 1); 223 of them were also typed by 5’NC sequencing (method 2) and 89 by hybridization (method 3) using 5’NC amplicons from Cobas Amplicor (Roche).
Sequencing and phylogenetic analysis of an NS5b fragment was used to resolve discrepant results. Suspected multiple genotype infections were confirmed by a method based on PCR-cloning and pyrosequencing of a 5’NC region. A genotype call was obtained by method 1 on 289 (97.97%) specimens, while one genotype 1, three genotypes 2, and two genotypes 3 were indeterminate. Concordance at the genotype level was high among methods 1 and 2 (214/220; Cramer’s V=0.849, p<0.005) and 1 and 3 (85/86; k=0.982, p<0.05), while concordance between methods 2 and 3 was total (17/17, k=1). Among 8 discordant results, five mixed-genotype infections were confirmed by cloning and pyrosequencing or Quality Control for Molecular Diagnostics (QCMD). Since method 1 can only detect 1a, 1b, 2a and 2b subtypes, concordance at the subtype level was not assessed for genotypes 3 to 5. Regarding genotype 1 subtyping efficiencies were 100%, 77.27%, and 73.91% for methods 1, 2, and 3, respectively. However, there were 11/101 discordants between methods 1 and 2. According to NS5b sequencing, method 1 was predominantly correct. Only 2/34 discordants were seen between methods 2 and 3. Regarding genotype 2, subtyping efficiencies were 100%, 45% and 92% by methods 1, 2 and 3, respectively. Analysis of discordant results (16/17) by NS5b sequencing revealed a putative new subtype within genotype 2, and that most subtype calls were not correct. Although only sequencing-based methods provide the possibility of identifying new variants, the real-time PCR method is quick, easy, and simple to interpret, thus providing a good single-step alternative to more time-consuming assays. While correlation with the other methods at the genotype level was high, being based on the NS5b for genotype 1 identification, the real-time PCR method performed better than the other two at assigning 1a and 1b subtypes.

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The following people have nothing to disclose: Elisa Martro, Victoria Gonzalez, Andrew Buckton, Veronica Saludes, Ramon Planas, Vicenç Ausina

1353
NATURAL HISTORY OF HEPATITIS C VIRUS INFECTION IN HIV-INFECTED INDIVIDUALS IN THE ERA OF HAART: SYSTEMATIC REVIEW AND META-ANALYSIS
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Our objectives were to estimate stage-specific hepatic fibrosis progression rates in HIV/hepatitis C virus (HCV) coinfected individuals and to determine factors associated with fibrosis progression in this population, including the impact of HIV on HCV disease progression. A systematic review of published prognostic studies was undertaken. Study inclusion criteria were: i) HIV and HCV infections determined by serological assays; ii) information about age at assessment of liver disease or HCV acquisition; iii) duration of HCV infection; and iv) histological fibrosis staging (F0-F4) and/or clinical diagnosis of cirrhosis. Annual stage-specific transition probabilities (F0→F1, F3→F4) were derived using the Markov maximum likelihood estimation method and a meta-analysis was performed using both fixed
and random effects models. The impact of covariates – study design (cross-sectional/retrospective, prospective, retrospective-prospective), study setting (clinical/community-based), age at/duration and mode of HCV infection, gender, alcohol consumption, HCV genotype and RNA, CD4 cell count, and highly active antiretroviral therapy (HAART) on fibrosis progression was evaluated using meta-regression models. Pre-HAART and HAART era cirrhosis rates were compared in studies reporting both HCV groups. Sixteen of 65 studies reviewed fulfilled the inclusion criteria. Mean (95% CI) fixed effects annual transition rates of the HIV/HCV coinfected individuals (82% injection drug users) were: F0→F1 0.119 (0.114, 0.125); F1→F2 0.118 (0.112, 0.125); F2→F3 0.156 (0.145, 0.169); and F3→F4 0.122 (0.109, 0.137). Random effects estimates showed comparable results. The mean (95% CI) probability of cirrhosis after 20 and 40 years was 25% (22%, 27%) and 77% (74%, 79%), respectively. All covariates of interest were significantly associated with fibrosis progression from F0→F1, while study setting and HCV RNA positivity were associated with progression from F3→F4. Overall, the rate ratio (95% CI) of cirrhosis between HIV/HCV coinfected and HCV monoinfected individuals was 2.1 (1.8, 2.5): 1.7 (1.1, 2.7) in the pre-HAART era (n=6); and 2.2 (1.9, 2.6) in the HAART era (n=10). In conclusion, our predicted estimates for cirrhosis in HIV/HCV coinfected individuals appear to be higher than published estimates for HCV monoinfection. This study improves on previous studies by using a method that does not require the assumption of constant transition rates between stages and taking into account the effects of study design and setting, clinical factors, and HAART on disease progression.

Disclosures: The following people have nothing to disclose: Hla Hla Thein, Qilong Yi, Gregory J. Dore, Murray D. Krah

1354 VALIDATION OF SIMPLE INDEXES (FIB-4, APRI, FORNS INDEX) AND PLATELET COUNT FOR NON INVASIVE PREDICTION OF LIVER FIBROSIS IN HIV-HCV COINFECTED FRENCH PATIENTS

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Background: Several non-invasive indexes using routinely available parameters have been proposed recently for the prediction of liver fibrosis in patients with chronic hepatitis C. However, little information regarding the validity of these indexes in human immunodeficiency virus (HIV)-HCV coinfected patients is available. Aim: to determine the diagnostic performance of FIB4, APRI, FORNS index and platelet count for the prediction of liver fibrosis in HIV/HCV coinfected patients. Methods: 200 HIV/HCV co-infected patients (male 66%, age 40±6 yrs) of the ANRS CO3 Aquitaine Cohort who underwent liver biopsy between 1999 and 2005 and had complete data to validate all the considered tests, were studied. Liver fibrosis was assessed using METAVIR scoring system and diagnostic performance by measuring areas under the ROC curve (AUROC). Results: Significant fibrosis (F2+F3+F4) was present in 157 patients (78.5%) and cirrhosis (F4) in 19.5%. Performance of the different methods using the published cut-offs are shown in the table. Conclusion: Overall, the diagnostic accuracy of these indexes was lower in HIV-HCV coinfected patients than in HCV monoinfected patients, particularly for the diagnosis of significant fibrosis. However, the use of FIB-4, APRI or platelet count may obviate the need for liver biopsy for the diagnosis of severe fibrosis-cirrhosis in up to 55% to 85 % of cases in HIV- HCV coinfected patients.
rhosis. The AUROC values for platelet count are similar to those for indirect fibrosis markers for F2 or more or F4 (Coco J Viral Hep 2007;14:360). The use of APRI is better than platelet count for non cirrhotic fibrosis, but has the same AUROC as platelet count in cirrhosis.

**Characteristic of patients**

<table>
<thead>
<tr>
<th></th>
<th>All patients (n=333)</th>
<th>Palermo pts (n=98)</th>
<th>London pts (n=135)</th>
<th>P</th>
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</thead>
<tbody>
<tr>
<td>Maltes</td>
<td>187/56/9 (6)</td>
<td>109/55/8</td>
<td>78/58/8</td>
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</tr>
<tr>
<td>Age (years)</td>
<td>55 (50-61)</td>
<td>56 (50-61)</td>
<td>54 (50-61)</td>
<td>n.s.</td>
</tr>
<tr>
<td>AST/ALT (U/L)</td>
<td>71 (60-112)</td>
<td>74 (60-112)</td>
<td>67 (50-161)</td>
<td>n.s.</td>
</tr>
<tr>
<td>ALT/ALT (U/L)</td>
<td>92 (79-135)</td>
<td>96 (79-135)</td>
<td>84 (60-135)</td>
<td>n.s.</td>
</tr>
<tr>
<td>Platelet count (per/µL)</td>
<td>189 (20-457)</td>
<td>168 (20-345)</td>
<td>281 (68-457)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>APRI</td>
<td>1.3</td>
<td>1.5</td>
<td>1.0</td>
<td>0.001</td>
</tr>
<tr>
<td>Stiffness (KPa)</td>
<td>13.0 (3-75)</td>
<td>14.6 (3-75)</td>
<td>10.83 (2-33)</td>
<td>0.001</td>
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<tr>
<td>Cirrhosis</td>
<td>106/32/10</td>
<td>74/37/10</td>
<td>32/24/9</td>
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The following people have nothing to disclose: Vincenzo Calvaruso, Sergio Mai-
mone, Fabrizio Bronte, Laura Mairelli, Vito Di Marco, Pinelopi Manousou, Alice Corbani, Elias Xirochakis, Alexandros Sigalas, Geoffrey M. Dusheiko, Antonio Crali; Andrew K. Burroughs

1356 LIVER STIFFNESS MEASUREMENT IN PATIENTS WITH CHRONIC HEPATITIS B IS NOT AS USEFUL AS THAT IN PATIENTS WITH CHRONIC HEPATITIS C FOR THE ASSESSMENT OF LIVER FIBROSIS

Yeon Seok Seo1, Eun Sun Kim1, Yong Dae Kwon1, Sanghun Park1, Bora Keum2, Beom Jin Park2, Yong Sik Kim1, Yoon Tae Jeen1, Hoon Tae Jeon1, Hoon Jai Chun2, Chang Duck Kim2, Ho Sang Ryu2, Soon Ho Um2; 1Internal Medicine, Korea University College of Medicine, Seoul, South Korea; 2Radiology, Korea University College of Medicine, Seoul, South Korea

Background/Aim: Recent studies suggested that liver stiffness measurement (LSM) using Fibroscan is noninvasive and useful for assessment of liver fibrosis. However, most studies only enrolled patients with chronic hepatitis C (CHC) and it is uncertain whether LSM would be useful in patients with chronic hepatitis B (CHB). This study was performed to evaluate the efficacy of LSM for the evaluation of liver fibrosis in patients with chronic hepatitis B and C. Methods: Patients with CHB or CHC who underwent liver biopsy were enrolled in this study. Both liver biopsy and LSM were performed in a day. Interpretation of the fibrosis score was carried out by an experienced pathologist using the Metavir scoring system (F0-F4). LSM was performed by Fibroscan. Efficacy of LSM and optimal cutoff values for fibrosis stage assessment were determined by a receiver-operating characteristics (ROC) curve analysis. Results: Ninety-one patients with CHB (n=64) or CHC (n=27) were enrolled. Median age was 40 (39±13; 14-68) years (M:F, 61:30). Fibrosis score on liver biopsy was F0-1 in 27 patients, F2 in 27, F3 in 26, and F4 in 11. Serum ALT and bilirubin level was 134.7±191.2 (median, 58) IU/L and 0.8±0.4 (median, 0.8) mg/dL, respectively. LSM was well correlated with fibrosis score (Kendall’s correlation coefficient: 0.617; p<0.001) in all patients. The areas under the ROC curve were 0.901 (95% CI, 0.839-0.963) for patients with significant fibrosis (F≥2), 0.899 (0.832-0.966) for patients with severe fibrosis (F≥3) and 0.882 (0.811-0.955) for patients with cirrhosis (F = 4). Optimal LSM cutoff values were 7.15 kPa for F≥2, 11.9 kPa for F≥3, and 13.85 kPa for F = 4. LSM was better correlated with fibrosis score in patients with CHC (0.773; p<0.001) than in patients with CHB (0.557; p<0.001). Furthermore, the areas under the ROC curve were larger in patients with CHC (0.944, 0.982, and 0.958 for F≥2, F≥3, and F4) than in patients with CHB (0.881, 0.863, and 0.850). The optimal cutoff values for F≥2 and F≥3 were similar in patients with CHC (7.05 and 11.4 kPa) and CHB (7.15 and 10.75 kPa), but sensitivity and specificity was superior in patients with CHC. Conclusions: LSM is a simple, safe and effective method for assessing liver fibrosis in patients with chronic viral hepatitis. The efficacy of LSM for the assessment of liver fibrosis was superior in patients with CHC than in patients with CHB.

Disclosures:
The following people have nothing to disclose: Yeon Seok Seo, Eun Sun Kim, Yong Dae Kwon, Sanghun Park, Bora Keum, Beom Jin Park, Yong Sik Kim, Yoon Tae Jeen, Hoon Jai Chun, Chang Duck Kim, Ho Sang Ryu, Soon Ho Um

1357 FIB4 USEFULNESS FOR MEASURING FIBROSIS CHANGES FOLLOWING ANTI HCV VIRAL TREATMENT

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Background: Liver biopsy still remains the key factor for evaluating the impact of anti-viral treatment on fibrosis. As FIB4 is a non invasive biological marker of fibrosis having a good discriminating power at time of initial diagnosis (i.e before any treatment), we wonder whether it could help to appreciate the pathological changes following therapy. Patients and methods: We selected from our file two hundred and twenty-nine HCV infected patients treated by combined therapy in whom a pre- and a post-treatment liver biopsy has been performed. They were 133 males and 96 females aged of 45 ± 11y. Mean time between treatment withdrawal and subsequent biopsy was 1.5 ± 1.1y and that between pre- and post-biopsy 2.6 ± 10days). FIB4 values before and after treatment were analysed according to changes in fibrosis score (Metavir) and to the response to treatment (positive or negative HCV RNA 6 months after therapy) at time of post-biopsy using variance analysis and non-parametric test for paired values. Results: As compared to pre-treatment values, mean post-treatment FIB4 values slightly decrease in sustained responders (n= 57 ; 1.4 ± 1.0 vs 1.2 ± 0.5, NS) while they significantly increased in non-responders (n= 172 ; 1.7 ± 1.7 vs 2.2 ± 2.2, p=0.004). Fibrosis score did not change in 117 (51%) patients. In this group, FIB4 values significantly increased in non responders (n= 85 ; 1.9 ± 0.3 vs 2.2 ± 0.20, p = 0.02) while it did not change in sustained responders (n= 32 ; 1.3 ± 0.3 vs 1.2 ± 0.4, NS). In 45 patients (19.6%), the fibrosis score increased of at least one unit: mean FIB4 value increased in the 41 non-responders to therapy (1.5 ± 1.0 vs 2.7 ± 2.3, p=0.001) and decreased in the 4 responders (2.2 ± 1.7 vs 1.1 ± 0.5, NS). Finally, in the 67 (29.2%) patients in whom the fibrosis score decreased of at least one unit, mean FIB4 value remained stable in non-responders (n= 46 ; 1.5 ± 1.5 vs 1.5 ± 1.4, NS) while it slightly decreased in responders to therapy (1.4 ± 0.8 vs 1.2 ± 0.5, NS). Multivariate analysis showed that the response to treatment was the single parameter significantly (p=0.007) associated to FIB4 variation after treatment. Conclusion: FIB4 variations following therapy are related to response to treatment, a model which fits well with the mathematical FIB4 formula mainly based on transaminase activities. However, individual changes in fibrosis scores related to treatment efficacy cannot be confidently evaluated using FIB4 in a given patient.
1358 EVOLUTION OF HEPATITIS C VIRUS (HCV) VIREMIA IN PROSPECTIVELY FOLLOWED INTRAVENOUS DRUG USERS (IVDUs) WITH DOCUMENTED ANTI-HCV ANTIBODY SEROCONVERSION PARTICIPATING IN A SWEDISH NEEDLE-EXCHANGE PROGRAM

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Background: The dynamics of HCV viremia following acute HCV infection have mostly been studied in cases of symptomatic acute hepatitis C, which represent a minor proportion of incident HCV infections. We used sera drawn approximately every 3-6 months for surveillance of blood-borne viruses among participants in a needle-exchange program (NEP) to assess the longitudinal evolution of HCV viremia in incident HCV infection. Methods: Out of 1150 newly registered participants in a NEP in Southern Sweden, 1997-2005, 198 cases of anti-HCV antibody body seroconversion were identified during longitudinal follow-up. The last anti-HCV negative, the first anti-HCV positive, and one sample obtained one year after seroconversion, were tested for HCV RNA load using real-time PCR (Roche Taqman) at 1/10 serum dilution (detection limit 150 IU/mL). Results: HCV RNA was detected in 81 (41%) of anti-HCV negative pre-seroconversion sera. HCV viremia was detected in the 1 year sample in 145 cases (73%), indicating probable establishment of chronic infection. Eight patterns of longitudinal HCV viremia were observed (Median viral load, see Table): 1) undetectable HCV RNA in all samples (17; 8.6%) 2) undetectable HCV RNA pre-seroconversion and at one year, detectable HCV RNA in seroconversion sample (16; 8.1%) 3) undetectable HCV RNA in first two samples, detectable HCV RNA at one year (19; 9.6%) 4) undetectable HCV RNA pre-seroconversion, detectable HCV RNA in both later samples (65; 33%) 5) detectable HCV RNA pre-seroconversion, both later samples undetectable HCV RNA (7; 3.5%) 6) detectable HCV RNA in first two samples, undetectable HCV RNA one year later (13; 6.6%) 7) detectable HCV RNA pre-seroconversion and at one year, undetectable HCV RNA in first post-seroconversion sample (17, 8.6%) 8) detectable HCV RNA in all samples (44; 22%). Conclusions: Several different patterns of HCV viremia were observed in acute hepatitis C in this cohort of IVDUs, most of whom had subclinical acute HCV infection. Chronic infection developed in 73% of cases and HCV viremia was detected in 41% of the last anti-HCV negative pre-seroconversion samples.

Median viral HCV RNA load*(IU/mL) during follow-up in IVDUs with anti-HCV antibody seroconversion.

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Disclosures: The following people have nothing to disclose: Bertrand Nalpas, Hélène Fontaine, Anais Vallet-Pichard, Vincent Mallet, Virginie Verkarre, Stanislas Pol

1359 FREQUENCY AND SIGNIFICANCE OF F-ACTIN ANTIBODIES (FAA) IN HIV/HCV CO-INFECTED PATIENTS

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Background: Autoantibodies are commonly observed in chronic HCV. Smooth muscle antibodies (SMA) are heterogeneous antibodies reactive to various cytoskeletal elements, such as actin, desmin, and tubulin. F-actin antibodies (FAA) are reportedly sensitive and specific for autoimmune hepatitis (AIH) and believed to be rare (<5%) in viral hepatitis. The presence of FAA predicts a subset of AIH patients with aggressive disease with increased mortality and liver failure requiring transplantation. The recent FDA approval of an ELISA detection system has made FAA testing available for routine clinical use. Hypothesis: Due to non-specific B-cell activation, HIV infection hypergammaglobulinemia and increased risk for autoimmune disorders. We hypothesized that HIV/HCV co-infected patients were more likely than HCV monoinfected controls to develop FAA and that FAA positivity would be associated with more advanced liver disease. Methods: 40 HIV/HCV co-infected patients and 51 HCV monoinfected controls were evaluated at a large academic hospital in New York City from June 2005 to June 2007. Patients randomly selected for inclusion had serologic testing for FAA and biopsy proven viral hepatitis without autoimmune features. Retrospective chart review was performed and extracted data included age, gender, HCV genotype, treatment history, serum protein-albumin gradient, ANA, and FAA. Using the Metavir scale, histologic data was evaluated for the presence of advanced inflammation (grade > 3) and fibrosis (stage > 3). Results: Co-infected patients and monoinfected controls possessed similar demographic and baseline characteristics with respect to mean age (49 vs. 51 years), genotype 1 (84% vs. 88%) and treatment status (84% vs. 88% treatment naive). Women were more prevalent in the coinfected group (65% vs. 45%). FAA were frequently observed in both co-infected (45%) and monoinfected controls (25%) (p =0.053). In HIV/HCV coinfected, the presence of FAA was associated with increased necroinflammatory grade > 3 (39% vs. 5%, OR 2.3, p = 0.02) and increased risk of advanced fibrosis stage > 3 (56% vs. 23%, OR 2.1, p = 0.03). For the HCV monoinfected patients, there was a trend towards increased disease activity with FAA positivity, however this association failed to reach statistical significance for both inflammation (31% vs. 23%, OR 0.5, OR > 0.5) and fibrosis (62% vs. 35%, OR 1.69, p > 0.05). Conclusion: FAA were observed in mono- and coinfected HCV patients at a frequency much higher than the reported literature. FAA may be markers of high grade inflammatory activity and/or fibrosis, especially in HIV/HCV coinfection.

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CAUSE OF DEATH IN PATIENTS WITH HEPATITIS C (HCV)-MIXED CRYOglobulinemia (MC) Vasculitis

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Patients and Methods: Inclusion criteria: 1) chronic HCV infection (HCV RNA+), 2) signs of MC vasculitis with the triad of purpura-arthritis-asthenia and sometimes renal or neurological involvement. The 23 patients who died between 1989 and 2006 were compared to 74 consecutive HCV MC patients followed during the same period in the same department who survived. Logistic regression analysis was used to determine independent prognostic factors. Results: Main characteristics of HCV MC patients who died were: 17 (65%) males, mean age 66 yrs, HCV infection duration 16 yrs (2-35), genotype 1 (n=17, 74%). Metavir score Activity=1.3 (0-3), Fibrosis=2.3 (0-4). Associated conditions: cardiovascular (7), alcoholism (4), autoimmune disease (2), chronic lung disease (2), depression (1). MC vasculitis signs at presentation were: peripheral neuropathy (n=19, 83%), purpura/skin ulcers (13, 57%), arthralgia (11, 48%), glomerulonephritis (9, 39%), central nervous system (3, 13%), digestive tract (2, 9%), poor general condition (11, 48%). MC was type II (n=18, 78%), creatininemia 162 micromol/l (58-630) with 9 patients having a renal insufficiency. After HCV treatment [IFN, PegIFN, Ribavirin], the virological response was: non-response (n=16), sustained (n=2), response-relapse (n=2). Other treatments included: steroids (n=15, 65%), plasmapheresis (n=11, 48%), immunosuppressors (n=9, 39%). After treatment, 17/23 patients had a clinical response of vasculitis features [complete (5), partial (12)]. Patients died 66 months (16-176) after the first sign of vasculitis. Main causes of death were [multiple for 6 patients]: infection (38%) [pulmonary (3), skin (2), digestive (3)], chronic liver disease (32%) [cirrhosis (2), gastrointestinal bleeding (1)], hepatocarcinoma (4), cardiovascular (18%) [myocardial infarction, pulmonary oedema, sudden death], vasculitis (18%) [cerebral, gastrointestinal], end stage renal failure (9%) and cancer (9%) [T cell lymphoma, lung]. In multivariate analysis, two factors were associated with the risk of death, i.e. a central nervous system involvement [HR=7.1; 95%CI 1.8-28.4; p=0.006], and immunosuppressors use [HR=4.4; 95%CI 1.8-10.8; p=0.001], whereas a good virological response to HCV treatment at month3 was protective [HR=0.4; 95%CI 0.1-0.9; p=0.04]. Conclusion: Death in patients presenting with HCV MC vasculitis is mainly due to infection, chronic liver disease, cardiovascular disease, and vasculitis. The risk of death is independently associated with a central nervous system involvement and the use of immunosuppressors, whereas an HCV virological response at month3 of HCV treatment is a positive prognostic factor.

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The following people have nothing to disclose: Samy Scerra, Dan Landau, David Saadoun, Mathieu Resche-Rigon, Aurélien Delluc, Damien Sene, Jean Charles Piete, Patrice Cacoub

INCIDENCE OF TYPE 2 DIABETES MELLITUS AND GLUCOSE ABNORMALITIES IN TREATED PATIENTS WITH CHRONIC HEPATITIS C INFECTION: RESULTS OF A CONTROLLED FOLLOW-UP STUDY

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Background: there is evidence that insulin resistance is an unfavourable prognostic factor in patients treated for chronic hepatitis C (CHC): incidence of type 2 diabetes mellitus (DM) and impaired fasting glucose (IFG) appear increased among patients not responding to antiviral therapy. Moreover, CHC is a high-risk condition for developing type 2 diabetes. Aim: to compare the incidence of glucose abnormalities (IFG or DM) between HCV+ patients with or without a long term virological response (LTR) after antiviral therapy. Methods: all 262 HCV+ patients enrolled by our Center in therapeutic randomised controlled trials with IFN/Peg-IFN +/- ribavirin from 1991 to 2001, whose baseline sera were stored at –80°C Celsius and who accepted to be retested, were considered. Patients with baseline diabetes or fasting blood glucose >100 mg/dl were excluded (n=38). All patients had baseline data regarding age, sex, viral load, genotype, histologic staging, glucose, cholesterol, triglycerides, BMI. Baseline insulin was measured in the stored serum and HOMA-IR was calculated. Patients were retested for transaminases, HCV-RNA, glucose, cholesterol, triglycerides; presence or absence of liver cirrhosis was detected by liver biopsy and/or liver elastography at the end of follow-up. Incidence of IFG or DM at the end of follow-up was compared between patients with LTR (HCV-RNA-negative 3 years after the end of therapy) and non-responders (NR, persistently positive HCV-RNA). Results: after a median follow-up of 8.0 years (range 3-16), the cumulative risk of DM (n=9) or IFG (n=22) among the 224 HCV+ included patients was 13.8% (95%CI=9.9-19.0), while the incidence rate (x 1000 person-years) was 18.6 (12.6-26.4). The risk was lower between LTRs (12/110, 10.9%) compared to NRs (19/114, 16.7%) (Odds Ratio, OR=0.61, CI=0.28-1.33, p=0.22). To adjust the comparison of LTRs vs NRs for potential confounders, we used a logistic regression model including baseline risk factors for diabetes and predictors of poor response (age, sex, HOMA-IR, BMI, familiarity for diabetes, HCV genotype-1, high viral load, cirrhosis). The adjusted OR for IFG or DM was 0.89 (CI=0.32-2.51, p=0.82). Other important risk factors for IFG-DMM were: age (OR=1.05 x year, CI=1.01-1.10), BMI (OR=1.21, CI=1.04-1.42) and familiarity for diabetes (OR=4.19, CI=1.77-9.95). Conclusions: after adjustment for baseline risk factors, incidence of glucose abnormalities was similar between LTRs and NRs. Our data didn’t add evidence to the hypothesis of a significantly higher incidence of diabetes among HCV patients not responding to therapy, but much larger studies are needed to exclude this possibility.

Disclosures:
The following people have nothing to disclose: Chiara Giordanino, Elisabetta Bugianesi, Antonina Smedile, Alessia Ciancio, Maria Lorena Abate, Antonella Olivero, Rinaldo Pellicano, Maurizio Cassader, Roberto Gambino, Giovannina Ciccone, Mario Rizzetto, Giorgio M. Saracco
1362

CPY1A1 POLYMORPHISM IS RELATED WITH FIBROSIS IN CHRONIC HEPATITIS C

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Background: Hepatitis C virus (HCV) infection is a worldwide health problem. Chronic HCV is characterized by progressive liver fibrosis; progression to cirrhosis takes place over a course of 20-30 years. There is evidence that genetic factors have an important role in the progression of liver fibrosis. Among these factors, cytochrome P-450 complex of enzymes (CYPs) seem to be important. CYPs are involved in the metabolism of drugs, chemicals and endogenous substrates. Genetic polymorphism of CYPs with modified activities have been showed to be associated with pathogenesis of several liver diseases. Aim: To analyze whether the polymorphisms of CPY1A1, CPY1A2, CPY2D6 and CPY2E1 genes are associated with liver fibrosis in chronic hepatitis C infection. Patients and Methods: One hundred and twenty five patients with chronic hepatitis C were included (87 males, 38 females, mean age ± SD: 49.9 ± 10.6 years). Grade and stage of liver fibrosis was assessed according to Knodell Index, ranging from F0 to F4. Patients were divided into 2 groups: Fibrosis 0-2 (n=90) and Fibrosis 4-3 (n=35). Genomic DNA was extracted from peripheral blood and polymorphisms of the CYP genes were analyzed by polymerase chain reaction (PCR) followed by restriction fragment length polymorphism (RFLP). The presence or absence of mutant alleles was correlated with a “slow” or “fast-acetylation” phenotype, respectively. CPY1A1: m1/m2 polymorphism in the 3'-noncoding region was detected by digestion with MspI, to differentiate the wild (m1) and the mutant (m2) alleles. CPY1A2: The transversion from A to C at position 734 in the first intron of CPY1A2 gene was analyzed by digestion with Apal. The presence of A or C represent the wild or mutant phenotype, respectively. CPY2E1: The 5'-flanking region of CPY2E1 was analyzed by restriction enzyme using PstI and RsaI. CPY2D6: The mutant allele CPY2D6*4 (G to A transition at position 1934) was studied by digestion with BsNI. Results: When CPY1A1 was analyzed, the frequency of wild-type and mutant alleles in group F0-2 was 85.5% (77/90) and 14.5% (13/90), while in group F3-4 was 63% (22/35) and 37% (13/35), respectively. The frequency of mutant phenotype was significantly higher in the F3-4 group when compared with F0-2 group (14.5 % vs 37%, p<0.007). When CPY1A1, CPY2E1 and CPY2D6 were analyzed, no differences between groups were obtained Conclusion: This preliminary results show an association between slow-acetylation status of CPY1A1 and liver fibrosis in chronic hepatitis C infection.

Disclosures: The following people have nothing to disclose: Gloria Moraleda, Rafael Barcena, Santos del Campo

1363

COMPARISON OF TWO REAL-TIME PCR BASED ASSAYS (REALTIME HCV, COBAS TAQMAN) WITH A SIGNAL AMPLIFICATION ASSAY (BDNA) FOR HCV RNA DETECTION

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Background: The prevalence of hepatitis C virus (HCV) infection in Africa varies between countries and communities. For most clinical testing currently in Uganda, commercially available rapid slide assays (RSA) are used to detect anti-HCV, rather than enzyme immunoassays (EIA). We determined the prevalence of HCV and the accuracy of these two testing modalities among patients admitted to a large city emergency medical ward (EMW) in Uganda. Methods: 380 patients admitted consecutively to Mulago Hospital, Kampala, were recruited after informed consent. Sera were tested for anti-HCV antibodies in Kampala using a Rapidtest® slide assay (Cortez Diagnostics, Calabasas, CA) and sent frozen to Dallas for blinded re-testing: anti-HCV antibodies by EIA and nucleic acid testing (NAT) (Siemens Centaur® and Versant® bDNA 3.0 respectively, Siemens Diagnostics, Tarrytown, NY), followed by polymerase time RT-PCR based tests have been introduced in routine diagnostics recently and have the potential of highly sensitive detection together with accurate quantification of HCV RNA in one assay. However, despite standardization to international units significant differences have been described between commercially available assays for HCV RNA quantification. Methods: In the present study, a new real-time PCR based assay (Abbott Realtime HCV, Abbott Molecular) was compared to the COBAS AmpliPrep/COBAS TaqMan HCV test (CAP/CTM, Roche Molecular Systems), and a signal amplification based assay (bDNA, Versant HCV Quantitative, Siemens). Results: Correlation of HCV RNA concentrations between the three different assays was estimated on repetitive testing of 66 clinical samples of patients infected with HCV genotypes 1 to 5. For the different HCV genotypes (gt) RealTime HCV showed mean differences of 0.02 (gt1a/b), 0.22 (gt2), 0.27 (gt3), 0.19 (gt4), and 0.03 (gt5) log10 IU/mL HCV RNA in comparison to bDNA and -0.75 (gt1a/b), -0.14 (gt2), 0.24 (gt3), 1.27 (gt4), -0.09 (gt5) log10 IU/mL HCV RNA. Genotype-specific assay sensitivity was estimated for RealTime HCV and CAP/CTM by multiple testing of HCV RNA concentrations between 3 and 50 IU/ml derived from diluted clinical samples. RealTime HCV showed a lower detection limit of 11.5 (gt1), 9.7 (gt2), 4.5 (gt3), 18.2 (gt4), and 3.6 (gt5) IU/ml whereas the lower detection limit for CAP/CTM was 14.5 (gt1), 46.8 (gt2), 29.2 (gt3), 56.3 (gt4), and 8.6 (gt5) IU/ml. For RealTime HCV, CAP/CTM, and bDNA HCV RNA quantification was linear between 4x10³ and 1x10¹⁰ IU/ml, with a correlation coefficient between expected and observed results of >0.98. Conclusion: In conclusion, highly sensitive detection and reliable HCV RNA quantification was observed for RealTime HCV, CAP/CTM, and the bDNA assay. However, up to 5-fold differences of absolute quantification were detected between the assays in genotype 1 infected patients which may cause significant problems for selection of patients suitable for treatment shortening.

Disclosures: The following people have nothing to disclose: Johannes Vermehren, Anette Wohnsland, Barbara Gärtner, Annegret Otto, Reinhold Gobel, Stefan Zeuzem, Christoph Sarrazin

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VALIDITY OF HEPATITIS C ANTIBODY TESTING IN UGANDA: A COMPARISON STUDY

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Background: The prevalence of hepatitis C virus (HCV) infection in Africa varies between countries and communities. For most clinical testing currently in Uganda, commercially available rapid slide assays (RSA) are used to detect anti-HCV, rather than enzyme immunoassays (EIA). We determined the prevalence of HCV and the accuracy of these two testing modalities among patients admitted to a large city emergency medical ward (EMW) in Uganda. Methods: 380 patients admitted consecutively to Mulago Hospital, Kampala, were recruited after informed consent. Sera were tested for anti-HCV antibodies in Kampala using a Rapidtest® slide assay (Cortez Diagnostics, Calabasas, CA) and sent frozen to Dallas for blinded re-testing: anti-HCV antibodies by EIA and nucleic acid testing (NAT) (Siemens Centaur® and Versant® bDNA 3.0 respectively, Siemens Diagnostics, Tarrytown, NY), followed by polymerase chain reaction together with accurate quantification of HCV RNA in one assay. However, despite standardization to international units significant differences have been described between commercially available assays for HCV RNA quantification. Methods: In the present study, a new real-time PCR based assay (Abbott Realtime HCV, Abbott Molecular) was compared to the COBAS AmpliPrep/COBAS TaqMan HCV test (CAP/CTM, Roche Molecular Systems), and a signal amplification based assay (bDNA, Versant HCV Quantitative, Siemens). Results: Correlation of HCV RNA concentrations between the three different assays was estimated on repetitive testing of 66 clinical samples of patients infected with HCV genotypes 1 to 5. For the different HCV genotypes (gt) RealTime HCV showed mean differences of 0.02 (gt1a/b), 0.22 (gt2), 0.27 (gt3), 0.19 (gt4), and 0.03 (gt5) log10 IU/mL HCV RNA in comparison to bDNA and -0.75 (gt1a/b), -0.14 (gt2), 0.24 (gt3), 1.27 (gt4), -0.09 (gt5) log10 IU/mL HCV RNA. Genotype-specific assay sensitivity was estimated for RealTime HCV and CAP/CTM by multiple testing of HCV RNA concentrations between 3 and 50 IU/ml derived from diluted clinical samples. RealTime HCV showed a lower detection limit of 11.5 (gt1), 9.7 (gt2), 4.5 (gt3), 18.2 (gt4), and 3.6 (gt5) IU/ml whereas the lower detection limit for CAP/CTM was 14.5 (gt1), 46.8 (gt2), 29.2 (gt3), 56.3 (gt4), and 8.6 (gt5) IU/ml. For RealTime HCV, CAP/CTM, and bDNA HCV RNA quantification was linear between 4x10³ and 1x10¹⁰ IU/ml, with a correlation coefficient between expected and observed results of >0.98. Conclusion: In conclusion, highly sensitive detection and reliable HCV RNA quantification was observed for RealTime HCV, CAP/CTM, and the bDNA assay. However, up to 5-fold differences of absolute quantification were detected between the assays in genotype 1 infected patients which may cause significant problems for selection of patients suitable for treatment shortening.

Disclosures: The following people have nothing to disclose: Johannes Vermehren, Anette Wohnsland, Barbara Gärtner, Annegret Otto, Reinhold Gobel, Stefan Zeuzem, Christoph Sarrazin
chain reaction (PCR) for bDNA negative samples. Recombinant immunoblot assay (RIBA) was used for NAT-negative samples. Anti-HCV antibody negative samples did not have NAT. Genotyping was determined after direct sequencing. Results: 49 patients (12.9%) were anti-HCV positive (n=43) or equivocal (n=6) by one or both methods. However, prevalence of anti-HCV antibodies was 5% and 6.8% by RSA and EIA respectively, showing very low concordance between the methods (only 3/49 positive in both tests; all 3 NAT positive). Of 16 RSA pos Kampala, but neg by EIA in Dallas, 3 were NAT pos, none were RIBA pos. Of 23 EIA pos in Dallas but RSA neg in Kampala, 5 were NAT pos; 2 were RIBA pos but NAT neg. RIBA testing performed on 32/49 HCV antibody positives revealed only 2/32 RIBA positive tests. 11/11 tested were genotype 1a. Summary: In this in-hospital prevalence study, 49/380 patients tested anti-HCV positive, but only 13/380 (3.4%) were NAT positive. Conclusions: Antibody testing yielded many apparent false positives using either RSA or EIA, with little overlap; rare false negatives were also observed. Previous studies in Africa have shown high false positive rates for anti-HCV, cause unknown, but presumably unique to this patient population and possibly due to cross-reactive antibodies with another flavivirus(es). These data indicate that the majority of reactive anti-HCV results are false positive. If confirmed in other sub-Saharan countries, the findings underscore the importance of confirmatory testing when establishing the diagnosis of chronic hepatitis C. Supported in part by James and Alinda Wikert Fund of the Southwestern Medical Foundation, Dallas TX and with lab support from Siemens Diagnostics, Tarrytown NY.

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1365 FIBROSIS STAGES DETERMINATION, REPRODUCIBILITY AND ROBUSTNESS OF BLOOD TESTS FOR LIVER FIBROSIS IN CHRONIC HEPATITIS C
Paul Cales1,2, Philippe Halff3, Yannick Bacq3, Vincent Leray4, Marie Christine Rousselet5, Marc Bourlière6, Anne de Muret7, Nathalie Sturm8, Gilles Hunault9, Guillaume Penaranda2, Marie Claude Brechot10, Candidce Trocme11, Jerome Bouruis12, 1Hepatology, University hospital, Angers, France; 2University Hospital, Grenoble, France; 3Alphablo, Marseille, France; 4Hepatology, University hospital, Tours, France; 5Pathology, University hospital, Angers, France; 6Hepatology, University hospital, Grenoble, France; 7Pathology, University hospital, Angers, France; 8Hepatology, University hospital, Tours, France; 9Biochimie, University hospital, Grenoble, France; 10HIFA laboratory, University hospital, Angers, France; 11University Hospital, Angers, France; 12Biochimie, Hospital, Tours, France

Aims. We evaluated new statistical tests describing the performance and reproducibility, including robustness, of blood test for liver fibrosis. Methods. Four blood tests were measured in 825 patients with chronic hepatitis C and liver biopsy. New statistical indices were: fibrosis stage probability for a precise diagnostic performance; reproducibility was evaluated with the overall reproducibility test index and 4 tests of robustness. Results. Performance - The fibrosis stage probability provides the percent chance of Metavir fibrosis stages as a function of its blood test value. The blood test can be divided into new bracket of fibrosis staging according to maximum probability. This graphic metric provides a new classification of fibrosis according to blood test stages. Reproducibility - The overall reproducibility is based on the coefficient of variation for the diagnostic cut-offs of blood tests among centers: FibroMeter: 4.2%, APRI: 24.0%, Fibrotest: 24.2%, Hepascore: 35.0%. The following indices evaluate the blood test robustness. Using published regression scores, the calculated a posteriori cut-offs and corresponding overall accuracy (gain against baseline a priori cut-offs) were in the whole population, respectively: FibroMeter: 0.51, 77.5% (0.8%), Fibrotest: 0.43, 74.1% (2.0%), Hepascore: 0.47, 72.7% (0.5%), APRI: 0.55 (vs 1.0), 73.9% (6.1%). The proportion of patients where the diagnosis of significant fibrosis consequently changed was: FibroMeter: 0.8% (p<0.02 vs baseline), Fibrotest: 5.8% (p=10-3 vs baseline and FibroMeter), Hepascore: 2.4% (p<10-3 vs baseline and p=0.02 vs FibroMeter), APRI: 18.2% (p=10-3 vs baseline and FibroMeter). The performance of new regression scores, calculated in the whole population, significantly increased compared to that of original-published-scores whereas the number of independent variables decreased for Fibrotest and Hepascore. The performance and the number of independent variables of FibroMeter were stable. The inclusion of all variables of all blood tests in stepwise binary logistic regression provided a new score for significant fibrosis including all the 7 variables of FibroMeter combined with sex (AUROC gain: 0.007) yielding an overall accuracy of 76.1% and an AUROC of 0.856 (p=0.02 vs FibroMeter). Conclusion. The fibrosis stage probability provides a new classification of fibrosis stages. The overall reproducibility test index offers the advantage of combining all the sources of variability. Thus, this is a global evaluation of the clinical applicability of a test. FibroMeter is the only test with a minimal variation in diagnostic performance and cut-off together with the highest robustness.

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The following people have nothing to disclose: Philippe Halff, Yannick Bacq, Vincent Leray, Marie Christine Rousselet, Marc Bourlière, Anne de Muret, Nathalie Sturm, Gilles Hunault, Guillaume Penaranda, Marie Claude Brechot, Candidce Trocme, Jerome Bouruis
eral reference population, matched for clinical and epidemiological features. By multivariate analysis, a reduced HRQOL was independently associated with a history of medical treatment against depression (low mental and physical summary scores of SF-36). A low physical summary score was associated with low working ability, whereas a low mental summary score was associated with age, IVDU and low social ability. HADS anxiety and depression scores were independently associated with a history of depression, low social and working ability. None of the SF-36 or HADS scores correlated with different levels of viral load or viral presence/absence. In particular, when comparing patients HCV RNA+ (n=555; 295 treatment naïve, 260 non-responders or responders-relapers to antiviral therapy) with those who did not have a detectable viral load (n=262; 42 with spontaneous clearance and 220 with sustained virological response in whom viral load was assessed ≥24 weeks after the end of treatment), we did not find any significant differences regarding any SF-36 or HADS scores between HCV RNA+ and HCV RNA- patients. In conclusion, anti-HCV+ persons have decreased HRQOL compared to controls. However, host and environmental factors, rather than viral, seem to impact on HRQOL level.

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### 1367

**THE APRI CAN NEGATIVELY PREDICT CIRRHOSIS IN PATIENTS WITH CHRONIC HEPATITIS C (HCV)**

Ned Snyder1, Rami Hawari1, Shu-Yuan Xiao2, John Petersen2; 1Internal Medicine, University of Texas Medical Branch, Galveston, TX; 2Pathology, University of Texas Medical Branch, Galveston, TX

Background: Radiologic screening for hepatocellular cancer in patients with HCV related cirrhosis is recommended. However, not all patients with HCV receive a liver biopsy, and the fibrosis stage of many patients is unclear. Several hepatitis fibrosis markers have been shown to be effective in separating mild from significant fibrosis in about 40-60% of patients, they are not as useful for predicting cirrhosis. We have previously found the AST Platelet Ratio Index (APRI) to be quite accurate in predicting mild and significant fibrosis, but there is overlap between F2-F4. (J Clin Gastroenter 40:535).

**Purpose:** We wanted to determine if the APRI could be used as a negative predictor of cirrhosis, and if so, what would be the best cut-off.

**Methods:** The patients studied are enrolled in a prospective study of hepatitis fibrosis markers in patients undergoing pretreatment staging liver biopsies for HCV. Blood was drawn on the day of the biopsy. The biopsies were read blindly by one pathologist using the Batts Ludwig criteria (F0-F4). The APRI was calculated as below*.

**Results:** 277 patients were studied, and 49 (17.7%) had cirrhosis while 88 (31.8%) had advanced fibrosis (F3-F4). The results are summarized in the table below. A cut-off of 0.54 correctly identified all 49 patients with F4 for a NPV of 100%, while a cut-off of 0.78 correctly identified 45 of 49 for an NPV of 97.0%. Using a cut-off of 0.78, unnecessary screening could have been avoided in 128 of the 228 patients (56.1%) without cirrhosis. A cut off of 0.54 identified 82 of 88 patients with advanced fibrosis for a NPV of 96.6%.

**Conclusion:** The APRI could be used to screen for patients with HCV that are unlikely to have cirrhosis, and therefore do not need screening for hepatocellular carcinoma.

<table>
<thead>
<tr>
<th>APRI</th>
<th>Total patients</th>
<th>F4</th>
<th>F0-F3</th>
<th>NPV</th>
<th>PPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>≥0.54</td>
<td>183</td>
<td>49</td>
<td>134</td>
<td>100.0%</td>
<td>26.8%</td>
</tr>
<tr>
<td>≥0.78</td>
<td>145</td>
<td>45</td>
<td>100</td>
<td>97.0%</td>
<td>31.0%</td>
</tr>
<tr>
<td>≥0.54</td>
<td>183</td>
<td>82</td>
<td>101</td>
<td>93.5%</td>
<td>41.8%</td>
</tr>
</tbody>
</table>

### 1368

**PROSPECTIVE COMPARISON OF TWO COMMERCIAL NON-INVASIVE FIBROSIS SERUM MARKER PANELS (FIBROSURE AND FIBROSPECT II) DURING INTERFERON-BASED COMBINATION THERAPY IN CHRONIC HCV GENOTYPE 1**

Keyur Patel1, Yves Benhamou2, Eric M. Yoshida3, Kelly D. Kaita4, Stefan Zeuzem5, Michael Torbenson6, Erik Pulkstenis7, John G. McTutchinson1, G M. Subramanian7; 1Duke Clinical Research Institute, Durham, MD; 2Hopital Pitie-Salpetriere, Paris, France; 3University of British Columbia, Vancouver, BC, Canada; 4University of Manitoba, Winnipeg, MB, Canada; 5W. Goethe-University Hospital, Frankfurt, Germany; 6Johns Hopkins University, Baltimore, MD; 7Human Genome Sciences, Rockville, MD

Background/Aim Noninvasive methods that follow changes in liver fibrosis after antiviral therapy could provide an alternative to liver biopsy for assessment of secondary histologic endpoints for CHC patients. Methods Comparisons between the two commercially available HCV fibrosis serum marker panels in the US (HCV FibroSure (Labcorp, Burlington, NC) and FibroSpect II (Prometheus Laboratories, San Diego, CA) were independently evaluated in serum samples obtained pre-treatment and at various intervals during the study (w12, 24, 48 on treatment and w12 and 24 follow up) in a Phase 2b, study of albinterferon alfa-2b or Peginterferon combination treatment in genotype 1 CHC, IFN-naïve patients. Pre-treatment liver biopsy specimens were graded for METAVIR fibrosis by a single pathologist. Results Pre-treatment serum marker panel tests were evaluated in 95 CHC patients (FibroSpect II, n=92; FibroSure, n=84), with mean biopsy length of 17.8 ± 8.0 mm and a prevalence of F2-F4=23% (22/95). Both panels indicated a very high sensitivity (FibroSure=1.00; FibroSpect II=0.95) in detecting stage F2-F4, a lower but comparable specificity (FibroSure=0.61; FibroSpect II=0.66), and a good AUROC (0.89 for FibroSure and 0.90 for FibroSpect). More notable changes were observed with FibroSpect II during therapy (panel A). Patients with SVR (n=45/68; 66.2%) had a trend towards greater improvement in index scores for both FibroSure (2.94 v. -20; p=0.14) and FibroSpect II (18.44 v. -6.82, p=0.05) (panel B). Conclusions Both marker panels demonstrate comparable performance characteristics for differentiating CHC patients with moderate-to-severe stage disease at baseline. Trends toward improvement in regression index scores with SVR suggest an improvement in liver fibrosis, and indicate the potential utility of non-invasive markers in assessing change in fibrosis with treatment.
1369
A PRACTICAL INDEX (FIBROINDEX) FOR THE PREDICTION OF SIGNIFICANT FIBROSIS IN PATIENTS WITH CHRONIC HEPATITIS C
Masahiko Koda, Yoshikazu Murawaki; 2nd Department of Internal Medicine, Tottori University, Yonago, Japan
The diagnosis of liver fibrosis stage in chronic hepatitis C is essential for prognostication and decision on antiviral therapy. This study constructed a simple model consisting of routine laboratory tests, then validated the model in cross-sectional and longitudinal studies. Consecutive treatment-naïve patients with chronic hepatitis C who underwent liver biopsy were divided into 2 cohorts: estimation set (n=240) and validation set (n=120). Longitudinal set consisted of 30 patients who underwent liver biopsies twice, before and after IFN treatment. FibroIndex was derived from platelet count, AST and gamma-globulin in the estimation set. The areas under the ROC curves of FibroIndex for the prediction of significant fibrosis were 0.83 and 0.82 for the validation set and better than those of Forns’ index and APRI. Using the best cut off values, the presence and absence of significant fibrosis were diagnosed with high positive predictive values and 35% of patients could avoid liver biopsy. In the longitudinal set, there was a significant decrease in FibroIndex among 14 patients whose fibrosis stage improved and a significant increase among 5 patients whose fibrosis stage deteriorated. Changes in the FibroIndex correlated significantly with variations in fibrosis stage. There was no such correlation for Forns’ index or APRI. In conclusion, FibroIndex is a simple and reliable index for the prediction of significant fibrosis in chronic hepatitis C, and could also be used as a surrogate marker during antifibrotic treatment for chronic hepatitis C.

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1370
ANTI-ARFP ANTIBODY POSITIVITY IS SIGNIFICANTLY ASSOCIATED WITH INCREASED HCV VIRAL LOAD IN END STAGE LIVER DISEASE
Jose L. Walewski1, Julio A. Gutierrez1, Arielle L. Klepper1, Ileana Aderca1, Michael R. Charlton2, Lewis R. Roberts2, Andrea D. Branch3; 1Liver Unit, Dept. of Gastroenterology, Tel Aviv Sourasky Medical Center, Tel Aviv, Israel; 2Cell Research and Immunology, Tel Aviv University, Tel Aviv, Israel
Intro: ARFPs stimulate specific immune responses in patients, but their functions are unknown. Because antibody levels can reflect disease states, (Nishizono, 1993; Baumert, 2000), we have developed a novel, specific and sensitive indirect ELISA for anti-ARFP antibodies in patient serum, seeking evidence of associations between anti-ARFP antibody levels and clinical parameters such as viral load, or presence of HCC. Synthetic core and ARFP peptides were used throughout. Methods: Serum samples were collected under Mayo Clinic or MSSM IRB approved consent. 1a Core peptide (aa 7-25), ARFP 1a consensus 70 mer peptide (aa 1-70) and ARFP middle peptide (aa71-89) were covalently linked to Reacti-Bind Maleimide Activated plates (Pierce). Serum samples were tested at a 1:2,000 dilution. All samples were measured in duplicate on each assay date, these values were averaged and BSA (ARFP 70mer) or PDCE2 (Core and middle ARFP) background values were subtracted. Threshold for positivity was the mean plus 3 St Devs. of 10 end-stage liver disease (ESLD) patients. 39 HCV ESLD samples were tested on each peptide. To be anti-ARFP positive, a sample had to test positive for both peptides (70mer and middle), both times each sample was tested. Statistical analyses were run in JMP 5.1. Results: All negative controls were negative for reactivity to core, 1a amino and middle ARFP peptides. There was no difference in anti-core positivity between the ARFP negative or positive samples. However, ARFP positivity was associated with a significantly higher HCV viremia, with log10 values of 1.71 +/- 0.43 in the ARFP negative samples, versus 3.53 +/- 0.73 (mean +/- SE) in the ARFP positive samples. This difference was statistically significant, p=0.026 by Wilcoxon Rank Sums test. Conclusions: While HCV ESLD were equally positive to core, ARFP positivity was significantly associated with higher HCV viremia in the ESLD. These results indicate that expression of the ARFP by HCV may vary by viral load, and that the detection of antibodies to this protein may provide clinically useful information regarding viral load to further evaluate patient status. (Supported by NIDA016156 and DDK066939)

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1371
SERUM LEVELS OF ASIALOGLYCOPROTEIN RECEPTOR SH2A CORRELATE WITH SUCCESS OF TREATMENT OF HCV PATIENTS
Yoav Lurie1, Elena Veselkin2, Maria Kondratyev2, Efrat Ron2, Moshe Santo1, Shimon Reif1, Irma Elashvili1, Lana Bar1, Ran Oren2, Gerardo Z. Lederkremer2; 1Liver Unit, Dept. of Gastroenterology, Tel Aviv Sourasky Medical Center, Tel Aviv, Israel; 2Cell Research and Immunology, Tel Aviv University, Tel Aviv, Israel
Study’s purpose: Using a novel sensitive marker of liver function, a soluble secreted form of the human asialoglycoprotein receptor (ASGPR sh2A) (see accompanying abstract) we have done a longitudinal study of therapeutic success of HCV patients. sh2A is secreted to the serum exclusively by hepatocytes and thus its levels reflect their functional state. Methods: Retrospective double blind study of serum samples analyzed for routine liver markers compared to sh2A, measured with a newly-developed ELISA assay. Results: A cohort of 17 HCV patients with mild fibrosis, followed monthly or biweekly during a year of treatment with ribavirin and interferon alpha showed a clear correlation of successful therapy (as measured through PCR to determine viral clearance) with a rapid recovery of sh2A to normal levels. In the cases where the therapy was not successful, sh2A levels remained abnormal or oscillated. In contrast, alanine and aspartate aminotransferases (ALT and AST) returned to normal values in all cases, as the treatment corrected the immediate causes of liver damage (reflected by the secretion of these enzymes) but did not necessarily lead to full...
recovery of hepatocyte function. Indeed, when sH2a levels returned to normal, this took place with a lag of 2-3 weeks after recovery of ALT to normal levels, as the recovery of hepatocyte function ensued reduction in damage. Albumin, prothrombin time and alkaline phosphatase show abnormal levels only for advanced disease and appeared normal throughout the treatment for almost all these mild patients. Conclusions: sH2a appears to be a unique sensitive marker, which could be well-suited for the non-invasive follow up of recovery of liver function in the course of therapy of HCV patients.

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1372  INFLUENCE OF INTERFERON (IFN)-BASED THERAPY ON LIVER FIBROSIS PROGRESSION (LFP) IN HIV/HCV CO-INFECTED PATIENTS
Yves Benhamou¹, Marc Antoine Valentín², ³, Stéphanie Dominguez, Marina Varastel, Adelina Zin, Christine Kattama², Thierry Poynard¹; ¹Hepatology, Hôpital Pitié-Salpêtrière, Paris, France; ²Infectious Diseases, Hôpital Pitié Salpêtrière, Paris, France; ³ClinSearch, ClinSearch, Bagneux, France

Background: LFP is more rapid in HIV/HCV co-infected patients compared to HCV mono-infected patients. The impact of IFN-based therapy on LFP in real life has been poorly studied in co-infected patients. Objective: To study impact of IFN-based therapy on LFP in HIV/HCV co-infected patients. Methods The data of all HIV/HCV co-infected patients who had undergone two liver biopsies were extracted from a cohort of HIV/HCV co-infected patients followed-up in our center. Patients who received IFN-based therapy were compared to those who never received anti-HCV therapy using the Chi-2 or Fisher’s exact test. Results The HIV/HCV co-infected cohort included 437 patients (75% male), aged 35±8 yrs at first visit: 230 patients had received anti-HCV therapy (≥3 months, mainly peginterferon plus ribavirin), 207 did not. Liver biopsy (METAVIR) results were available in 187 (81%) and 114 (55%) of the treated and untreated patients, respectively. Hepatitis C was more severe in the treated vs. untreated group: liver activity was graded A2/A3 in 51% vs. 22% (p<0.001) and fibrosis was scored F3/F4 in 38% vs. 13% (p<0.001) of the cases at the first biopsy. LFP was evaluated in 44% of treated patients and 14% of untreated patients who had repeated biopsies (table). The median time from first to last biopsy was 3.9 and 4.3 years, respectively (p=0.858). The LFP rate, expressed in METAVIR F points per year, was lower in treated patients achieving a sustained virological response (SVR) to anti-HCV therapy compared to patients with no SVR and untreated patients (p=0.024). Treated patients had a reduced LFP rate compared to untreated patients (0.18±0.59 vs. 0.22±0.46). Patients with SVR had a negative mean LFP rate indicating regression of fibrosis. Conclusion Anti-HCV therapy in HIV/HCV co-infected patients tended to slow down liver fibrosis progression and was associated with liver fibrosis improvement in patients achieving a SVR. Near half of the untreated patients with mild liver fibrosis at baseline may have at least 1 point worsening in liver fibrosis after a median period of 4 years. This research was supported by Schering Plough.

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The following people have nothing to disclose: Marc Antoine Valentín, Stéphanie Dominguez, Marina Varastel, Adeline Zin, Christine Kattama, Thierry Poynard

1373  HIGHER LEVEL OF ALANINE AMINOTRANSFERASE MASS IN LIVER DISEASE PATIENTS THAN NORMAL POPULATION MEASURED BY SANDWICH ELISA
Eui Y. Choi¹, Hyun-Jung Kim¹, Dong J. Kim², Kyung-Joon Kang¹, Se-Hyun Bang¹, Sang W. Oh²; ¹Biomedical Sciences, Hallym University, Chuncheon, South Korea; ²Internal Medicine, Hallym University, Chuncheon, South Korea; ³Biology Education, Chunbuk University, Chunju, South Korea

Alanine aminotransferase (ALT1, GPT) mediates the reversible transamination between alanine and alpha-keto-glutarate to form pyruvate and glutamate, and it has been used clinically as an indicator of liver function test. However, the catalytic activity of this enzyme is not corresponded well to a degree of hepatocellular damage. To resolve this issue, we developed an immunoassay procedure for measurement of ALT1 mass. ALT1 cDNA was amplified by RT-PCR from human liver mRNA and cloned into an expression vector. Recombinant ALT1 protein was produced in a bacterial expression system and injected for the production of monoclonal antibodies (mAbs) against ALT1. After selection of mAb pairs by epitope mapping, we fabricated a sandwich ELISA system. ALT1 mass with sera from patients of liver diseases was compared to that of ALT1 enzymatic activity and analyzed for clinical use by applying to ROC curve. The enzymatic activity assay, as expected, discriminated well acute hepatitis (N=46, 789.4±1080.2 IU/L) from normal (N=30, 21.5±10.6 IU/L), but not other diseases including liver cirrhosis (N=61, 25.9±31.2 IU/L). To the contrary, the ALT1 immunoassay did show a much difference between normal group (70±31.8 ng/ml) and patient groups, particularly liver cirrhosis group (276.5±297.9 ng/ml). It suggested that the ALT1 immunoassay by mass is a more accurate diagnostic method than the enzymatic activity assay in discriminating liver disease patient from normal, particularly liver cirrhosis. And, we also measured the mass of autoantibody from the serum with each liver disease by indirect ELISA method to check whether autoantibody affects on the measurement of enzymatic activity. A higher concentration of both IgA-free and IgA-complex autoantibody were detected in serum of liver cirrhosis than that of acute hepatitis, indicating that the enzymatic activity of ALT1 in hepatocarcinoma and liver cirrhosis could be inhibited by the autoantibody. The presented results showed that the immunological measurement for ALT1 mass can be an excellent tool in establishing of discernible clinical test for patients of liver diseases.

<table>
<thead>
<tr>
<th>Variation of fibrosis from first to last biopsy, METAVIR F point (4, %)</th>
<th>Un-treated for HCV</th>
<th>Treated for HCV</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤0</td>
<td>1 ±0.5</td>
<td>15 (51.7)</td>
<td>9 (31.0)</td>
</tr>
<tr>
<td>8</td>
<td>1 ±0.5</td>
<td>31.8 ng/ml</td>
<td>10.6 IU/L</td>
</tr>
</tbody>
</table>

Liver fibrosis progression rate (METAVIR F point per year)
mean±sd (median, %) CI
0.23±0.46 | 0.04±0.39 | 0.30±0.63 | 0.51±0.46 | -0.02±0.45 | 0.00 | 0.00 |

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Dong J. Kim - Grant/Research Support: Schering-Plough
Kyung-Joon Kang - Grant/Research Support: Schering-Plough
Se-Hyun Bang - Grant/Research Support: Schering-Plough
Sang W. Oh - Grant/Research Support: Schering-Plough


### Characteristics of the patient groups with liver diseases

<table>
<thead>
<tr>
<th>Patient group</th>
<th>Numbers</th>
<th>ALT (U/L)</th>
<th>Max (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute Hepatitis</td>
<td>46</td>
<td>789±1480 (278-5970)</td>
<td>96±875 (114-395)</td>
</tr>
<tr>
<td>Chronic Hepatitis</td>
<td>54</td>
<td>64-2366 (75-289)</td>
<td>107-910 (43-502)</td>
</tr>
<tr>
<td>Hepatic carcioma</td>
<td>45</td>
<td>57.5±61 (74-560)</td>
<td>132±614 (543-733)</td>
</tr>
<tr>
<td>Liver cirrhosis</td>
<td>61</td>
<td>25.5±31 (2.2-67)</td>
<td>276±297 (9.31-1296)</td>
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<tr>
<td>Control</td>
<td>30</td>
<td>27.8±27 (9.5-16)</td>
<td>70±31 (8.1-118)</td>
</tr>
</tbody>
</table>

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### IN VIVO DETECTION OF CASPASE ACTIVITY IN PATIENTS WITH CHRONIC HEPATITIS C AS A NOVEL BIOMARKER OF DISEASE SEVERITY

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Background: Assessment of liver fibrosis in patients with hepatitis C infection (HCV) is currently critical for further management decision. We have recently demonstrated that determination of caspase 3-generated cytokeratin 18 (CK-18) fragments’ level in blood is an independent predictor of advanced fibrosis in patients with nonalcoholic steatohepatitis. While others have shown that these levels may be increased in patients with HCV. Thus the aim of the present study was to determine the value of this novel biomarker for assessing fibrosis in patients with HCV. Methods: Prospectively, 72 consecutive HCV-infected patients referred to our liver biopsy clinic were enrolled. On the biopsy day, demographic and clinical data were collected along with blood samples. Histology of all liver biopsies was assessed blindly by an experienced hepatopathologist and used as the gold standard method for fibrosis staging using the Batts-Ludwig scoring system. CK-18 fragments’ level was measured in duplicates using a specific sandwich immunoELISA assay. Results: CK-18 fragments’ level was high in patients with advanced fibrosis (Stage 3-4, n=16) compared to those without advanced fibrosis (Stage 0-2, n=56) (Median (Q25, Q75): 276 U/L (146, 559), 168 U/L (96, 343)) respectively, although the difference did not reach statistical significance (P =0.08). The odds of having advanced fibrosis increased with increasing CK-18 levels. For every 50 U/L increase in the plasma level of CK-18 fragments, the likelihood of having advanced fibrosis increased 10%. In multivariate analysis, CK-18 fragments’ level was found to be marginally associated with advanced fibrosis (P = 0.054) after adjusting for several factors including age and presence of steatosis. Conclusion: Measurement of plasma CK-18 fragment level appears to be useful to identify HCV patients with severe fibrosis. Future larger studies are warranted.

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### COMMON PATHWAYS IN ENDOGENOUS DEPRESSION AND IN IFN-INDUCED DEPRESSION IN HCV PATIENTS

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Aims and background: Combination therapy with pegylated interferon-alpha (IFN-α) and ribavirin for chronic hepatitis C can induce depressive side effects in up to one third of patients. To date, it is unclear if IFN-induced depression and endogenous depression have common causes. Patients and methods: A total of 50 Caucasian patients with histologically proven chronic hepatitis C were treated with standard combination therapy consisting of pegylated IFN-α2a (Pegasys, 180µg once weekly) and ribavirin (800-1200 mg daily) for 6 or 12 months. RNA was isolated from peripheral blood which was collected 12h before and 12h after the first injection of IFN-α. The transcriptional profile was analysed using human genomic microarrays (Affymetrix, HG U133A) and quantitative real-time RT-PCR. Array data were normalized and fold change values were subjected to class prediction analysis to identify genes which are differentially regulated in patients with or without IFN-induced depression. Furthermore, PBMC from 10 HCV-negative patients hospitalized for severe major depression and from 11 healthy controls were cultivated in vitro with (100 and 1.000 U/mL) or without pegylated IFN-α2a for 16h to assess the expression of target genes using quantitative RT-PCR. Results: 11/50 HCV patients (22%) developed clinically relevant IFN-induced depression. Using class prediction analysis, the development of depression could be predicted with 91% accuracy by 16 genes which were significantly higher induced in patients with IFN-associated depression compared to patients without depression during IFN treatment. Interestingly, 6 of these genes (DYNLT1, GCH1, TOR1B, DISC1, MEF2A, ST3GALS) have previously been described as being associated with recurrent major depression or with neuronal development in the brain, whereas the remaining genes were identified as classical IFN response genes. These data could be verified by quantitative RT-PCR. After in vitro IFN stimulation the gene response of 5/6 depression-associated genes was significantly higher in PBMC from 10 patients hospitalized for severe major depression as compared to 11 healthy controls. Conclusions: These data suggest that selective hyper-responsiveness to exogenous IFN therapy or endogenous (endogenous depression) type 1 IFNs may lead to the development of depressive symptoms. The functional analysis of the differentially regulated target genes that were identified in this study could lead to the discovery of novel therapeutic approaches to improve the efficacy of and adherence to HCV therapy and, possibly, to treat major endogenous depression.

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Differential pattern of HCV-specific T cell responses distinguishes SVR and relapse patients receiving standard antiviral therapy plus therapeutic IC41 vaccination

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Virological relapse after end of therapy is observed in 20-40% of patients responding to PEG-IFN/RBV based standard therapy (ST) of chronic hepatitis C. Enhancing HCV-specific T cell responses by therapeutic vaccination might be an option to prevent relapse. However, effects of ongoing ST on vaccine responses are largely unknown. IC41 consists of five synthetic peptides harboring HCV T-cell epitopes and the synthetic adjuvant poly-L-arginine. IC41 can induce HCV-specific IFN-gamma-secreting CD4+ and CD8+ T cells in healthy volunteers and chronic HCV patients refractory to ST. Here we aimed to investigate IC41 during ST and its ability to reduce relapse rates in an open label, single arm phase II study. 35 patients with chronic HCV genotype 1, HCV-RNA-negative (Cobas Amplicor test, detection limit 100 copies/ml) after 12 weeks of ST received 6 s.c. vaccinations of IC41 in 4 weeks intervals from ST-week 28 to 48. HCV peptide-specific T cell responses were assessed at treatment weeks 28 (prior first vaccination), 48, FU-4, FU-12 and FU-24 applying IFN-gamma ELISPOT, proliferation and HLA-A2 tetramer assays. From 23 patients fulfilling all criteria of the study protocol (HLA-A2 positive, RNA negative at week 48), 14 achieved SVR, whereas 8 relapsed (36%), for 1 patient follow-up data were missing. T cell responses were weak or absent at week 28 before vaccination and were significantly more frequent and stronger at the end of antiviral therapy and during follow-up in 8/23 patients. Interestingly, we found a clear-cut difference between patients who relapsed and patients with SVR in terms of ELISPOT at weeks 48 and FU-4: while T cells were barely detectable in relaper, over 40% of SVR patients showed robust T cell responses against the vaccine peptides. Finally, ELISPOT response rates and strength were compared to previous studies applying the same vaccine dose and schedule in healthy volunteers (n=120) and chronic non-responder/relaps patients (N=60). Patients in the current trial (HCV-RNA negative and receiving interferon therapy) had slightly higher T cell response rates as compared to RNA-positive nonresponders patients, matching closely response rates in healthy volunteers. In conclusion, IC41 vaccination did not prevent virological relapse in patients receiving standard PEG-IFN/ribavirin therapy of chronic hepatitis C. However, T cell responses were inducible in individuals with ongoing interferon treatment and these T cell responses were associated with lower relapse rates. Future trials need to explore whether optimized vaccine responses may enhance sustained response rates to standard therapy of chronic hepatitis C.

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A phase 1 study to evaluate the safety, tolerability, pharmacokinetics (PK) and pharmacodynamics (PD) of escalating single doses of R7025, a novel pegylated interferon α compared to placebo and peginterferon α2a (40KD) (PEGASYS®) in healthy volunteers

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Background: Pegylated interferon (PEG-IFN) in combination with ribavirin is the standard of care for patients with chronic hepatitis C (CHC). However, treatment is not yet optimal in patients infected with HCV genotype 1 with sustained viral response rates of ~50%. R7025 is a novel human pegylated IFNα molecule generated using DNA Shuffling (Maxygen, Inc.) technology that exhibits ~50-fold higher antiviral activity compared to PEG-IFNα-2a, but only 2- to 10 fold greater antiproliferative activity in vitro. R7025 contains the identical branched chain 40KD PEG molecule used in the synthesis of PEG-IFNα-2a. The primary objective of this study was to evaluate the safety, tolerability, PK and PD activity of single ascending doses of R7025 in healthy volunteers. Methods: In each cohort, subjects were randomized to receive a single dose administered subcutaneously in the abdomen according to the following scheme: 8 R7025, 2 placebo, 2,180 µg PEG IFNα-2a. Subjects were evaluated frequently for safety, tolerability, PK and PD assessments. The following dose levels were evaluated: 1, 5, 20, 40, 80 and 120 µg. Results: 72 subjects were enrolled and completed the study. The PD markers, serum neopterin and 2′,5′-oligoadenylate synthetase (2,5 OAS) levels were increased in subjects who received a 20-120 µg dose of R7025. Doses of 40 µg and higher produced a similar level of PD induction as a 180 µg dose of PEG IFNα-2a. Serum exposure (Cmax and AUC) of R7025 increased with dose and was sustained over 168 hrs, supporting a minimum of once weekly dosing. Doses up to 80 µg were well tolerated with most adverse events rated (AEs) as mild or moderate. No serious AEs have been reported. In the 120 µg group, 4/8 R7025 treated subjects experienced severe flu-like symptoms, which were adequately controlled with analgesics and fully reversible. Most subjects who received 20-120 µg of R7025/placebo exhibited a reversible decrease in neutrophils but only grade 1/2 changes in neutrophils were observed. Conclusion: R7025 doses up to 80 µg were well tolerated with 20 µg being the lowest pharmacologically active single dose studied. R7025 doses of 40 µg and greater produced a level of serum neopterin and 2,5 OAS induction which was comparable to a 180 µg dose of PEG IFNα-2a. Most AEs were mild and consistent with those observed after administration of IFNα. A dose of 120 µg transient severe flu-like symptoms were induced in 2 of 8 patients.
observed. Changes in hematological parameters observed across all doses were mild and reversible. The findings from this study warrant further evaluation of R7025 in CHC patients.

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1378 PHOSPHOROTHIOATE OLIGONUCLEOTIDE IS A POTENT ENTRY INHIBITOR OF HEPATITIS C VIRUS INFECTION IN VITRO AND IN VIVO
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Current treatment of chronic hepatitis C based on combination of peginterferon and ribavirin is only effective in about half of the patients and is accompanied by substantial side effects. Developing new classes of drugs against HCV is crucial. Phosphorothioate oligonucleotides (PS-ONs) have a sequence-independent antiviral activity against HIV-1 by inhibiting virus-cell fusion. Because viral entry is a highly conserved mechanism, this antiviral action of PS-ONs may be effective against infection by other enveloped viruses with type I or II fusion mechanisms. The aim of this study is to assess whether PS-ONs inhibit HCV infection and to evaluate the antiviral mechanism of action of PS-ONs. Methods: Various forms of PS-ONs and the control phosphodiester oligonucleotides (PO-ONs) were synthesized and evaluated in infectious HCV cell culture systems. To test the efficacy of PS-ONs in vivo, human hepatocytes transplanted uPA/SCID mice were inoculated with infectious HCV and treated with the PS-ON. Results: The PS-ONs exhibited potent inhibitory activities in both cell culture-generated HCV-JFH1 (HCVcc) and HCV pseudo-particles (HCVpp) systems. This inhibitory activity was size and phosphorothioate dependent but sequence independent. The control PO-ONs had no inhibitory activity against HCV infection. The PS-ONs had no effect on viral replication in the HCV replicon system and binding of HCV-LPs to cells, indicating that the target of inhibition by PS-ONs is at the post-binding, cell-entry step. In human hepatocyte-engrafted uPA/SCID mice, the PO-ONs also appeared to efficiently block de novo HCV infection. Conclusion: The PS-ONs (amphipathic DNA polymers) are promising new class of antiviral compounds that inhibit HCV fusion and entry.

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1379 IDENTIFICATION OF MODULATORS OF HCV REPLICATION USING A HIGH-THROUGHPUT SCREEN
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Lee F. Peng, Sun-Suk Kim, Wenyu Lin, Naoya Sakamoto, Nobuyuki Kato, Masanori Ikeda, Stuart Schreiber, Raymond T. Chung Background/Aims: Small organic molecules (SOMs) can exert meaningful biological effects, as evidenced by the fact that the majority of drugs in clinical use today are SOMs, including ribavirin. We have established an efficient, high-throughput automated method to rapidly screen the effects of SOMs on HCV replication. We therefore sought to identify novel SOMs capable of regulating HCV replication. Methods: We optimized the subgenomic pRep-Feo replica Huh7 cell line for the 384-well microplate format, and used this line to previously screen the Bioactives Library (Kim SS et. al., Gastro 2007; 132:311-320). We next used pRep-Feo to screen the DOS Set (a large library of novel diversity-oriented synthesis [DOS] compounds). After identifying several molecules capable of either stimulating or inhibiting HCV replication in this primary screen, we then validated hit compounds using a full-length HCV replicon cell line (OR6) in secondary screens. Results: We identified a number of novel antiviral (41) and pro-viral (20) agents from the DOS Set. Twenty-one of the antiviral compounds came from only two specific libraries of novel epoxide DOS compounds. The antiviral activity of these epoxides was then validated, with the most active compound having an IC50 in the 500 nM range. Using structure-activity relationship analysis, we were able to synthesize a compound with an IC50 approximately 50% of the most potent lead compound of the library from which it was derived. There was no significant cytotoxicity observed at the IC50 for these compounds. Conclusions: We have developed a simple, reproducible, and reliable cell-based high-throughput screening (HTS) assay system using an HCV replicon model to identify novel epoxide compounds that inhibit HCV replication. This method can be used to identify not only putative antiviral agents, but also cellular regulators of viral replication that can be used as probes to study viral biology. Studies to identify cellular targets of these compounds are underway.

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1380 DENDRITIC CELLS AND REGULATORY T CELLS AS DECISION MAKERS FOR THE DURATION OF PEGYLATED INTERFERON-α AND RIBAVIRIN THERAPY IN CHRONIC HEPATITIS C PATIENTS
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Backgrounds and aim: The ultimate goal of peg-IFNα and ribavirin therapy in chronic hepatitis C patients is the prevention of hepato-carcinogenesis by eradication of HCV. One of the reasons for treatment failure is high relapse rate after the completion of 48-week treatment. The treatment extension up to 72 weeks may suppress HCV re-appearance in such potential relapsers. Attaining persistently normal ALT levels (biochemical response, BR) is alternatively important, since the long-term incidence of hepatocellular carcinoma is comparably suppressed in BR with those in SVR. According to the 24-week stopping rule, combination therapy may be discontinued in patients who fail to attain negative HCV RNA by week 24. However, some of them become BR if the therapy is continued up to week 48. Thus, the continuation of treatment from 48 to 72 weeks in potential relapsers or 24 to 48 weeks in biochemical responders may give them higher chance of attaining SVR or BR.
respectively. Currently, no reliable marker is available for defining such patients. We thus aimed to evaluate the feasibility of the frequency and function of immune cells, including dendritic cells (DC) and regulatory T cells (Treg), for the predictors of therapeutic outcome. Methods: Twenty-five CHC patients with HCV genotype 1 and high titer were enrolled in this study. They received 48 weeks of PEG-IFNα2b and ribavirin therapy. During the treatment, frequencies of myeloid DC (MDC), plasmacytoid DC (PDC), Treg (CD4+CD25high+) and their changes were determined by means of flow cytometry. The ability of patient DC to stimulate allogenic CD4+ T cells was assessed at the end and after the therapy as reported previously. Results: Among 25 patients who completed the treatment, 11 patients achieved SVR, 11 were transient response (TR) and 3 were non-response (NR), respectively. In comparison among the SVR, TR and NR groups, MDC, PDC and Treg frequencies did not differ throughout the therapy. At week 48 and thereafter, allostimulatory capacity of DC in TR was sustained to be lower than those in SVR patients (p<0.05). By tracing the changes from the beginning to week 24, Treg frequency significantly increased in BR compared to those in non-BR group (p<0.05). Even in patients who failed to clear HCV by week 24, Treg frequency in BR was higher than those in non-BR (p<0.05). Conclusions: In 48 weeks of PEG-IFNα2b and ribavirin therapy, end-of-treatment DC dysfunction and the early phase increase of Treg are served as predictors of relapse and BR, respectively. The extension of treatment needs to be considered in such patients, from the duration based on the rule of early virological response.

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1381
DEVELOPMENT OF INTERGENOTYPIC CHIMERIC REPLICONS FOR BROAD-SPECTRUM ANTIVIRAL ACTIVITY DETERMINATION OF HEPATITIS C VIRUS POLYMERASE INHIBITORS
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To address the need for broad-spectrum antiviral activity characterization of HCV polymerase inhibitors, we created a panel of intergenotypic chimeric replicons containing NS5B sequences from genotypes (gt) 2b, 3a, 4a, 5a and 6a HCV isolates. Viral RNA extracted from non-gt 1 HCV patient plasma was subjected to reverse transcription. The NS5B region was amplified by nested PCR and cloned into the corresponding region of the gt 1b (Con-1) subgenomic reporter replicon by SOEing PCR. Stable cell lines were generated with replication competent chimeras for in vitro antiviral activity determination of HCV non-nucleoside polymerase inhibitors (NNPI) that target different regions of the protein. The NNPI1 and NNPI2 binding sites exist in the thumb domain, while the NNPI3 and NNPI4 sites are in the palm domain of the polymerase. Compounds that bind to the NNPI2 and NNPI3 allosteric sites showed 8- to >1280-fold reductions in antiviral activity against non-gt 1 NS5B chimeric replicons compared to the gt 1b replicon. Smaller reductions in susceptibility, ranging from 0.2- to 33-fold, were observed for the inhibitor binding to the NNPI1 site. The inhibitor binding to the NNPI4 site showed broad-spectrum antiviral activity against all chimeric replicons evaluated in this study. Sequence analysis and computer modeling suggest that residues L419 and L482 may facilitate the interactions between gt 1 polymersases and inhibitors targeting the thumb domain. Indeed, introduction of L419 and L482 found in non-gt 1 polymerases into the gt 1b polymerase, either alone or in combination, resulted in varying levels of reduction in susceptibility to inhibitors that bind to the NNPI1 and NNPI2 sites, but no change in susceptibility to interferon and other NNPIs. In conclusion, evaluation of HCV NNPIs against intergenotypic chimeric replicons showed differences in activity spectrum for inhibitors that target different regions of the enzyme, some of which could be associated with specific residues that differ between gt 1 and non-gt 1 polymerases. Our study demonstrates the utility of chimeric replicons for broad-spectrum activity determination of HCV inhibitors.

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week. Increased serum 2’,5’-OAS activity and neopterin levels were observed between 4 and 24 hours post administration and both remained above baseline levels for at least 10 days. In conclusion, R7025 demonstrates greatly enhanced in vitro properties ideally suited to the treatment of CHC and a unique ratio of antiviral/immunomodulatory to antiproliferative activity. Further, in vivo, the molecule exhibits PK/PD properties consistent with at least once weekly dosing intervals.

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1383
BOTANICAL MEDICINES FOR HEPATITIS C

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Background: A striking feature of hepatitis C virus (HCV) infection is its propensity to establish a chronic disease state. Because state-of-the-art antiviral treatments are costly, have serious side-effect profiles, and moderate probabilities for durable cures, many patients opt for complementary and alternative medicine (CAM)-based approaches to improve liver health. However, mechanisms of action studies are lacking to support the use of CAMs for management of chronic hepatitis C. Methods: In the current study, we examined two widely used botanicals, silymarin and sho-saiko-to (SST), for antiviral and immunomodulatory actions. Results: Silymarin inhibited expression of TNF-α in anti-CD3 stimulated human PBMC and NF-κB dependent transcription in human hepatoma HuH7 cells. Moreover, both silymarin and SST dose-dependently inhibited infection of HuH7 and HuH7.5.1 cells by JFH-1 virus. Both compounds also displayed prophylactic and therapeutic effects against JFH-1 infection, and when combined with IFN-α, inhibited HCV replication more than IFN-α alone. Antiviral effects induced by Silymarin involved Jak-Stat dependent and independent signaling, while SST enhanced ISRE transcription via p38 MAP kinase activation. HPLC fractionation of the herbal preparations permitted identification of the components eliciting antiviral actions. Conclusions: The data demonstrate that standardized silymarin and SST have antiviral action against in vitro HCV infection, and that silymarin has immunomodulatory and anti-inflammatory actions. Therefore, CAM-based approaches may assist in the management patients with chronic hepatitis C.

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1384
SVRSCORE: A NOVEL AND HIGHLY PREDICTIVE SCORE FOR SVR PREDICTION IN CHRONIC HEPATITIS C GENOTYPE 1 INFECTED PATIENTS

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Background: Previous studies demonstrated that viral load (VL) is an important predictor for treatment outcome in patients with chronic hepatitis C virus (CHC). We looked at pre-treatment (BL) VL and week 4 VL (W4) to assess a predictive score achieving probability of sustained virological response (SVR) in CHC genotype 1 patients: the SVRScore. Patients-Methods: 113 CHC genotype 1 patients were included in the study among which 86 patients achieved complete 48 weeks treatment (54 had an SVR). Patients were treated with PEG IFN alpha 2b+RBV. Serum HCV RNA was measured using COBAS TaqMan HCV Test [TaqMan HCV; Roche Molecular Systems Inc., Branchburg, N.J.]. Binary logistic regression analysis was assessed with BL and W4 VL as predictors of SVR, to obtain the SVRScore. Results: In univariate analysis BL VL and W4 VL were both associated with SVR (<0.001 and p<.01 respectively). In multivariate analysis, BL VL and W4 VL were both independent predictors of SVR (respective odds ratio 0.29 [95% CI 0.09-0.95], and 0.39 [0.24-0.63]. From the binary logistic regression, we calculated the SVRScore combining BL VL and W4 VL (cf. formula below). Area under the ROC curve for SVR prediction was 0.89 and positive predictive value of SVR was 86% (44/51). Conclusions: We developed the SVRScore, a new, simple, and highly predictive score for SVR prediction in CHC genotype 1 infected patients. With high predictive values the SVRScore remain useful for the treatment management of CHC infected patients. A near future analysis will be conducted to validate the SVRScore on an independent cohort.

\[
\text{SVRScore} = \frac{0.6031 - 1.2442(\log_{10} BL\ VL) - 0.9309(\log_{10} W4\ VL)}{1 + 0.6031 - 1.2442(\log_{10} BL\ VL) - 0.9309(\log_{10} W4\ VL)}
\]

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Background and Aims: Following lead optimization of a novel series of substituted imidazopyridines, GS-9190 was identified as a promising drug candidate based on its potent inhibition of in vitro HCV RNA replication and excellent selectivity index. The aim of this study was to characterize the biological activity of GS-9190 against HCV replicons of different genotypes and against various drug resistant mutants. GS-9190 was also studied in combination with known anti-HCV agents. Methods: The antiviral activity of GS-9190 was evaluated in HCV genotypes 1b, 1a and 2a replicon cells, in the JFH1 (genotype 2a) infectious system, and against several related and unrelated viruses. Cytotoxicity of GS-9190 was determined in a panel of cell lines. A transient replication assay using Lunet cells (Huh-7) was used to characterize susceptibility of known drug resistant mutants to GS-9190. Combinations of GS-9190 with other HCV agents were evaluated in the genotype 1b (Con-1) replicon cells. Results: GS-9190 is a potent inhibitor of HCV genotype 1b and genotype 1a replicon replication (EC50 = 0.6 and 2.5 nM, respectively). The 50% cytostatic concentration of GS-9190 in various cell lines is > 50µM, illustrating a high degree of selectivity (SI > 20,000). GS-9190 has lower potency against a genotype 2a replicon or against the JFH1 infectious virus (EC50 ~ 1 µM) and is inactive against a number of related and unrelated viruses. Replicons resistant to various HCV protease and polymerase inhibitors remained fully susceptible to GS-9190. The combination of GS-9190 with either IFN-α or several HCV protease and polymerase inhibitors resulted in an additive antiviral activity. The combination of GS-9190 with a protease inhibitor was more efficient in clearing hepatoma cells from their replicon than either agent alone. Following long-term culture with increasing concentrations of GS-9190, resistant replicons were selected. Transfection of naïve Lunet cells with total RNA isolated from the GS-9190res replicon cells transferred drug resistance, indicating that resistance is associated with the viral genome. In biochemical assays, GS-9190 is inactive against HCV NS3 serine protease, NS3 RNA helicase, and NS5B polymerase (either in initiation or elongation assays).

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1387
A Hepatitis C T-Cell Vaccine Candidate Covering HCV Genotype Complexity and HLA Type Diversity

Annegret Van der Aa1, Vera Goossens1, Koen Allosery1, Gert Verheyden1, Scott Southwood2, Carol Dahlberg2, Denise McKinney2, Mark Newman2, Helmut M. Diepolder3, Geert Maertens3, Marie-Ange Buyse1; 1Innogenetics NV, Zwijnaarde, Belgium; 2Pharmexa-Epimmune Inc., San Diego, CA; 3Immusystems GmbH, München, Germany

Background: Viral clearance of HCV has been shown to be associated with multi-specific, strong CD8+ T-cell (CTL) responses together with strong CD4+ T-cell (HTL) responses. A candidate therapeutic polypeptide T-cell vaccine able to induce such potent specific T-cell responses could ultimately lead to viral clearance. Also, by taking human HLA as well as HCV genotype diversity into account, such a vaccine should be applicable to the majority of infections. Methods: HCV-derived CTL and HTL epitopes for a wide range of HLA class-I and HLA-DRB1 alleles were identified applying different algorithms on the full HCV genome. Further selection was made based on in vitro HLA binding affinity of the peptides, immunogenicity in HLA transgenic mice, and recall reactivity of CD8+ and CD4+ T-cells in an IFNγ-ELISPOT assay using HCV patient samples. Several DNA plasmids containing different sets of selected epitopes were designed, constructed, and evaluated for immunogenicity in HLA transgenic mice. Finally, epitopes were included in the candidate HCV polypeptide therapeutic vaccine based not only on their immunological properties but also their conservancy within and between HCV genotypes, as well as their HLA restriction. In addition, the polypeptide construct was expressed as protein in E. coli, purified using affinity chromatography and tested for immunogenicity. Results and discussion: A plasmid vector was constructed that contains 34 HCV-derived CTL epitopes, 13 HCV-derived HTL epitopes, and the pan-DR helper T-cell epitope PADRE®. Theoretical population coverage calculations show that the selected set of CTL and HTL epitopes can provide recognition of at least four CTL and three HTL epitopes in any HCV genotype 1-infected subject, irrespective of HLA type. Vigorous CTL and HTL responses could be induced using the pDNA or protein formats of the vaccine candidate in HLA-A01, HLA-A02, HLA-A11, and HLA-A24 transgenic mice. Conclusion: A set of HCV-derived CTL and HTL epitopes was identified that showed excellent affinity, immunogenicity, and cross-reactivity, and formed the basis for the development of a universal T-cell vaccine candidate for treatment of chronically infected HCV patients. The T-cell vaccine candidate has now been shown to be immunologically active both in the form of pDNA and protein.

Disclosures:
The following people have nothing to disclose: Kilian Weigand, Franziska Voigt, Christoph Eisenbach, Birgit Hoyler, Wolfgang Stremmel, Jens Encke

1388
Using Dendritic Cells as Therapeutic or Prophylactic Vaccine Against HCV

Kilian Weigand, Franziska Voigt, Christoph Eisenbach, Birgit Hoyler, Wolfgang Stremmel, Jens Encke; Gastroenterology and Hepatology, University of Heidelberg, Heidelberg, Germany

Dendritic cells (DCs) are a promising tool for therapeutic and/or prophylactic vaccination experiments, considering their multiple functions in immune modulation. We used bone-marrow derived dendritic cells from BALB/c mice loaded with pseudo particles from the hepatitis C virus (HCVpp) to vaccinate naive BALB/c mice. HCVpp consists of the two (genotype 1b) envelope proteins E1 and E2, covering a non-HCV core structure. Thus, not a single epitope, but the whole "viral surface" induces immunogenicity. HCV E1/E2 pseudo particles were successfully established, demonstrated by Huh-7 infection and production of neutralizing antibodies. Then, syngenic BALB/c DCs were ex vivo loaded with HCVpp, after a course of 8 day maturation with GMCS-F and IL-4. This resulted in strong activation of dendritic cells ex vivo, as shown by FACS analyses. For vaccination mature and activated DCs were injected intra peritoneal. To increase immune responses, the BALB/c mice were boosted by the same procedure two weeks after the first immunisation event. Humoral and cellular immune responses were measured by ELISA, ELISPOT and CTL assay. Our results indicate dendritic cells as promising vaccination model to be tested further, for example by surrogate infection experiments.

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The following people have nothing to disclose: Kilian Weigand, Franziska Voigt, Christoph Eisenbach, Birgit Hoyler, Wolfgang Stremmel, Jens Encke

1389
Efficacy of Interferon β Combined Cyclosporine A Treatment in the Retreatment of Chronic Hepatitis C - Promising Aspect of Host Factor Targeting Therapy

Kazuaki Inoue, Tsunamasa Watanabe, Masaya Yamada, Makoto Yoshiha; Gastroenterology, Showa University Fujigaoka Hospital, Yokohama, Japan

Background/Aim: A significant proportion of chronic hepatitis C patients fails to achieve sustained virological response (SVR) even after the treatment with pegylated interferon (IFN) alpha combined ribavirin. The effective treatment for patients who previously failed combination standard IFN plus ribavirin or pegylated IFN plus ribavirin has not been well established. Management of these patients is the most challenging task and new compounds targeting NS3 protease or NS5B polymerase are now under evaluation. Cyclophilins are essential host factors for HCV replication. We have originally developed IFN combined cyclosporine A treatment and also reported its favorable anti-HCV effect. We report here the efficacy of divided administration of IFNβ plus cyclosporine A in the treatment of chronic hepatitis C patients who failed Peg-IFN or IFN combined ribavirin. Patients and method: We prospectively included 59 patients (median age, 63) with 1) histologically proven chronic hepatitis C, 2) genotype 1b and 3) non-responders and relapsers to combination IFN plus ribavirin or combination pegylated IFN plus ribavirin. We conducted the present study to confirm the efficacy, safety and tolerability of our protocol. Serum HCV RNA level was 3900 KIU/ml. The treatments consisted of an induction therapy, an intensified therapy and a maintenance therapy. The induction therapy comprised intravenous 1 MU IFNβ every 4 hours for the first 3 days, 1.5 MU IFNβ every 6 hours for the next 4 days and 2 MU IFNβ every 8 hours for the following 3 weeks, totaling 168 MU of IFNβ. The intensified therapy was induction therapy shortened to 2 weeks. The maintenance therapy comprised of pegylated IFNα2b and ribavirin. CsA was given 4 times daily for a total dose of 200 mg during the induction and the intensified therapies. Ribavirin was given twice daily for a total dose of 800 mg (body weight over 60 kg) or a total dose of 600 mg (body weight equal to or less than 60 kg) during the maintenance therapy. The institute review board approved this protocol. Results: The end treatment response and sustained virological response rate of the present study were 73% (43/59) and 59% (35/59).
Disclosures:
The following people have nothing to disclose: Kazuaki Inoue, Tsunamasa Watanabe, Masaya Yamada, Makoto Yoshioka

1390 IN VITRO ACTIVITY AND PRECLINICAL PHARMACOKINETICS OF THE HCV PROTEASE INHIBITOR, TMC435350
Kenneth Simmen1, Oliver Lenz1, Tse-l Lin1, Greg Fanning1, Pierre Raboisson1, Herman de Kock1, Gerben van ’t Klooster1, Åsa Rosenquist2, Michael Edlund2, Magnus Nilsson2, Lotta Vrang2, Bertil Samuelsson3; 1Tibotec Pharmaceuticals Ltd., Eastgate Village, Little Island, Cork, Ireland; 2Medivir AB, Box 1086, SE-141 22 Huddinge, Sweden

Background: As a class, HCV NS3/4A protease inhibitors have shown promise in clinical trials for the treatment of chronic hepatitis C virus infection. TMC435350 is a novel and potent macrocyclic NS3/4A protease inhibitor. To further assess the potential of TMC435350, we characterized the in vitro activity of TMC435350 alone or in combination with different classes of HCV inhibitors. The plasma pharmacokinetics and tissue distribution were also studied in vivo. Methods: The effect on HCV RNA level and the emergence of drug-resistant colonies was analyzed in the replicon model with TMC435350 alone, or in combination with interferon alpha, ribavirin, and different HCV polymerase inhibitors. Pharmacokinetic profiles were evaluated following single or repeated dosing in rats. The tissue distribution of TMC435350 was studied in male rats at time points from 0.5 up to 31 hours after a single oral dose of 40 mg/kg. Results: In biochemical HCV NS3/4a protease assays, TMC435350 exhibited Ki values <0.1nM for subtypes 1a (H77) and 1b (conl) enzymes. TMC435350 was found in the subgenomic genotype1b replicon model to have an EC50 of 8nM and a selectivity index [SI] of > 1000. The combination of TMC435350 with different classes of HCV inhibitors further increases its activity in reducing HCV RNA in an additive to synergistic manner, and further reduced the emergence of resistant replicon colonies. After single oral administration of a PEG400-based solution of TMC435350 at 40 mg/kg the mean peak plasma concentration [Cmax] was 1430ng/ml and was observed at two hours post-dose [Tmax]. The absolute bioavailability of TMC435350 was calculated at 44% after single oral administration of a 40 mg/kg dose. TMC435350 was found to be extensively distributed to the liver, small- and large intestines (tissue/plasma ratios >35). Concentrations in other organs were similar to plasma. Notably, TMC435350 was still quantifiable in the liver tissue up to 31 hours post-dosing. Conclusions: TMC435350 is a novel potent and specific HCV protease inhibitor, with good oral bioavailability and a favorable liver distribution. In addition, in vitro studies support the potential use of TMC435350 in combination with other HCV inhibitors.

Disclosures:
The following people have nothing to disclose: Kenneth Simmen, Oliver Lenz, Tse-l Lin, Greg Fanning, Pierre Raboisson, Herman de Kock, Gerben van ’t Klooster, Åsa Rosenquist, Michael Edlund, Magnus Nilsson, Lotta Vrang, Bertil Samuelsson

1391 HCV POLYMERASE (NM107) AND PROTEASE (BOCEPREVIR) INHIBITORS IN COMBINATION SHOW ENHANCED ACTIVITY AND SUPPRESSION OF RESISTANCE IN THE REPLICON SYSTEM
David N. Standring1, Vadim Bichko1, Robert Chase2, Max LaColla2, Lisa Lallos1, Angela Skelton2, Mary Soukasakos2, M. Tausek3, Xiao Tong2, Robert Ralston2; 1Idenix Pharmaceuticals, Cambridge, MA; 2Schering-Plough Research Institute, Kenilworth, NJ

Background: Combination of small-molecule antiviral agents directed against different molecular targets offer an attractive strategy for the effective therapy of hepatitis C. As monotherapies, valopicitabine (NM283) (Idenix/Novartis), an investigational nucleoside NS5B polymerase inhibitor, and boceprevir (SCH 503034) (Schering-Plough), an investigational NS3 protease inhibitor, have demonstrated antiviral activity against hepatitis C virus (HCV) in clinical studies. Using cell culture replicon studies we show that the combination of polymerase and protease inhibitors leads to greater anti-HCV activity, no cross-resistance and the suppression of treatment emergent-resistance, compared to monotherapy with each agent. Methods: The combined antiviral effect of the polymerase and protease inhibitors was evaluated in genotype 1b HCV replicon cells by real time RT-qPCR or ELISA. Possible cross-resistance of these compounds was evaluated using replicon variants carrying single protease inhibitor resistance mutations (T54A, A156S, V170A, A156T), or the polymerase inhibitor resistance mutation (S282T). The selection of resistant cell colonies was carried out for 3-4 weeks in the presence of various concentrations of one or both drugs. Results: The combination of SCH 503034 and NM107 showed dose-dependent enhancement of replicon inhibition, compared with the effect of each inhibitor used alone. No cytotoxicity was observed. In cross-resistance studies, NM107 showed similar antiviral activity (EC50 1.5-2 µM) against wild-type and SCH 503034-resistant replicons. SCH 503034 showed similar activity (EC50 0.3-0.4 µM) against wild-type and NM107-resistant replicons. When tested against their known resistance mutations, each compound showed a 5- to 125-fold loss in susceptibility. In selection experiments, the combination of SCH 503034 and NM107 significantly reduced the frequency of resistant colonies in a dose-dependent manner, compared to each inhibitor used alone. Conclusions: In these in vitro studies, the combination of the polymerase and protease inhibitors showed enhanced anti-replicon activity with no cross-resistance and a greater genetic barrier to resistance. These results support clinical evaluation of this combination in patients with chronic hepatitis C.

Disclosures:
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ANA598, A NOVEL NON-NUCLEOSIDE INHIBITOR OF HCV NS5B POLYMERASE, EXHIBITS FAVORABLE PHARMACOKINETIC PROPERTIES IN MULTIPLE PRECLINICAL SPECIES

INTRODUCTION: Inadequate responses to current HCV therapy create an urgent unmet need for new antiviral agents to complement today’s standard of care, particularly for treatment of genotype 1 infections. Probable future HCV therapies will add multiple direct-acting antiviral agents taken from differing functional classes to enhance response and suppress the emergence of resistant HCV variants. The essentiality of the NS5B polymerase predicts that inhibitors of this enzyme should be attractive additions to current standard of care. However, first generation development candidates targeting this essential enzyme suffer notable defects in potency, metabolism, or pharmacokinetic properties that limit their potential. We describe here the superior pharmacokinetic properties of ANA598, a novel, orally available and potent “palm site” inhibitor of HCV genotype 1 NS5B polymerase. RESULTS AND DISCUSSION: ANA598 demonstrated high oral bioavailability in all four species evaluated. ANA598 plasma exposures were lower in the rat than the monkey; exposure did not differ significantly for solution and suspension formulations in these species. ANA598 demonstrated a greater than proportional increase in plasma exposures with increasing dose in monkey and rat; in the mouse exposures were approximately dose-proportional up to a 1000mg/kg. An oral dose of 5mg/kg ANA598 to monkeys provided C12 and C24 levels (21,600nM and 7,600nM, respectively) that were substantial multiples of the replicon EC50 (31nM for genotype 1a and 3nM for genotype 1b) even after adjustment for protein binding. ANA598 accumulated in rat liver and reached a liver-to-plasma ratio of ~20 at 12 hours after an oral 5mg/kg dose, suggesting significant additional antiviral coverage in the primary target organ for HCV replication. CONCLUSIONS: Preclinical studies of ANA598 in four species have demonstrated high oral bioavailability and plasma and liver trough levels that are substantial multiples of replicon EC50. The favorable combined characteristics of ANA598 support additional investigation of the compound and clearly differentiate it from other molecules of this functional class.

Plasma PK parameters of ANA598 after a single oral dose of 5mg/kg

<table>
<thead>
<tr>
<th>Species</th>
<th>Tmax (h)</th>
<th>AUC(0-∞) μM*h</th>
<th>C12 (μM)</th>
<th>C24 (μM)</th>
<th>Tmax (h)</th>
<th>T1/2 β</th>
<th>Liver/Plasma at 12h</th>
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</thead>
<tbody>
<tr>
<td>Cyclomollics Monkey</td>
<td>65</td>
<td>602</td>
<td>21.6</td>
<td>7.6</td>
<td>42</td>
<td>5.5</td>
<td>8</td>
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<tr>
<td>Beagle Dog</td>
<td>85</td>
<td>441</td>
<td>13.8</td>
<td>3.7</td>
<td>40</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>*CD-1 Mouse</td>
<td>45</td>
<td>42 / 114</td>
<td>0.101 / 0.74</td>
<td>11 / 22</td>
<td>0.5 / 14</td>
<td>3.4</td>
<td></td>
</tr>
<tr>
<td>Sprague Dawley Rat</td>
<td>-115</td>
<td>94</td>
<td>0.3 / 2.0</td>
<td>24</td>
<td>0.4</td>
<td>5</td>
<td>~20</td>
</tr>
</tbody>
</table>

* - mean values in rat and mouse are given separately for males and females, respectively

** - C12 and C24 - plasma levels at 12 and 24 hours post dose, respectively

Disclosures:

The following people have nothing to disclose: Leo Kirkovsky, Yuefen Zhou, Daniel Norris, Ellen Okamoto, Thomas G. Nolan, Darian Bartkowski, Julia Khandurina, Maria Sergeeva, Douglas Murphy, Benjamin Ayida, Alan Xiang, David Ellis, Julie Blazel, Zhongxiang Sun; Anadys Pharmaceuticals, Inc., San Diego, CA

ME3738 INHIBITS HEPATITIS C VIRUS REPLICATION BY ENHANCING INTERFERON-β

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Background/Aims: We previously reported that ME3738, a derivative of soyasapogenol B, has effects against hepatitis C virus (HCV) in cells derived from hepatocytes. Our established model of full-length genotype 1a HCV replication supports replication not only in interferon-β (IFN-β) induction-deficient HuH7 cells, but also in HepG2 cells. Therefore, we used our model to examine whether or not ME3738 modifies innate antiviral activity. We also assessed the effects of ME3738 combined with interferon-α (IFN-α) on HCV. Methods: HepG2 or HuH7 cells were transfected with a full-length HCV cDNA plasmid (pH77), and infected with a replication-defective adenoviral vector expressing T7 polymerase (Ad-T7pol). Cells were infected with Ad-T7pol, then ME3738 (0.1-10µM) and/or 100 IU/ml of IFN-α was added. Protein and RNA were harvested from the cells on day 1, 2, 3, 5, 7 and 9 post-infection. To knock down IFN-β expression, 20 nM of siRNA targeting IFN-β was transfected into the cells 1 day before pH77. We measured HCV positive and negative strands as well as mRNA levels of innate antiviral response-related genes using real-time RT PCR.

We assessed HCV core protein expression by ELISA. Results: The anti-HCV effect of ME3738 was more pronounced in HepG2 than in HuH7 cells. ME3738 enhanced mRNA levels of IFN-β in HepG2 cells. The mRNA levels of 2′-5′ oligoadenylate synthetase and MXA were stimulated by IFN-β in HepG2 cells. When the IFN-β was knocked-down by siRNA, the anti-HCV effect of ME3738 was abrogated. In HuH7 cells, the IFN-β mRNA levels triggered by viral double-stranded RNA were increased slightly, and neither IFN-β nor IFN-stimulated gene mRNA expression by ME3738 was enhanced. ME3738 combined with IFN-α elicited synergistic anti-HCV effects in HepG2 cells (HCV/GAPDH copy ratio (Day 3); 2.58±1.60×10^-6 (ME3738+IFN-α) vs. 3.55±1.56×10^-5 (ME3738), p<0.05). Conclusions: The anti-HCV effect of ME3738 was more potent in HepG2 cells than in cells deficient in IFN-β induction. The enhancement of endogenous IFN-β by ME3738 suggests that this agent exerts innate antiviral action more effectively along the type I IFN pathway. Adding IFN-α synergistically enhanced the anti-HCV action of ME3738. Thus, ME3738 might be a useful anti-HCV agent either with or without IFN-α.

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The following people have nothing to disclose: Yoichi Hiasa, Yashio Tokumoto, Ichiro Konishi, Bunzo Matsusura, Kojiro Michitaka, Raymond T. Chung, Morikazu Onishi

PF-00868554, A POTENT NON-NUCLEOSIDE INHIBITOR OF THE HEPATITIS C VIRUS RNA POLYMERASE

Stephanie Shi, Koleen Herlihy, Rebecca Irvine, Susan Binford, Cristina Lewis, Jim Nonomiya, Amy Patrick; Pfizer, San Diego, CA

PF-00868554 is a non-nucleoside inhibitor of the HCV RNA polymerase, which exerts its inhibitory effect by binding to the
thumb-base domain of the protein. It is a potent and selective inhibitor of the enzyme, with a mean IC50 of 0.0096 µM against genotype 1 polymerases in biochemical assays. To determine the in vitro antiviral activity of PF-00868554 against various HCV laboratory and clinical strains, a panel of chimeric replicons was generated in which polymerase sequences derived from genotype 1a and 1b clinical isolates were cloned into the genotype 1b (Con1 strain) subgenomic reporter replicon. The antiviral activity of PF-00868554 was evaluated against this panel of chimeric replicons in the transient replication assay in Huh7.5 cells using the luciferase reporter end-point. Results indicate that PF-00868554 has potent in vitro antiviral activity against a majority (95.8%) of genotype 1a and 1b replicons, with overall mean EC50 and EC90 values of 0.059 and 0.33 µM, respectively. PF-00868554 showed no cytotoxic effect in several human cell lines up to the highest concentration evaluated (320 µM). Furthermore, the antiviral activity of PF-00868554 was retained in the presence of human serum proteins. Our results demonstrate that PF-00868554 has potent and broad-spectrum antiviral activity against genotype 1 HCV strains, supporting its use as an antiviral agent in HCV-infected patients.

Disclosures:
The following people have nothing to disclose: Stephanie Shi, Koleen Herlihy, Rebecca Irvine, Susan Binford, Cristina Lewis, Jim Nonomiya, Amy Patrick

1395 MECHANISTIC CHARACTERIZATION OF GS-9190, A NOVEL NON-NUCLEOSIDE INHIBITOR OF HCV NS5B POLYMERASE WITH POTENT ANTIVIRAL ACTIVITY AND A UNIQUE MECHANISM OF ACTION

Hung Shih1, Inge Vliegen2, Betty Peng3, Huiling Yang1, Jan Paeshuyse2, Gerhard Purstinger3, Martijn Fenaux1, Eric Mabery3, Gina Bahador1, Laura S. Lehman1, Steven Bondy1, Winston Tse1, Hans Reiser1, William A. Lee1, Johan Neyts1, Weidong Zhong1, 2Gilead Sciences, Inc., Foster City, CA; 3Rega Institute, Leuven, Belgium; 4University of Innsbruck, Innsbruck, Austria

Background and Aims: GS-9190 is a drug candidate currently being evaluated for safety, tolerability, pharmacokinetics, and antiviral efficacy in chronically infected HCV patients. It is a novel imidazopyridine analogue with potent antiviral activity in HCV replicon cells, especially against genotype 1 HCV. GS-9190 has lower potency against a genotype 2a (JFH1) subgenomic replicon or against the JFH1 infectious virus. The aim of this study was to define the mechanistic action of GS-9190.

Methods: Chimeric replicons carrying sequences from genotype 1b (Cont1-1) and genotype 2a (JFH1) were used to identify viral determinants of susceptibility to GS-9190. A cell-based, strand-specific qRT-PCR replicase assay was employed to analyze the inhibitory profile of GS-9190. Drug resistance selection with GS-9190 was performed in Cont1 replicon cells. Results: To investigate the mechanism of action, we utilized the susceptibility difference between genotype 1b and genotype 2a (JFH1) subgenomic replicon by comparing the viral genome including several inside the beta-hairpin of NS5B. Subsequent studies showed that only those mutations in NS5B were able to confer resistance to GS-9190 when introduced into a wild-type replicon, whereby the total number of mutations correlated with the degree of resistance. Collectively, these data demonstrate that GS-9190 represents a novel non-nucleoside NS5B inhibitor whose binding pocket involves beta-hairpin and is in close proximity to the catalytic active site of NS5B. Detailed mechanistic characterization of GS-9190 and its interaction with NS5B will be presented.

Disclosures:
The following people have nothing to disclose: Stephanie Shi, Koleen Herlihy, Rebecca Irvine, Susan Binford, Cristina Lewis, Jim Nonomiya, Amy Patrick

1396 MODELING THE POTENTIAL COST-EFFECTIVENESS OF ADDING A NOVEL STAT-C AGENT TO CURRENT STANDARD THERAPY IN PATIENTS WITH GENOTYPE 1 CHRONIC HEPATITIS C INFECTION

Sumedha P. Galhenage1, Gillian D. Sanders2,3, Keyur Patel1,4, Kevin A. Schulman5,6, John G. McHutchison1,4; 1GI/Hepatology, Duke Clinical Research Institute, Durham, NC; 2Outcomes Research and Assessment Group, Duke Clinical Research Institute, Durham, NC; 3Division of Clinical Pharmacology, Duke University Medical Center, Durham, NC; 4Division of Gastroenterology and Hepatology, Duke University Medical Center, Durham, NC; 5Center for Clinical and Genetic Economics, Duke Clinical Research Institute, Durham, NC; 6Division of General Internal Medicine, Duke University Medical Center, Durham, NC

Background: Current standard of care for chronic hepatitis C (CHC) infection with peginterferon (PEG-IFN) and ribavirin (RBV) (P+R) is costly, and effective in only half of all treated cases. Recent data have shown that the addition of novel Specifically Targeted Antiviral Therapy for HCV (STAT-C) to standard of care has the potential to advance the treatment of genotype 1 CHC infection. Thus, we modeled this new strategy to assess the economic impact of improved viral response on costs and outcomes. Methods: A Markov model was developed to compare standard therapy with 4 treatment strategies (a STAT-C agent for 12 weeks combined with P +/− R for 12-48 weeks). The model followed treatment-naïve individuals with mild CHC infection (Metavir FO-F1) from treatment initiation until death. Costs of PEG-IFN α-2a 180mcg weekly and RBV 1000-1200mg daily were based on average wholesale prices. Base cost of STAT-C therapy was assumed equivalent to 48 weeks of P + R. Rates of SVR and adverse effects were estimated. Natural history of CHC infection, treatment patterns, healthcare costs and utilities for all disease states were obtained from published data. Analyses
Princesa”, Madrid, Spain; 3Memorial Sloan-Kettering Cancer Cen-
tered, New York, NY; 4R&D Department, Industrial Farmaceútica

The following people have nothing to disclose: Sumedha P. Galhenage, Gillian D. Sanders, Keyur Patel, Kevin A. Schultz

1397
AM3 INHIBITS HCV REPLICATION THROUGH ACTIVA-
TION OF PERIPHERAL BLOOD MONONUCLEAR CELLS

Pedro L. Majano 2, Samuel Martín-Vílchez 1, Yolanda Rodríguez-
Munoz 1, Paloma Sanz Cameno 1, Francisca Molina-Jimenez 2, Jose L. Alonso 4, Salvador Gonzalez 2, Manuel Lopez-Cabrera 2, Ricardo Moreno-Otero 4, Paloma Sanz Cameno 1, Francisca Molina-Jimenez 2, Salvador Gonzalez 2, Manuel Lopez-Cabrera 2, Ricardo Moreno-Otero 4

INTRODUCTION Immunoferon® is a drug whose active princi-
ple is a glycoconjugate of natural origin composed of a glu-
cosyranosyl polysaccharide from Candida utilis and a storage
protein from nongerminated seeds of Ricinus communis. The
glycoconjugate that constitutes the active principle of
Immunoferon® (hereafter referred as AM3) has been shown to
modulate regulatory and effector functions of the immune sys-
tem by acting on peripheral blood mononuclear cells (PBMCs)
and modifying the expression of extracellular mediators, such
as tumor necrosis factor alpha (TNF-α) and interleukin-1 beta.
Previously, it has been shown that AM3 has antiviral effect
against HBV due to stimulation of secretion of molecules with
antiviral properties by PBMC. OBJECTIVE: To analyze whether
AM3 has antiviral properties against HCV replication in vitro.
MATERIALS AND METHODS: Reagents: AM3 was prepared
according to the methods described in patents P9900408 (Spain)
and PCT/ES99/00338. The phosphorylated glu-
cosylmannon polysaccharide and the protein were combined in a
5:1 (wt/wt) polysaccharide/protein proportion. Cell culture: We
used two clones derived from Huh-7 cell with genomic bicistronic,
full-length HCV genome. PBMCs were purified from peripheral
blood cells from healthy donors by Ficoll density
centrifugation and cultured at 106 cells/ml for 24 h prior to
analysis. PBMC were stimulated or not with AM3 (1 µg/ml) for
48 or 96 h and then cell supernatants were collected. Huh7/HCV clones were treated for 24 or 48 h with the PBMC
supernatant (conditioned medium; 1:2 proportion, with normal
culture medium). To analyse whether AM3 has a direct antiviral
effect on HCV replication, Huh7/HCV clones were incubated
with various doses of AM3 for 48 h. Methods: Total RNA
Huh/HCV clones were isolated and HCV RNA was analyzed
by real-time RT-PCR. Additionally, core and non-structural pro-
tein NS5a levels were determined by western blot. RESULTS:
HCV replicons cells treated with supernatant derived from AM3
stimulated PBMC exhibit lower HCV RNA and protein accumu-
lation when compared to untreated controls. However, AM3
did not affect directly the expression of RNA or protein in any
of the cell clones tested. CONCLUSION: In this report, we
found that AM3 inhibited HCV RNA and protein expression by
an indirect mechanism. We found that AM3 lacked intrinsic
antiviral properties, and that the antiviral effect of the glyco-
conjugate was due to stimulation of secretion of molecules with
antiviral properties by PBMC. Our data suggest that the
employment of AM3 as an adjuvant administered simultane-
ously with conventional antiviral drugs may potentiate the
endogenous response against viral infection.

Disclosures: Pedro L. Majano - Grant/Research Support: Other
Jose L. Alonso - Employee: Other

The following people have nothing to disclose: Samuel Martin-Vilchez, Yolanda Rodriguez-Munoz, Paloma Sanz Cameno, Francisca Molina-Jimenez, Salvador Gonzalez, Manuel Lopez-Cabrera, Ricardo Moreno-Otero

1398
PRECLINICAL PHARMACOKINETIC CHARACTERIZATION OF GS-9190, A NOVEL NON-NUCLEOSIDE HCV NS5B
POLYMERASE INHIBITOR

Chris Yang, Yujin Wang, Lani Wieman, Eugene Eisenberg, Melody
Lee, Bernard Murray, Leah Tong, Adrian Ray, Steven Bondy, Winston
Tse, Amy Coluci, Matthew Wright, Laura S. Lehman, William A. Lee, Gerald Rhodes; Gilead Sciences, Foster City, CA

Background and Aims: GS-9190 is a novel non-nucleoside HCV RNA polymerase inhibitor with potent in-vitro antiviral
activity and a high selectivity index. GS-9190 is currently being
evaluated for safety/tolerability, pharmacokinetics and antiviral
efficacy in chronically infected HCV patients. It is a novel imi-
dazopyridine analogue with potent antiviral activity against
 genotype 1 HCV replicon cells. The aim of these studies was to
characterize the pharmacokinetic profile of GS-9190 in rele-
vant preclinical models prior to administration in humans. Meth-
ods: Single dose pharmacokinetic studies were conducted
following intravenous and oral administration of GS-9190 in
rats, dogs and cynomolgus monkeys. The distribution and
routes of excretion were determined in rats following oral
administration of 14C-GS-9190. The absorption, metabolic sta-
bility, protein binding and the CYP450 inhibition and induction
profile of GS-9190 were evaluated in vitro in systems derived
from human and, where appropriate, preclinical species. Results:
GS-9190 was stable to in vitro metabolism in microso-
lates, dogs and cynomolgus monkeys. The distribution and
clearance and a volume of distribution comparable to
body water. Oral bioavailability was greater than 30 % in

1397
AM3 INHIBITS HCV REPLICA
all preclinical species and was not limited by hepatic first pass extraction. In the rat, the majority of the 14C-radiolabelled dose was excreted slowly into the bile with only minor excretion in the urine. Recovery of radioactivity was essentially complete 48 hr after administration of 14C-GS-9190. The available human in vitro data project a favorable clinical pharmacokinetic profile, with high oral bioavailability for GS-9190 in human subjects. As a result it is predicted that plasma concentrations of GS-9190 sufficient to drive pharmacological effect can be achieved with relatively modest doses in humans.

Disclosures:
The following people have nothing to disclose: Chris Yang, Yujin Wang, Lani Wie,man, Eugene Eisenberg, Melody Lee, Bernard Murray, Leah Tong, Adrian Ray, Steven Bondy, Winston Tse, Amy Colucci, Matthew Wright, Laura S. Lehman, William A. Lee, Gerald Rhodes

**LB6**

E. Jenny Heathcote1, Ed Gane2, Robert DeMan3, Sam Lee4, Robert Flisiak5, Michael P. Manns6, Konstantin Tchernev7, Oya Kurdas8, Mitchell L. Shiftman9, Jeff Sorbel10, Jane Anderson10, Elsa Mondau10, Franck Rousseau10, 1University of Toronto, Toronto, ON, Canada; 2Middlemore Hospital, Auckland, New Zealand; 3Erasmus MC University Medical Center Rotterdam, Rotterdam, Netherlands; 4University of Calgary, Calgary, AB, Canada; 5Medical University, Sofia, Bulgaria; 6Haydarpaşa Numune Hospital, Istanbul, Turkey; 7Virginia Commonwealth University, Richmond, VA; 8Gilead Sciences, Durham, NC

**Background:** Tenofovir Disoproxil (TDF) is a nucleotide analogue approved for the treatment of HIV-1 with activity against hepatitis B virus (HBV). The primary objective of this Phase III, 5 year study was to compare the safety and efficacy of 300 mg TDF versus 10 mg ADV at 48 weeks in subjects with HBsAg + CHB. **Methods:** This was a double-blind, active-controlled, study of mono-infected subjects with HBsAg+ CHB who were randomized in a 2:1 ratio to TDF:ADV. Entry criteria included subjects 18-69 years of age with compensated liver disease, ALT>2xULN, HBV DNA>10^6 copies/mL and a Knodell necroinflammatory score without worsening in the midzone accompanied by accentuated elevation of plasma ALT levels as compared to alcohol-fed wt mice (95% 18-21). This pathology resembled necrosis commonly frequent in the TDF group (19%) vs ADV group (10%). Renal safety was excellent; no TDF subject had a confirmed 0.5 mg/dL creatinine increase or creatinine clearance <50 mL. TDF was well tolerated with no TDF subject discontinuing due to an AE and with the majority completing primary endpoint assessments (90%TDF,93%ADV). Primary and secondary efficacy endpoints are summarized below. **Conclusion:** TDF, at a dose of 300 mg QD, was well tolerated and demonstrated superior efficacy to ADV through 48 weeks as illustrated by the higher percentage of HBeAg+ subjects achieving the primary efficacy endpoint and most secondary endpoints.

<table>
<thead>
<tr>
<th>Efficacy Endpoints</th>
<th>TDF 300 mg N=176</th>
<th>ADV 10 mg N=90</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary Composite Endpoint</td>
<td>67%</td>
<td>12%</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Histologic Response</td>
<td>74%</td>
<td>68%</td>
<td>NS</td>
</tr>
<tr>
<td>% HBV DNA&lt;69 c/mL (LLQ)</td>
<td>69%</td>
<td>9%</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>% HBV DNA&lt;10^6 c/mL</td>
<td>74%</td>
<td>12%</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Normal ALT</td>
<td>69%</td>
<td>54%</td>
<td>0.018</td>
</tr>
<tr>
<td>HBeAg Seroconversion</td>
<td>21%</td>
<td>18%</td>
<td>NS</td>
</tr>
<tr>
<td>HBsAg Loss</td>
<td>3%</td>
<td>0%</td>
<td>0.018</td>
</tr>
</tbody>
</table>

**Disclosures:**
E. Jenny Heathcote - Consultant/Adviser: Gilead
Robert DeMan - Grant/Research Support: Gilead
Sam Lee - Grant/Research Support: Gilead; Consultant/Adviser: Gilead; Speaker’s Bureau: Gilead
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Jeff Sorbel - Employee: Gilead
Jane Anderson - Employee: Gilead
Elsa Mondau - Employee: Gilead
Franck Rousseau - Employee: Gilead

The following people have nothing to disclose: Ed Gane, Robert Flisiak, Konstantin Tchernev, Oya Kurdas

**LB7**
ACTIVATION OF TLR4-TRA6-FK1 PATHWAY INDUCES HEPATOCELULAR CARCINOMAS BY SYNERGISTIC INTERACTIONS BETWEEN ALCOHOL AND HEPATITIS C VIRUS NSSA

Keigo Machida1, Hidekazu Tsukamoto2, Hasnik Mkrtchyan2, Alla Dynyk2, Helene Liu3, Jiaohong Wang3, Ranjit Ray3, Ratna Ray4, Michael M. Lai1; 1Molecular Microbiology and Immunology, University of Southern California Keck School of Medicine, Los Angeles, CA; 2Pathology, University of Southern California Keck School of Medicine, Los Angeles, CA; 3Internal Medicine, St. Louis University, St. Louis, MO; 4Pathology, St. Louis University, St. Louis, MO

**Background:** Alcohol exacerbates hepatitis C virus (HCV)-induced liver disease as exemplified by their synergistic effects on the incidence of hepatocellular carcinoma (HCC). Inflammation plays an important role in HCC oncogenesis. HCV NSSA protein induces expression of TLR4, which mediates inflammation in response to endotoxin. It is yet to be tested whether TLR4 induction plays a role in the alcohol-HCV synergism for oncogenic potential. **Objectives** We aimed to determine whether chronic alcohol intake causes synergistic induction of HCC by HCV NSSA in mice. **Methods** HCV NSSA transgenic (Tg) and wild type (wt) mice were fed with Lieber-DeCarlie alcohol diet for 12 months or infused with alcohol diet for 4 week by intragastric infusion model. **Results** HCCs in NSSA Tg mice were induced by 12-month-chronic alcohol feeding (0% vs. 25%). Further, 4-week-ethanol-infused NSSA Tg mice displayed sub-massive necrosis and inflammation in the midzone accompanied by accentuated elevation of plasma ALT levels as compared to alcohol-fed wt mice (95%±18 vs. 181±21). This pathology resembled necrosis commonly observed in chronically ethanol-fed rodents given acute lipopolysaccharide (LPS) and is consistent with TLR4-mediated injury in NSSA Tg mice. Further, weekly enteral administration
of LPS to ethanol-fed NS5A Tg mice resulted in an additional increment in ALT levels and pathology. LPS injection in NS5A Tg mice increased mortality, liver damage, and TNF-α production through TGF-β-activated kinase 1 (TAK1) and TNFR-associated factor 6 (TRAF6) complexes and phosphorylation of JNK in liver. TAK1-TRAF6 complex formation was induced in HCCs in NS5A Tg mice fed with alcohol. These results demonstrate that alcohol feeding induces HCCs in the liver of NS5A Tg mice through induction of TLR4. The TLR4 signaling molecules, involving TAK1, TRAF6, and JNK, are involved in alcohol-induced oncogenesis in NS5A Tg mice. We conclude that there is a synergistic interaction between HCV proteins and alcohol because of the enhanced endotoxin-induced inflammation through TLR4.

Disclosures:
The following people have nothing to disclose: Keigo Machida, Hidekazu Tsukamoto, Hasimik Mrkchtian, Alla Dynnyk, Helene Liu, Jiaohong Wang, Ranjit Ray, Ratha Ray, Michael M. Lai

LB8
EVALUATION OF VIRAL VARIANTS DURING A PHASE 2 STUDY (PROVE2) OF TELAPREVIR WITH PEGINTERFERON ALFA-2A AND RIBAVIRIN IN TREATMENT-NAIVE HCV GENOTYPE 1-INFECTED PATIENTS
Taral Kiefer1, Yi Zhou1, Eileen Zhang1, Michelle Marcial1, Randal Byrn1, Thomas Pfeiffer1, Janice Miller1, Ann Tigges1, Doug Bartels1, Ann Kwong1, Peter Ferenci2, Geoffrey Dusheiko2, Stefan Zeuzem3, Jean-Michel Pawlotsky4, Infectious Diseases, Vertex Pharmaceuticals Incorporated, Cambridge, MA; 2Department of Internal Medicine III, Medical University of Vienna, Vienna, Austria; 3Centre for Hepatology, Royal Free Hospital, London, United Kingdom; 4Department of Medicine I, J.W. Goethe University Hospital, Frankfurt, Germany; 5Department of Virology, Henri Mondor Hospital, University of Paris XII, Créteil, France

Background: The VX05-950-104EU study (PROVE2) is a Phase 2 study of an HCV protease inhibitor, telaprevir (VX-950, TVR) 750 mg q8h, in combination with Peg-IFN-alfa-2a (P) 180 µg/week and ribavirin (R) 1000-1200 mg/day in treatment-naive subjects with genotype 1 hepatitis C. Subjects were randomized into 4 groups that received: 1) TVR/P/R for 12 weeks, 2) TVR/P for 12 weeks, 3) TVR/P/R for 12 weeks followed by 12 weeks of P/R, and 4) P/R for 48 weeks (control group). Viral breakthrough (increase of > 1-log above HCV RNA nadir or increase to > 100 IU/mL in previously undetectable patients) was observed in some subjects during the first 12 weeks of therapy. Viral sequencing was used to evaluate the contribution of TVR resistance to antiviral response. Methods: Plasma samples for viral sequencing were taken at baseline and at each HCV RNA assessment. The NS3-4A RNA region was amplified by nested RT-PCR and sequenced in samples with HCV RNA > 1,000 IU/mL. Prospectively defined criteria for identifying potential resistance mutations were applied using a Poisson distribution. Results: HCV RNA was undetectable (LOD 10 IU/mL) for viral sequencing were taken at baseline and at each HCV RNA assessment. The NS3-4A RNA region was amplified by nested RT-PCR and sequenced in samples with HCV RNA > 1,000 IU/mL. Prospectively defined criteria for identifying potential resistance mutations were applied using a Poisson distribution. Results: HCV RNA was undetectable (LOD 10 IU/mL) at Week 4 in 14% of subjects in the P/R group, 74% in the TVR/P/R groups (p<0.001), and 53% in the TVR/P group (p<0.001); and at Week 12 in 43% of subjects in the P/R group, 79% in the TVR/P/R groups (p<0.001), and 63% in the TVR/P group (p<0.015). During the first 12 weeks on treatment, 6 of 152 subjects (4%) in the TVR/P/R groups, 19 of 76 subjects in the TVR/P group (25%), and 1 of 77 subjects (1%) in the P/R group had viral breakthrough. The breakthroughs were associated with wild-type virus in the P/R group and previously identified TVR-resistant variants in the TVR groups TVR/P/R: genotype 1a, n=4: mainly V36M and R155K; genotype 1b, n=2: mainly A156T; TVR/P: genotype 1a, n=12: mainly V36M and R155K; genotype 1b, n=7: mainly V36A, T54A, R155K, and A156S/T). Conclusions: In this interim analysis, TVR/P/R produced a significantly greater antiviral response at Weeks 4 and 12 compared to TVR/P and P/R in treatment naive subjects with genotype 1 HCV. In the TVR groups, viral breakthroughs were associated with the selection of known TVR-resistant variants. The breakthrough rate in the TVR/P group was higher than in the TVR/P/R groups, suggesting that suboptimal inhibition of viral replication provides a greater probability for selection of TVR-resistant variants. Some subjects with viral breakthrough on TVR had a subsequent decline in HCV RNA during treatment with P/R, indicating that emergence of TVR-resistance does not preclude response to P/R. *on behalf of the PROVE2 Study Team

Disclosures:
Tara Kieffer - Employee: Vertex; Yi Zhou - Employee: Vertex; Eileen Zhang - Employee: Vertex; Michelle Marcial - Employee: Vertex; Randal Byrn - Employee: Vertex; Thomas Pfeiffer - Employee: Vertex; Janice Miller - Employee: Vertex; Ann Tigges - Employee: Vertex; Doug Bartels - Employee: Vertex; Ann Kwong - Employee: Vertex; Peter Ferenci - Speaker: Roche; Consultant/Adviser: Roche; Grant/Research Support: Roche; Grant/Research Support: Human Genome Sciences; Consultant/Adviser: Novartis; Grant/Research Support: GlaxoSmithKline; Geoffrey Dusheiko - Grant/Research Support: Roche; Grant/Research Support: Vertex; Consultant/Adviser: Roche; Consultant/Adviser: Vertex; Stefan Zeuzem - Consultant/Adviser: Roche; Consultant/Adviser: Vertex; Speaker: Vertex; Speaker: Roche; Grant/Research Support: Vertex; Grant/Research Support: Roche; Consultant/Adviser: Vertex; Speaker's Bureau: Gilead; Speaker's Bureau: Human Genome Sciences; Speaker's Bureau: Indenix; Jean-Michel Pawlotsky - Grant/Research Support: Roche; Consultant/Adviser: Roche; Consultant/Adviser: Vertex; Consultant/Adviser: Roche; Consultant/Adviser: Vertex; Grant/Research Support: Vertex; Consultant/Adviser: Vertex; Speaker's Bureau: Vertex; Speaker's Bureau: Vertex.

LB9
ANTIVIRAL ACTIVITY, PHARMACOKINETICS, SAFETY, AND TOLERABILITY OF R7128, A NOVEL NUCLEOSIDE HCV RNA POLYMERASE INHIBITOR, FOLLOWING MULTIPLE, ASCENDING, ORAL DOSES IN PATIENTS WITH HCV GENOTYPE 1 INFECTION WHO HAVE FAILED PRIOR INTERFERON THERAPY
Rajender Reddy1, Maribel Rodriguez-Torres2, Ed Gane3, Richard Robson4, Jacob Lalezari5, Gregory T. EVerson6, Edwin DeJesu5, John G. McHutchison8, Hugo E. Vargas8, Amanda Bead9, Carlos A. Rodriguez11, George Z. Hill11, William Symonds10, Michelle Berrey10,12, University of Pennsylvania, Philadelphia, PA; 2Fundacion de Investigacion de Diego, Santurce, PR; 3Auckland Clinical Studies Limited, Auckland, New Zealand; 4CCST, Christchurch, New Zealand; 5Quest Clinical Research, San Francisco, CA; 6University of Colorado, Aurora, CO; 7Orlando Immunology Center, Orlando, FL; 8Duke Clinical Research Institute, Durham, NC; 9Mayo Clinic, Phoenix, AZ; 10Pharmasset, Inc., Durham, NC; 11Roche, Palo Alto, CA

Background: R7128 is a prodrug of PSI-6130, an oral cytidine nucleoside analog polymerase inhibitor, currently in development for the treatment of HCV. This multiple ascending dose study assessed safety, tolerability, pharmacokinetics, and preliminary antiviral activity of R7128 in subjects with HCV genotype 1 infection. Methods: Multiple oral doses of R7128 monotherapy were administered for 14 days to 40 HCV-infected patients (n=10 per cohort with 8 active + 2 placebo) of 750mg QD, 1500mg QD, 750mg BID & 1500mg BID. PK, safety and virology assessments were conducted throughout the study period. Data are available for the 750mg QD, 1500mg QD and 750mg BID cohorts. The 1500mg BID cohort is fully...
enrolled. **Results:** All subjects had HCV genotype 1 (29 – 1a; 11 – 1b), had previously failed alpha-interferon and were non-cirrhotic. There were no SAEs reported and no AEs required dose modification; no clinically significant changes in vital signs, ECGs, hematology, renal or other laboratory parameters occurred. Preliminary data on adverse events (AEs) reported during treatment in subjects receiving R7128 include a total of 32 events in 14 of 24 subjects, most of mild intensity as compared to 26 events in 5 subjects receiving placebo. 18 AEs were reported in 7 subjects that received 750mg QD, 3 AEs in 3 subjects receiving 1500mg QD, and 11 AEs in 4 subjects receiving 750mg BID. The most frequently reported AEs for patients receiving R7128 were headache (8), dry mouth (2), nausea, (2) and upper respiratory infection (2); in subjects on placebo, headache (3) and diarrhea (3) were most commonly reported. The baseline HCV RNA concentrations, ranging from 5.2 to 7.4 (log10 IU/mL), and mean change from baseline observed, ranging from -0.7 to -2.9 (log10 IU/mL) for active, are summarized in the table below. Plasma exposure to the prodrug, R7128, was negligible; exposure to PSI-6130 and a uridine metabolite, PSI-6206, increased with increasing doses of R7128. Terminal half-life was approximately 5 h for PSI-6206, increased with increasing doses of R7128. Terminal half-life was approximately 5 h for PSI-6130 and 20 h for psi-6206. **Conclusion:** R7128 monotherapy was generally well-tolerated and resulted in significant, dose-dependent suppression of HCV replication following 14 days of monotherapy. These results support continued development of R7128 for the treatment of HCV infection in combination with pegylated interferon and ribavirin. Results for the 1500 BID cohort will be available for presentation.

<table>
<thead>
<tr>
<th>Dose</th>
<th>Placebo (n=8)</th>
<th>750mg QD (n=8)</th>
<th>1500mg QD (n=8)</th>
<th>750mg BID (n=8)</th>
<th>1500mg BID (n=8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean baseline HCV RNA concentration (log10 IU/mL)</td>
<td>6.7 (6.3 to 7.2)</td>
<td>6.6 (5.2 to 7.4)</td>
<td>6.6 (5.8 to 6.9)</td>
<td>6.5 (5.7 to 7.3)</td>
<td>Ongoing</td>
</tr>
<tr>
<td>Mean (range) change from baseline on day 15 (log10 IU/mL)</td>
<td>-0.3 (-0.7 to 0.1)</td>
<td>-0.9 (-1.7 to -1.1)</td>
<td>-1.5 (-0.9 to -2.5)</td>
<td>-2.1 (-1.8 to -2.9)</td>
<td>Ongoing</td>
</tr>
<tr>
<td>Proportion with ≥2 log decline by day 15</td>
<td>±0%</td>
<td>±3%</td>
<td>±7%</td>
<td>±8%</td>
<td>Ongoing</td>
</tr>
</tbody>
</table>

Disclosures:
Rajender Reddy - Employee: Other
Maribel Rodriguez-Torres - Employee: Other
Ed Gane - Grant/Research Support: Other
Richard Robson - Employee: Other
Jacob Lalezari - Grant/Research Support: Other
Gregory T. Everson - Employee: Other
Edwin DeJesus - Grant/Research Support: Other
John G. McHutchison - Grant/Research Support: Other; Consultant/Adviser: Other
Hugo E. Vargas - Grant/Research Support: Other
Amanda Beard - Employee: Other
Carlos A. Rodriguez - Employee: Roche
William Symonds - Employee: Other
Michelle Berrey - Employee: Other

The following people have nothing to disclose: George Z. Hill

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**LB10**

**PHASE 2A STUDY TO EVALUATE THE SAFETY AND TOLERABILITY AND ANTI-VIRAL OF 4 DOSES OF LOCTERON**

Iryna Dzyublyk1, Tetiana Yegorova2, Larisa Moroz3, Oleksandra Popovych3, Igor Zaytsev4, Vladimir Miroshnichenko5, Eva Herrmann5, Stefan Zeuzem6, Ewoud J. van Hoogdalem7, John E. Humphries8; 1PL Shupyk National Medical Academy of Postgraduate Education, Kyiv, Ukraine; 2Kiev City Hospital, Kiev, Ukraine; 3M.I. Pyrogov Vinnitsa National Medical University, Vinnitsa, Ukraine; 4M. Gorky Donetsk State Medical University, Donetsk, Ukraine; 5Saarland University Hospital, Homburg/Saar, Germany; 6Saarland University, Homburg/Saar, Germany; 7Octoplus, N.V., Leiden, Netherlands; 8Biolet Therapeutics, Pittsboro, NC

Background: Controlled-release recombinant interferon-alfa 2b (Locteron) is a novel approach to delivery of interferon (IFN) given every 2 weeks with improved tolerability combined with a high level of hepatitis C virus (HCV)RNA reduction. Methods: A phase 2a, open-label, dose-ranging study was conducted in treatment-naive patients with genotype 1 chronic HCV infection to evaluate the safety, tolerability and anti-viral effect of Locteron. 32 patients were randomized to receive subcutaneous injections of Locteron 14 days apart over 12 weeks in 4 dose cohorts (8 per cohort) of 160, 320, 480 and 640 µg, with the 640 µg group starting after safety evaluation of the other cohorts. All subjects received weight-based ribavirin. HCV RNA reduction modeling was performed. Results: The mean HCV RNA reduction at week 4 for the 160, 320, 480 and 640 µg groups were 1.1, 3.1, 2.9 and 3.1 logs, respectively. Percent of HCV negative subjects at week 4 were 0, 25, 38 and 25, respectively. Average viral reduction after 12 weeks for the 3 lower doses of Locteron (160, 320 and 480 µg) was 1.8, 4.5 and 4.2 logs, respectively. 63% of subjects at the 2 middle doses (320 & 480 µg) were HCV negative (LLOQ < 28 IU/mL) at 12 weeks. Early viral response (EVR: 12-week ≥ 2-log drop in HCV RNA) was achieved in 88% and 100% of subjects in the 320 and 480 µg Locteron dose cohorts, respectively. Modeling of HCV kinetics demonstrated dose-dependent biphasic kinetics reflected by mean and maximum efficiency in blocking viral production from day 1 to day 28 of treatment. Mean and maximum efficiency were 41%±23% and 54%±27%, respectively, in the 160 µg cohort, 58%±19% and 71%±21% in the 320 µg cohort, 72%±20% and 84%±11% in the 480 µg cohort, and 73%±24% and 80%±22% in the 640 µg cohort. The median T1/2 of free HCV RNA was 2 weeks and the median T1/2 of infected cells was 2.8 days. Clinical adverse events were almost exclusively mild in intensity with only 1 severe adverse event in the 3 lower dose cohorts. The most common adverse events were arthralgia (50%), weakness (50%), myalgia (38%) and headache (33%), with chills, nausea and diarrhea each reported in fewer than 5% of subjects. Fever (Temp ≥ 38.0°C) occurred in only 1 subject in these first 3 cohorts. Full data on all 4 cohorts for all 12 weeks will be available by the AASLD meeting. Conclusions: In this study, Locteron, a controlled-release formulation of unmodified IFN-alfa 2b, administered every 2 weeks to treatment-naive patients with chronic hepatitis C (genotype 1) demonstrated strong anti-viral activity combined with an improved safety and tolerability profile compared to currently marketed IFNs and those in development.

Disclosures:
Ewoud J. van Hoogdalem - Employee: Other
John E. Humphries - Employee: Other
Antiviral activity of the non-nucleoside polymerase inhibitor, VCH-759, in chronic hepatitis C patients: results from a randomized, double-blind, placebo-controlled, ascending multiple-dose study.

Curtis Cooper1, Eric J. Lawitz2, Peter Ghali3, Maribel Rodriguez-Torres4, Frank H. Anderson5, Samuel S. Lee6, Louise Proulx7
1Department of Internal Medicine, Division of Infectious Disease, The Ottawa Hospital, Ottawa, ON, Canada; 2Alamo Medical Research, San Antonio, TX; 3Department of Gastroenterology and Hepatology, McGill University Health Center, Royal Victoria Hospital, Montréal, QC, Canada; 4Fundación de Investigación de Diego San Juan, PR; 5Liver and Intestinal Research Center, Vancouver, BC, Canada; 6Liver Unit, University of Calgary, Calgary, AB, Canada; 7ViroChem Pharma Inc, Laval, QC, Canada

Background: VCH-759 is a novel, orally bioavailable non-nucleoside inhibitor of hepatitis C virus RNA-dependent RNA polymerase. It has demonstrated sub-micromolar IC50 against the HCV replicons of genotype 1a and 1b. Methods: This multiple ascending dose study was designed to assess the effect on viral kinetics, viral resistance, pharmacokinetics, safety and tolerability of VCH-759 given as monotherapy for 10 days with a 14-day follow-up period. Three cohorts of treatment-naive chronic hepatitis C patients infected with HCV genotype 1 received either placebo, 400 mg tid, 800 mg tid or 800 mg bid. Viral loads were determined using the Roche Amplicor assay (lower limit of quantification=600 IU/ml). VCH-759 plasma levels were assessed over 6 hours for the tid regimen and over 12 hours for the bid regimen on Days 1 and 10 and daily, prior to the morning dose, on Days 1 to 11. Results: Thirty-two (32) subjects were enrolled and completed the study: 9: placebo, 9: 400mg tid, 9: 800 mg tid, 5: 800 mg bid. VCH-759 was rapidly absorbed with peak plasma levels of 1857 ± 773 ng/ml, 3675 ± 2213 ng/ml and 4627 ± 94 ng/ml at Day 1 for the 400 mg tid, 800 mg tid and 800 mg bid doses respectively. All subjects had more than a one log reduction with values ranging between 1.4 and 2.6 log10 for the 400 mg tid dose, 1.5 and 2.9 log10 for the 800 mg bid dose and 1.2 and 3.3 log10 for the 800 mg tid dose. The mean maximal decrease in HCV RNA log10 was 1.9, 2.3 and 2.5 for the 400 mg tid, 800 mg bid and 800 mg tid doses respectively. The plasma levels at trough (before the morning dose) were higher than the IC90 (420 ng/ml) between Days 2 and 11 for 800 mg tid and between Days 2 and 7 for 800 mg bid. VCH-759 was well tolerated with the most frequent adverse events being gastrointestinal disorders (most likely due to the formulation vehicle used) reported in both the active and the placebo groups. Conclusions: VCH-759 achieved a 2 log10 or larger decline in HCV RNA at doses of 800 mg tid and bid. VCH-759 was well tolerated with no serious adverse events and no discontinuation. Genetic sequencing of NS5B is ongoing. Further studies combining VCH-759 with current therapies are warranted.

Restoration of the defective innate immune system following treatment with the probiotic Lactobacillus casei Shirota in patients with alcoholic cirrhosis: a proof of concept study

Vanessa Stadlbauer, Rajeshwar P. Mockereeje, Stephen Hodges, Gavin A. Wright, Nathan Davies, Rajiv Jalan; The Institute of Hepatology, University College London, London, United Kingdom

Introduction: Our recent studies showed that in patients with alcoholic cirrhosis, neutrophil phagocytic dysfunction is associated with increased incidence of infection and mortality, and this dysfunction is possibly mediated by endotoxaemia. As probiotics are known to alter gut flora and decrease gram-negative organisms, we hypothesized that probiotic treatment in patients with alcoholic liver disease restores neutrophil function. Methods: In an open-label study, twelve patients with alcoholic cirrhosis received food supplementation with Lactobacillus casei Shirota (6.5×109) 3 times a day for 4 weeks. Neutrophil oxidative burst, phagocytosis, toll-like receptor (TLR) expression, plasma cytokines and cytokine production after ex vivo stimulation were measured. Data are given as mean±SEM in table 1. Results: Baseline neutrophil phagocytic capacity was significantly lower in patients compared with controls (73% versus 98%, p<0.05), which normalized after a 4 week treatment with probiotics (73% increasing to 100%, p<0.05). Stimulated lev-
els of TNFR1, TNFR2 and IL-10 significantly decreased following treatment (42%, 43% and 34% respectively, p<0.05). TLR2 (p<0.05), TLR4 (p<0.01) and TLR9 (p<0.05) expression were significantly higher in patients than in controls. Probiotic therapy resulted in significant reduction in TLR4 (p<0.05) surface expression compared to baseline. During treatment with the probiotic no adverse events were noted; compliance to the study medication was 86%. In conclusion, we make the novel observation of marked neutrophil functional defect in well-compensated alcoholic cirrhosis and a predominant anti-inflammatory cytokine production ex vivo which may make the patients with alcoholic cirrhosis more susceptible to infection. Treatment with Lactobacillus casei Shirota for four weeks is safe and, normalises the phagocytic capacity and cytokine response to lipopolysaccharide. Our data suggests an important mechanistic role for TLR 4 in the mediation of the neutrophil functional defect, and establish a proof-of-concept that probiotics restore innate immune function in cirrhosis, warranting further randomized, well powered, controlled studies.

**Table 1:**

<table>
<thead>
<tr>
<th></th>
<th>control</th>
<th>baseline</th>
<th>end of the study</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phagocytic capacity (%)</td>
<td>98.6±6.3</td>
<td>73.2±5.1*</td>
<td>100±14.65</td>
</tr>
<tr>
<td>ex vivo TNFR1 (pg/ml)</td>
<td>123.9±12.0</td>
<td>306.3±32.2*</td>
<td>179.1±40.65</td>
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<tr>
<td>ex vivo TNFR2 (pg/ml)</td>
<td>159.2±39.7</td>
<td>561.3±79.6*</td>
<td>316.5±69.35</td>
</tr>
<tr>
<td>ex vivo IL-10 (pg/ml)</td>
<td>5.5±1.3</td>
<td>311.0±110.9**</td>
<td>59.3±15.55</td>
</tr>
<tr>
<td>TLR 2 (MFU)</td>
<td>19.7±7.7</td>
<td>34.4±4.7*</td>
<td>44.2±9.5</td>
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<tr>
<td>TLR 4 (MFU)</td>
<td>74.6±8.2</td>
<td>201.0±34.6**</td>
<td>78.7±15.48</td>
</tr>
<tr>
<td>TLR 9 (MFU)</td>
<td>55.4±18.7</td>
<td>659.4±216.1*</td>
<td>321.1±60.3</td>
</tr>
</tbody>
</table>

* p<0.05 compared to control

** p<0.01 compared to control

** LB14 FIRST EVIDENCE THAT ALBUMIN CAN DIRECTLY IMPROVE CARDIAC CONTRACTILITY IN CIRRHOTIC RATS**

Giulio Ceolotto, Italia Papparella, Maurizio Cavalli, Antonietta Sticca, Lorenzo Franco, Sergio Bova, Andrea Semplicini, Angelo Gatta, Paolo Angeli; Dept Clinical and Exp medicine, University of Padova, Padova, Italy

A low cardiac output due to cirrhotic cardiomyopathy could be a critical factor in the pathogenesis of hepatorenal syndrome (HRS) in cirrhosis. Albumin together with vasoconstrictor drugs is widely used in the management of HRS however the mechanisms are still unclear. Aim of our study was to verify whether albumin infusion may restore a normal cardiac contractile response to β-adrenergic agonists and to improve β-adrenergic signal transduction in an animal model of cirrhosis with ascites. 12 Wistar Kyoto rats were treated for 15 weeks by inhalation with carbon tetrachloride (CCl4) to induce cirrhosis and 12 rats were used as control animals. Three days and one day before the sacrifice, albumin was administered as saline solution at a dose of 3g/kg B.W. i.v. to 8 cirrhotic and to 8 control rats. In 4 cirrhotic rats the same amount of saline solution was administered at the same times. Left ventricular contractility was determined in isolated hearts with a β-agonist, isoproterenol. Cardiac gene expression of β-adrenergic signalling was performed by Real-Time PCR. Plasma antioxidant capacity was evaluated with Electron Spin Resonance spectroscopy. The maximal response of cardiac contractility induced by isoproterenol (10-8 mol and 10-6 mol) was significantly reduced in the left ventricular tissue of cirrhotic rats in comparison to controls (p<0.01). In albumin-treated cirrhotic rats, isoproterenol-induced cardiac contractility was stronger in comparison to saline-treated cirrhotic rats (p<0.01) and it was not significant
different than controls. In hearts from saline-treated cirrhotic rats, gene expression analysis showed a significant overexpression of G protein alpha inhibiting subunit 2 (Gai2) (p<0.01), of regulator of G-protein signalling (RGS2) (p<0.01) and of phosphodiesterase-2a (PDE), as compared to control rats. In albumin-treated cirrhotic rats, gene expression of RGS2 and PDE2a was lower in comparison to saline-treated cirrhotic rats alone (p<0.01) and was similar to control animals. No difference was observed for Gai2 expression between saline-treated and albumin-treated cirrhotic rats. Plasma antioxidant capacity was decreased in cirrhotic rats compared to the control rats (to 57% ± 8%, p<0.01). The administration of albumin almost completely restored the antioxidant level in cirrhotic rats. These results demonstrate for the first time that the administration of albumin improve cardiac contractility and β adrenergic signal transduction by modulating the expression of RGS2 and PDE2a in cirrhotic rats. All these effects were accompanied by an increase of the level of antioxidant status in these animals.

Disclosures:
The following people have nothing to disclose: Giulio Ceolotto, Italia Papparella, Maurizia Cavalli, Antonietta Sticca, Lorenzo Franco, Sergio Bova, Andrea Semplinici, Angelo Gatta, Paolo Angeli

**LB15 EFFICACY AND SAFETY OF VALOPICITABINE IN COMBINATION WITH PEGYLATED INTERFERON-α (PEG IFN) AND RIBAVIRIN (RBV) IN PATIENTS WITH CHRONIC HEPATITIS C**

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Background: Future HCV therapy will likely combine drugs with different modes of action and complementary resistance profiles. We investigated the pharmacokinetics, antiviral activity and safety of valopicitabine, an investigational NS5B polymerase inhibitor, combined with PegIFN and RBV. Methods: 117 treatment-naïve HCV genotype 1 patients with compensated liver disease were randomized among 3 arms: (1) valopicitabine 200 mg QD + PegIFNα2a 180 µg QW, (2) RBV 1000-1200 mg daily + valopicitabine 200 mg QD + PegIFNα2a 180 µg QW, or (3) RBV 1000-1200 mg daily + valopicitabine placebo QD + PegIFNα2a 180 µg QW. Patients received valopicitabine (or placebo) for 1 week prior to PegIFN with or without weight-based RBV on Day 8, and continued treatment for 12 weeks. After Week 12, patients received 36 weeks of PegIFN/RBV. Results: There was no PK drug-drug interaction detected between valopicitabine and RBV. As-treated analysis demonstrated a mean HCV RNA difference between the triple combination group and PegIFN /RBV of 0.45-0.66 log10 IU/mL; this was maintained at each study visit. HCV RNA PCR-negativity rates at Weeks 4, 8 and 12 were also greater in the triple combination group (16%, 56%, 72%, respectively) vs PegIFN/RBV (5%, 37%, 62%) and PegIFN/valopicitabine (11%, 33%, 44%). By ITT analysis, HCV RNA PCR-negativity rates at Week 12 were 67%, 62% and 44% for PegIFN/RBV/valopicitabine, PegIFN/RBV, and PegIFN/valopicitabine, respectively. Three patients, all recipients of valopicitabine triple combination therapy, discontinued prematurely, one for vomiting attributed to valopicitabine. One serious adverse event was reported in a PegIFN/RBV/valopicitabine recipient and attributed to PegIFN. Through Day 36, GI-related adverse events were reported in 77% of PegIFN/RBV/valopicitabine recipients and 67% of PegIFN/valopicitabine recipients, vs 49% of the PegIFN/RBV group that did not receive valopicitabine. Increases in mean AST, CK, pancreatic amylase and lipase were observed in the valopicitabine-containing arms (AST and CK subsequently decreased by the end of treatment in the triple combination arm); but these did not correlate with clinical adverse events. Conclusion: Valopicitabine combined with PegIFN/RBV demonstrated additive antiviral activity. HCV RNA PCR-negativity rates at all timepoints were consistently greater in the triple regimen compared with PegIFN/RBV. However, the incidence of GI-related adverse events and lab abnormalities was higher in the valopicitabine-containing arm. After comprehensive review of the valopicitabine clinical program, the development of this molecule has been discontinued due to its overall risk/benefit profile.

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The following people have nothing to disclose: Eric J. Lawitz, Norman Gitlin, Terry Box, Tuan Nguyen

**1399 SONIC HEDGEHOG IS AN AUTOCRINE VIABILITY FACTOR FOR MYOFIBROBLASTIC HEPATIC STELLATE CELLS**

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Background/Aims: Factors released during liver injury, such as platelet derived growth factor-BB (PDGF) promote the accumulation of myofibroblastic hepatic stellate cells (MF-HSC) that play a pivotal role in the pathogenesis of cirrhosis. The Hedgehog pathway regulates remodelong of other injured tissues. Our aim was to evaluate the hypothesis that autocrine production of Sonic hedgehog (Shh) promotes MF-HSC growth. Methods: Primary rat HSC were cultured and treated without or with PDGF, a pharmacologic inhibitor of PDGF-regulated kinases, adenovirus expressing activated or dominant negative AKT, or Hedgehog signaling inhibitors. Shh production and HSC growth were assessed. Results: By day 7 during spontaneous culture-induced activation, HSC produced 6 fold more biologically active Shh protein than freshly isolated HSC (P<0.05). Neutralizing Shh antibodies activated caspases 3/7(P<0.05 vs control IgG) and induced apoptosis of cultured HSC. Adding PDGF(20ng/ml) to culture-activated HSC further increased expression of Shh mRNA and protein (both P<0.05 vs untreated) and enhanced MF-HSC growth (BrDU incorporation 5-fold increased, P<0.01). Both of the latter processes followed activation of AKT and were abrogated by AKT inhibitors. Even without exogenous PDGF, adenoviral delivery of activated AKT up-regulated Shh expression 6 fold (P<0.05 vs control adenovirus), demonstrating a direct role for AKT in regulating Shh expression in HSC. Moreover, Shh neutralizing antibodies and other Hh pathway inhibitors (cyclopamine and A99944) reduced the mitogenic actions of PDGF by at least 50% (P<0.05 vs control). Conclusion: These results identify Shh as an
autocrine growth factor for MF-HSC and suggest a role for HH signaling in the pathogenesis of cirrhosis.

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1400 AUTOCRINE SIGNALING BY SDF-1α THROUGH ITS RECEPTOR, CXCR4, MEDIATES HEPATIC STELLATE CELL ACTIVATION IN VIVO AND IN VITRO

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Chemokine interactions with their receptors have been implicated in hepatic stellate cell (HSC) activation. The hepatic expression of CXCR4 is increased in Hepatitis C (HCV)-cirrhotic livers and plasma levels of its endogenous ligand, Stromal-derived factor-1α (SDF-1α), correlate with increased fibrosis in these patients. The expression of CXCR4 by HSCs has not been reported. We hypothesize that HSCs express functional CXCR4 and that receptor engagement by SDF-1α promotes HSC activation, fibrogenesis and proliferation. Methods: To determine whether activated HSCs express CXCR4 in vivo, human liver biopsy specimens from HCV patients were examined for co-expression of CXCR4 and α-SMA using confocal microscopy. Expression of CXCR4 mRNA, protein, and surface expression in LX2 cells, a HSC line, was determined by RT-PCR, Western, and FACS analyses, respectively. Primary HSCs isolated from normal liver were culture-activated and CXCR4 expression examined by sequential FACS analyses. To explore the functional role of CXCR4 on stellate cells, LX2 cells were treated with SDF-1α and expression of α-SMA by Western, collagen I expression by qRT-PCR, and proliferation by 3H thymidine incorporation were examined. Involvement of the ERK1/2 signaling pathway, known to be important in HSC biology, was characterized by treating cells with SDF-1α or diluent (+/- AMD3100, a CXCR4 inhibitor) and immunoblotting for phospho-ERK1/2. ELISAs for SDF-1α were performed on media from both LX2 and primary HSCs. Effect of TGFβ1, a potent fibrogenic cytokine, on CXCR4 expression in LX2 and primary HSCs was examined by Western and FACS. Results: Activated HSCs express CXCR4 in vivo in patients with HCV. Both LX2 and primary HSCs express CXCR4 which increased with progressive culture-induced activation. SDF-1α (100-1000ng/ml) induced: α a dose-dependent increase in α-SMA expression (p<0.005); b) a 2-fold increase in collagen I (α1) mRNA expression (p<0.007); and c) a significant increase in proliferation (p<0.05) in LX2 cells. SDF-1α stimulates CXCR4-dependent ERK-1/2 phosphorylation, as increases were blocked by AMD3100 pretreatment. TGFβ1 increased CXCR4 expression (>2-fold; p<0.005) in HSCs at all stages of activation. LX2 and primary HSCs produce SDF-1α implicating both paracrine and autocrine regulation of HSC activation via CXCR4. Conclusions: 1) Activated HSCs express functional CXCR4 receptor in vivo and in vitro; 2) CXCR4 receptor activation by SDF-1α is pro-fibrogenic through its effects on HSC activation, fibrogenesis, and proliferation. 3) The availability of small molecule inhibitors of CXCR4 make this receptor a novel target for antifibrotic approaches.

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1401 HUMAN AND RAT HEPATIC STELLATE CELLS EXPRESS OPIOID RECEPTORS AND ARE STIMULATED BY ENDOGENOUS OPIOID PEPTIDES IN A PARACRINE MANNER

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Background: Endogenous opioid peptides (EO) modulate the growth of nervous and non-nervous cells. Hepatic stellate cells (HSC) are the main cell phenotype involved in liver fibrogenesis, they display molecular markers of nervous cells and respond to neurotransmitters. Aim: to evaluate the eventual effect of EO on human HSC (hHSC) biology and liver fibrogenesis. Methods: hHSC were isolated from excess liver after colonic metasases resection, rat HSC (rHSC) from normal rats. Human liver biopsies were obtained from patients with chronic liver diseases. Liver fibrosis was induced in rats by dimethylnitrosamine (DMN) administration. Results: By western blot and reverse transcriptase-PCR, quiescent and activated hHSC and rHSC expressed the “classical” opioid receptor isoforms (DOR, MOR, and KOR). Opioid receptor activation by specific agonists increased hHSC and rHSC proliferation and type I collagen synthesis. By using specific intracellular kinase inhibitors, these effects were induced through a calcium-dependent PKCα/ERK1/PI3K pathway activation. The general opioid receptor antagonist naloxone blocked the effect of EO on HSC proliferation and type I collagen synthesis, with no effects on either PDGF-induced proliferation or TGFβ1-induced type I collagen accumulation, confirming that the effects of EO on HSC were mediated by a specific agonist-receptor interaction. In the course of human cholestatic liver diseases, cholangiocytes synthesize met-enkephalin, the most important endogenous opioid peptide. Met- enkephalin was also expressed by hepatocytes in liver biopsies of patients with chronic hepatitis C and alcoholic liver diseases. Furthermore, parenchymal met-enkephalin expression in the DMN model of hepatic fibrosis preceded the process of rHSC activation. In liver samples from both human and rat chronic liver diseases, opioid receptors co-localized with alpha-smooth muscle actin (α-SMA) and glial fibrillar acidic protein (GFAP), as markers of hHSC and rHSC respectively. Finally, met- enkephalin induced cell proliferation and type I collagen synthesis in both hHSC and rHSC. Conclusions: In chronic liver diseases, the cellular targets of the hepatic injury produces the main endogenous opioid peptide, met- enkephalin. The synthesis of met-enkephalin in an experimental model of hepatic fibrosis precedes the process of HSC activation. In liver samples from both human and rat chronic liver diseases, opioid receptors co-localized with alpha-smooth muscle actin (α-SMA) and glial fibrillar acidic protein (GFAP), as markers of hHSC and rHSC respectively. Finally, met- enkephalin induced cell proliferation and type I collagen synthesis in both hHSC and rHSC. Conclusions: In chronic liver diseases, the cellular targets of the hepatic injury produces the main endogenous opioid peptide, met- enkephalin. The synthesis of met-enkephalin in an experimental model of hepatic fibrosis precedes the process of HSC activation towards a myofibroblastic phenotype. The expression of opioid receptors and the effect of met-enkephalin on hHSC and rHSC indicates that EO stimulate in a paracrine manner the process of hepatic fibrogenesis during chronic liver injury.

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1402
EFFECT OF SIMULATED MICROGRAVITY ON RAT HEPATIC STELLATE CELLS AND THEIR INTERACTION WITH EXTRACELLULAR MATRIX PROTEINS
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Background: Hepatic stellate cells (HSC) are the major cell type responsible for liver fibrosis. HSC become activated when plated on plastic and express activation markers such as smooth muscle actin (SMA) and procollagen A (PCA). It is recognized that cells grown in a two-dimensional environment tend to dedifferentiate and lose the specialized features of their original tissues. In this study we used a rotary cell culture system (RCCS) to produce a 3D culture of HSC which more closely mimics the in vivo situation. RCCS produces a microgravity-like environment in which HSC undergo free-fall as they rotate and form aggregates of cells.

Aim: To analyze gene expression of HSC activation in 3D environment in simulated microgravity for quiescent and chronically activated HSC.

Methods: HSC were isolated from male Sprague-Dawley rats by pronase collagenase digestion followed by sucrose density gradient centrifugation. HSC were plated on plastic in Ham's DMEM media and trichrome staining of 10 day microgravity aggregates of HSC was performed. In situ perfusion of rat liver was performed. The hepatic capsule was removed and hepatic parenchymal tissue was mechanically dissociated from portal tracts. Portal tract tissue was dispersed and subsequently plated on type I collagen. Primary PF were cultured using an explant-culture technique for 72 h. The

Results: Quiescent and activated HSCs express both isoforms of the insulin receptor and about 1.5 times more IRS2 than IRS1. Interestingly, we observed that protein steady state levels of the insulin receptor, but not its corresponding mRNA, were downregulated with increasing concentrations of insulin in the culture medium. Expression of IRS3, IRS4 and GLUT4 was very weak. Insulin induces phosphorylation of AKT in quiescent and activated HSCs, but nearly no phosphorylation of ERK1/2. Moreover, insulin causes no significant increase in the gene expression of the transcription factor SREBP-1c and the lipogenic enzyme fatty acid synthase. Other typical insulin responsive genes such as PEPCK, glucokinase and the transcription factor HNF-4α were poorly expressed in the absence and presence of insulin. In addition, we did not find a direct effect of insulin on α-SMA, a positive marker for HSC activation and GFAP, a negative marker. Conclusions: Although the main components of the insulin receptor signaling pathways are present in mouse HSCs, transcription of genes, known to be affected by insulin in other cell types, was not affected. This suggests the presence of one or more negative regulators of the insulin signaling pathways, and hence insulin resistance of mHSCs, which is enhanced by downregulation of the receptor under the influence of its ligand. The mechanism by which insulin receptor protein decreases following exposure to insulin is the current focus of our research.

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1403
MOUSE HEPATIC STELLATE CELL INSULIN RESISTANCE IS CAUSED BY DOWNREGULATION OF INSULIN RECEPTOR PROTEIN AND DEFECTIVE DOWNSTREAM SIGNALING
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Background & aims: NAFLD is characterized by the occurrence of insulin resistance and hyperinsulinemia. In the progression of this disease, activated hepatic stellate cells (HSCs) play an important role. We investigated insulin signaling in mouse HSCs and the effects of insulin on HSC activation. Methods: Transdifferentiation of HSCs was obtained by culturing primary mouse HSCs on polystyrene dishes in presence of 10% fetal bovine serum. Quiescent 2-day cultured cells were compared with activated 13-day old HSCs. The presence of key molecules of the insulin receptor signaling pathways was determined by quantitative RT-PCR and Western blot. Effects of different insulin concentrations (1-100 nM) on HSC activation and on the activation of the PI3K- and MAPK-pathway were investigated. Results: Quiescent and activated HSCs express both isoforms of the insulin receptor and about 1.5 times more IRS2 than IRS1. Interestingly, we observed that protein steady state levels of the insulin receptor, but not its corresponding mRNA, were downregulated with increasing concentrations of insulin in the culture medium. Expression of IRS3, IRS4 and GLUT4 was very weak. Insulin induces phosphorylation of AKT in quiescent and activated HSCs, but nearly no phosphorylation of ERK1/2. Moreover, insulin causes no significant increase in the gene expression of the transcription factor SREBP-1c and the lipogenic enzyme fatty acid synthase. Other typical insulin responsive genes such as PEPCK, glucokinase and the transcription factor HNF-4α were poorly expressed in the absence and presence of insulin. In addition, we did not find a direct effect of insulin on α-SMA, a positive marker for HSC activation and GFAP, a negative marker. Conclusions: Although the main components of the insulin receptor signaling pathways are present in mouse HSCs, transcription of genes, known to be affected by insulin in other cell types, was not affected. This suggests the presence of one or more negative regulators of the insulin signaling pathways, and hence insulin resistance of mHSCs, which is enhanced by downregulation of the receptor under the influence of its ligand. The mechanism by which insulin receptor protein decreases following exposure to insulin is the current focus of our research.

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purity of cells was assessed by immunocytochemistry with fibulin-2 for liver fibroblasts, GFAP (glomerular fibrillary acidic protein) for HSCs, and CK-19 for cholangiocytes. Cytokine microarray was performed with culture supernatants from both PF and HSCs and compared by quantitative assay. Differences in expression of key cytokines were confirmed by extracting cell lysates and performing immunoblot analysis. RESULTS: PF were confirmed in culture by positive immunostaining with fibulin-2; PF cells were both GFAP and CK-19 negative. Conversely, lobular HSCs stained negative for fibulin-2 and positive for GFAP. In the initial cytokine array analysis, we designed a platform and tested nineteen of the most common cytokines associated with liver fibroblast biology. When compared to HSCs, PF arrays demonstrated significant overexpression of TIMP-1 (tissue inhibitor of metalloproteinase-1), VEGF (vascular endothelial growth factor), MCP-1 (monocyte chemoattractant protein-1), and CINC-2 and CINC-3 (cytokine-induced neutrophil chemoattractant). Immunoblot with antibodies to these proteins confirmed increased expression in fibulin-2 positive cells when compared to GFAP-positive, fibulin-2 negative cells. CONCLUSIONS: This study indicates that successful isolation and culture of PFs demonstrate key biologic differences between PF and HSCs. Importantly, cytokine array is a useful way to further distinguish key physiological differences with lobular myofibroblasts since upon activation such cells secrete a large number of cytokines. Such differences in biologic profiling may account for the mechanism of enhanced biliary fibrosis in rodent models.

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The following people have nothing to disclose: Xiaokun Ding, Neeraj Saxena, Frank A. Anania

1405
NOX2 AND RAC1 PLAY IMPORTANT ROLES IN LIVER FIBROGENESIS BY FACILITATING PHAGOCYTOSIS OF APOPTOTIC BODIES AND PRODUCTION OF ROS BY STELLATE CELLS

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We have previously shown that hepatic stellate cells (HSC) are activated by the phagocytosis of apoptotic bodies (AB) from hepatocytes. Phagocytosis induces NADPH oxidase (NOX) resulting in production of superoxide (O2-) and pro-collagen α 1(I) upregulation. As NOXs are central to the fibrogenic process, and NOX2 is described as the phagocytic NOX, our hypothesis is that NOX2 activation plays a role in phagocytosis of AB and subsequent O2- production. Furthermore, the small GTP-ase Rac1, a regulatory element of NOXs could also involved in phagocytosis of AB, and in downstream signaling leading to HSC activation. Methods: Primary rat HSC were transfected with NOX2-specific siRNA, exposed to AB, and the phagocytic rate, O2- production (lucigem assay) and the pro-collagen α 1(I) expression (real-time PCR) were tested. NOX2-dependent Rac1 activation was evaluated by the Rac-GTP pull-down assay after exposing HSC to AB in the presence or absence of 100 μM apocynin (NOX inhibitor), or 20 μM LY294002 (PI3K inhibitor). LX-2 cells were transfected with the constitutively active Rac1 (G63L), then exposed to AB and the rate of phagocytosis, and changes in the actin cytoskeleton (phallolidin staining) were evaluated. Results: The rate of phagocytosis in NOX2 siRNA-treated cells decreased to 0.70-fold (±0.20), compared to scrambled siRNA-transfected cells. The O2- production of NOX2 siRNA transfected-cells decreased to 0.41-fold (±0.11) and the pro-collagen α 1(I) expression to 0.64-fold (±0.32). Phagocytosis of AB induced Rac1 GTP-ase activation, and this was inhibited by apocynin suggesting that Rac1 activation occurs in concert with NOX2 activity. Inhibition of PI3K did not affect Rac1 activity. To determine if Rac1 is directly involved in the phagocytosis of AB, HSC were transfected with Rac1-Q63L, and we found that this increased the phagocytic rate by 1.65-fold (±0.16), when compared to cells transfected with the empty vector. There was also a significant reorganization in the actin cytoskeleton surrounding the phagosomes suggesting that Rac1 mediates its effects on phagocytosis via regulation of the actin cytoskeleton. In conclusion, NOX2 is involved in phagocytosis of AB by HSC and is responsible for O2- production and upregulation of procollagen α 1(I) expression. NOX-dependent Rac1 activation leads to an increase in phagocytosis with reorganization of the actin cytoskeleton in HSC, perpetuating the fibrogenic activity.

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1406
REGULATION OF HEPATIC STELLATE CELL GROWTH BY NUCLEAR AND CYTOSOLIC CALCIUM SIGNALS

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Background. Hepatic stellate cells (HSC) are important mediators of liver fibrosis. Many HSC mitogens linked to calcium signals have been identified, yet the downstream mediators of HSC proliferation have not been well characterized. Recent data suggest that nuclear, but not cytosolic calcium signals are critical regulators of growth in hepatocytes. Thus, the aims of this project were to determine whether nuclear and/or cytosolic calcium was important in the regulation of HSC growth.

Methods. LX-2 spontaneously immortalized human hepatic stellate cells (kindly provided by Dr. Scott Friedman) were used for all studies. Blockade of calcium signals within nuclear and extranuclear compartments was obtained using plasmid constructs expressing the calcium chelator parvalbumin linked to either a nuclear-localization sequence or a nuclear exclusion-sequence and further linked to the fluorescence protein DsRed (PV-NLS-DsRed and PV-NESS-DsRed, respectively). LX-2 cells were transfected with DsRed alone, PV-NLS-DsRed, or PV-NESS-DsRed. Appropriate targeting of plasmids was determined using confocal microscopy to identify DsRed fluorescence. Determination of calcium signals was determined using confocal microscopy to observe fluo-4 fluorescence. Cell proliferation was assessed by bromo-deoxyuridine incorporation. Results. Transfection of LX-2 cells with constructs resulted in appropriate targeting of DsRed (throughout the cell), PV-NLS-DsRed (to the nucleus alone), and PV-NESS-DsRed (to the extra-nuclear cytosol). Calcium signals induced by extracellular ATP (100 μM) were not inhibited in cells transfected with DsRed. However, calcium signals induced by extracellular ATP were blocked only within the nucleus in LX-2 cells transfected with PV-NLS-DsRed, and calcium signals induced by extracellular ATP were blocked only within the cytosol in LX-2 cells transfected with PV-NESS-DsRed. LX-2 proliferation was not changed in cells transfected with DsRed alone. However, LX-2 proliferation was decreased to 21.2% of control (p < 0.0001) in LX-2 cells transfected with PV-NLS-DsRed and to 60.5% (p < 0.0005) in LX-2 cells transfected with PV-NESS-DsRed (n = 10).

Conclusions. Hepatic stellate cell mitogens acting via calcium signals do so in a fashion distinct from hepatocyte mitogens, since both nuclear and extranuclear calcium are important in this process. The signal
transduction mechanisms by which these processes occur have yet to be defined.

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The following people have nothing to disclose: Elwy M. Soliman, Michelle Rodrigues, Davidson A. Gomes, Jin Yu, Michael H. Nathanson, Jonathan A. Draff.

1407
APOTOPIC HEPATOCYTE DNA INHIBITS HSC CHEMOTAXIS AND STIMULATES HEPATIC STELLATE CELL DIFFERENTIATION VIA TLR9
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Background: Hepatocyte apoptosis is known to result in hepatic stellate cell (HSC) differentiation, but the molecular signals are not well defined. Mammalian DNA from apoptotic cells is enriched in Cpg motifs, and can activate B cells via TLR9. We hypothesized that apoptotic mammalian DNA can activate HSC via TLR 9. Methods: cDNA was prepared from human HSC line LX-2 and mouse HSC and amplified with primers specific for TLR9. LX-2 cells and primary mice HSC were cultured with CpG (5µg/ml), apoptotic DNA (50µg/ml) or healthy DNA (50µg/ml). After 24 hours, quantitative real time PCR was performed for TGF-β and collagen1, and western blotting for α-smooth muscle actin. We tested the ability of apoptotic DNA to inhibit PDGF induced HSC chemotaxis, and tested if the mechanism of TLR9 inhibition of PDGF induced increase in cytosolic Ca2+ was by inhibition of IP3 mediated signaling. We performed intra-peritoneal injection for carbon tetrachloride (CCl4) (850µl/kg) to wild-type and TLR9 KO mice for 8 weeks. Liver samples were stained with Sirius Red. Results: LX-2 cells and mouse HSC express mRNA for TLR9. TLR9 agonist and mammalian DNA from apoptotic hepatocytes induce up-regulation of TGF-β and collagen1 mRNA, and inhibited PDGF induced chemotaxis. These effects are blocked by TLR9 antagonist and were not present in TLR9 KO mice. TLR9 agonist induces up-regulation of α-smooth muscle actin by western blotting. Activation of TLR9 prior to exposure to PDGF significantly diminished the peak and plateau of the increase in cytosolic Ca2+ (P<0.02). Pre-treatment of LX-2 cells with apoptotic hepatocyte DNA inhibited IP3 mediated increase in cytosolic Ca2+. TLR9 KO mice compared to wild-type had significantly reduced CCI4 induced liver fibrosis. Conclusions: Apoptotic mammalian DNA can signal cellular apoptosis to HSC via TLR9, resulting in actin reorganization, inhibition of chemotaxis and HSC differentiation.

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1408
SYMPATHETIC NERVOUS SYSTEM SIGNALLING IN HUMAN PRIMARY HEPATIC STELLATE CELLS: A NOVEL ANTI-FIBROTIC PATHWAY
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Background: Emerging evidence suggests that sympathetic nervous system (SNS) signalling regulates hepatic fibrogenesis. Mouse hepatic stellate cells (HSC) synthesize and release the SNS neurotransmitter (SNSNT), norepinephrine (NE) and are regulated by NE in culture. Mice lacking NE have impaired fibrogenic responses. The relevance of these animal studies to human fibrogenesis is unclear. Aim: To investigate the effects of the SNSNTs NE and neuropeptide Y (NPY) on human primary HSC (hHSC) proliferation, collagen gene expression and release of pro-fibrogenic cytokines. Methods: Primary hHSC were isolated from healthy liver segments of patients (n=3) undergoing resection of metastases. Quiescent hHSC autofluoresced, with abundant expression of glial fibrillary acidic protein (GFAP). Myofibroblastic transformation in culture reduced GFAP and increased α-smooth muscle actin plus collagen-α1 expression. hHSC conditioned media was analyzed for NE by HPLC. RNA was extracted from hHSC and analyzed for expression of adrenergic receptors by QRT-PCR. Basal proliferation of hHSC with, time in culture, prazosin (PRZ, α1-adrenoceptor antagonist) or propranolol (PRL, β-adrenoceptor antagonist), or stimulated by NPY and NE+PRZ/PRL was assessed by a colorimetric, tetrazolium assay. Apoptosis was assessed by annexin V. Involvement of intracellular pathways was assayed by culture of hHSC with NE or NPY in the presence of specific inhibitors: pertussis toxin(G-protein inhibitor); wortmannin(Pi3-kinase inhibitor); SB202190(p38 MAP kinase inhibitor); PD98059(MEK inhibitor) and RO-32-0432(Protein kinase C inhibitor). Induction of collagen-α1 gene by NE or NPY was assessed by RT-PCR. To see if NE and NPY induced collagen expression directly, interleukin (IL)-4, IL-13, leptin and transforming growth factor-β1 were assayed by ELISA. Results: hHSC synthesize, release NE and express α1A, β1, β2, β3 adrenoceptors. NE is an autocrine growth and survival factor since basal growth and survival is reduced by PRZ±PRL by ≥50%, p<0.05. Time reduces basal proliferation: cells at day30 cells grow less than day15 (p<0.001). PRZ inhibited exogenous NE induced hHSC proliferation by 80±5%, p<0.02. NE and NPY induced hHSC proliferation involves p38 MAP kinase and Pi-3 kinase respectively. NE but not NPY induced collagen gene expression through TGF-β1 with no involvement of IL-4, IL-13 or leptin. Conclusion: hHSC synthesize, release NE and express α1A, β1, β2, β3 adrenoceptors. PRZ inhibits NE induced hHSC proliferation. NPY induces hHSC growth through Pi-3 kinase. NE but not NPY upregulates collagen gene expression through TGF-β1. SNS signalling is a novel anti-fibrogenic pathway in humans.

Disclosures:
The following people have nothing to disclose: Barbara Sigala, Florence Potter, Clare Selden, Myrddin Rees, Anthony Wierzbicki, Humphrey J. Hodgson, Jude A. Oken.

1409
ENDOGLIN PHOSPHORYLATION BY TGF-β RECEPTORS IN TRANSDIFFERENTIATING HSC
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Background: Transdifferentiation of hepatic stellate cells (HSC) is regulated by two different TGF-β type I receptors, i.e. ALK5 and ALK1. In endothelial cells it has been shown that endoglin modulates signaling via these two type I receptors in an opposite manner. The functional interplay of endoglin with the TGF-β type II and type I receptor (ALK1) at the surface of the cell is characterized by specific phosphorylation of the C-terminal domain of endoglin by these receptors which in turn modulate the function of endoglin. When the receptor is internalized type II receptor serine-phosphorylation is lost. We have previously...
shown that endoglin is highly expressed in HSC and that it is exposed on the surface. Results: We show here that endoglin is engaged in a TGF-β1 binding receptor-complex in rat HSC by TGF-β1 cross linking experiments. To monitor a direct interaction of these receptors we analyzed the serine phosphorylation of endoglin by the TGF-β type II receptor at the C-terminus of rat endoglin (ser-626/ser-627). By this approach we could demonstrate a strong phosphorylation of endoglin in early and late HSC which declines sharply in MFB. Experiments with siRNA to endoglin in HCS and reconstitution of this receptor system in COS-7 cells confirmed the specificity of this RII phosphorylation. In addition the surface expression of RII paralleled that of the endoglin phosphorylation which in turn may reflect at least in MFB the insensitivity to TGF-β. Furthermore RII phosphorylated endoglin is not exclusively exposed at the surface, because no biotinylated endoglin could be precipitated from surface biotinylated cells cultured for 1 day or 2 days, although these cells showed a high amount of phosphorylated endoglin. On the other hand in MFB we could detect significant amounts of endoglin in the membrane but only a marginal phosphorylation level. Conclusion: We conclude that endoglin is involved in TGF-β signaling in HSC as could be demonstrated by binding of the ligand and phosphorylation by the type II receptor. The corresponding type one receptor involved is most likely ALK1, because we found that a population of the serine phosphorylated endoglin is in addition threonine phosphorylated by immunoprecipitation experiments. Finally we speculate that the surface exposed endoglin in MFB fulfills a function which is independent of TGF-β.

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1410

OXIDATIVE STRESS INDUCE COLLAGEN SYNTHESIS BY C-ABL ACTIVATION IN HUMAN HEPATIC STELLATE CELLS

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Accumulating data support the hypothesis that oxidative stress plays a key role in the development of hepatic fibrosis. However, the molecular events directly involved in oxidative stress-induced collagen gene expression are still debatable. The following people have nothing to disclose: Tommaso Mello, Elisabetta Ceni, Simone Polvani, Laura Cioni, Francesca Lisì, Barbara Ottanelli, Francesca Buccolierò, Mirko Tarocchi, Stefano Milani, Andrea Galli

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MESENCHYMAL β-CATENIN SIGNALING REGULATES HEPATIC STELLATE CELL FATE

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Background: In the adult liver, differentiation of quiescent hepatic stellate cells (HSCs) to activated, extracellular matrix and growth factor-producing myofibroblastic cells in part supports liver regeneration through promotion of hepatocyte proliferation. In the embryonic liver, fibroblast growth factor (FGF) 10 is secreted by embryonic hepatic stellate cells (HSC) and activates the canonical WNT signaling pathway in hepatoblasts to maintain their proliferation (Berg et al., Hepatology, 2007). We also observe the similar paracrine role of HSC-derived FGF-10 in adult liver regeneration after partial hepatectomy (Estrada et al., in progress). Previously we have shown that mesenchymal β-catenin signaling play an important role in expanding the pool of Fgf10 expressing mesenchymal progenitor cells (De Langhe et al., unpublished). Aim: To further characterize the development of embryonic HSCs, we used mice in which β-catenin expression is abrogated in the mesenchyme with the use of the mesenchymal specific Derm-1 promoter. Results: Dermo1-Cre+/-/β-cateninflox/- conditional knockout (CKO) embryos largely phenocopy Pitx1-/-/Pitx2-/- double knockout embryos (De Langhe et al., unpublished), and exhibit smaller livers with defective architecture, characterized by larger blood vessels located more proximally similar to that of Pitx2-/- mice. We find in the embryonic liver, that lack of mesenchymal β-catenin signaling leads to a differentiation of embryonic HSCs into myofibroblasts characterized by increased smooth muscle actin and collagen type I expression. CKO embryonic livers also feature abnormal and leaky blood vessels with pools of blood visible within the liver. This phenotype correlates with an increase in Flik1 expression, indicating a defect in blood vessel maturation. Conclusion: Based on our results, we speculate that embryonic stellate cells typically manifest canonical WNT activation, which is critical for normal liver and HSC differentiation and development.

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The following people have nothing to disclose: Tove A. Berg, Stijn De Langhe, Joaquin J. Estrada, Hidekazu Tsukamoto, Saverio Belluscio, Kasper Wang
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MOUSE HEPATIC STELLATE CELLS EXPRESS THE INTERMEDIATE FILAMENT PROTEIN SYNCOILIN
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Background: Hepatic stellate cells (HSCs) are important in several (patho)physiological conditions. In response to chronic injury, HSCs are activated and change from quiescent to myofibroblast-like cells with contractile properties. This makes HSCs an interesting target in the study of portal hypertension. The shift in phenotype is accompanied by a dramatic change in expression of intermediate filaments (IFs). In contrast to most differentiated cell types, HSCs express a broad, but variable spectrum of IFs. Aim: Investigate the expression and functions of the IF protein syncoilin in HSCs. Methods: Syncoilin expression in isolated and cultured mouse HSCs was studied by quantitative reverse transcription polymerase chain reaction, Western blotting, and fluorescence immunocytochemistry (confocal microscopy). We generated different syncoilin antibodies for these purposes. The function of syncoilin in HSC contraction was studied by means of optical recording using the fluorescent calcium indicator, Fluo-4. Results: Syncoilin mRNA was present in HSCs, skeletal muscle, heart, brain, and stomach and was upregulated during HSC activation. While in quiescent HSCs no syncoilin protein could be detected, this protein was strongly upregulated during in vitro activation. We verified the cellular localization of syncoilin using immunocytochemistry. We observed filamentous staining in HSCs undergoing in vitro transdifferentiation. The syncoilin IF bundles did not colocalize with alpha smooth muscle actin or desmin filaments. To assess the function of syncoilin we performed calcium measurements in HSCs transfected with control or syncoilin siRNAs. In cells transfected with syncoilin siRNA, intracellular calcium release following stimulation with endothelin-1 was reduced. Conclusion: To our knowledge, we are the first group to demonstrate the presence of the IF protein syncoilin in mouse HSCs. This IF protein is strongly upregulated during in vitro activation. We show that syncoilin is an important player in intracellular calcium signaling.

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1413
ATRIAL NATRIURETIC PEPTIDE (ANP) PREVENTED LIVER FIBROSIS IN RAT USING NEW TREATMENT SYSTEM
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[Aim/Background]: The atrial natriuretic peptide (ANP) has been used as the acute heart failure treatment in clinical and it is reported the suppression of fibrosis in the heart and the lung recently. The aim of this study was to examine the preventive effect of atrial natriuretic peptide (ANP) on liver fibrosis both in vitro and in vivo using new continuous infusion pump treatment system. [Methods]: In vitro, isolated hepatic stellate cells (HSC) were treated with ANP (0, 10, 50, 100 µg/ml). We examined α-SMA (alpha smooth muscle actin) expression by Western blot analysis and fibrotic gene mRNA expression was analyzed by RT-PCR. In vivo, Wister rats were injected with dimethylthiourea (DMN) twice a week via intraperitoneal injection (i.p.) for 4 weeks to develop the liver fibrosis. After one-week treatment with DMN, rats were randomly divided to three groups (each group n=6); Then rats were treated with ANP (0=saline, 0.1 and 1.0 mg/kg/min) using 24 hour continuous infusion pump for another 3 weeks. At the end of the study, the percent area of liver fibrosis, α-SMA-positive lesions were measured with computer analyzing system. [Results]: In vitro study, expression of α-SMA, type I collagen and TIMP-1 mRNA was inhibited with ANP in a dose-dependent manner. In vivo study, high dose ANP treated group showed significantly decreased serum AST (mean value: 81.8 vs 2000 IU/l, P<0.01) and ALT level (mean value: 63.8 vs 2000 IU/l, P<0.01). ANP significantly (P<0.01) decreased liver fibrosis area as well as α-SMA-positive lesions in a dose-dependent manner. In whole liver, ANP significantly inhibited type I collagen, MMP2 and TIMP-1 mRNA expression. [Conclusion]: Our results showed that the atrial natriuretic peptide (ANP) inhibited liver fibrosis by direct inhibition of hepatic stellate cell activation and with hepatocyte protection.

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1414
THE SYNTHETIC COLLAGEN ANALOG (GPO)10 STIMULATES PROLIFERATION, MIGRATION AND EXPRESSION OF N-CADHERIN IN HEPATIC STELLATE CELLS VIA ACTIVATION OF PROMMP-2
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Introduction: Matrixmetalloproteinase-2 (MMP-2) degrades denatured collagens and plays a key role in liver fibrogenesis and fibrolysis. We previously showed that proMMP-2 binds to nonsubstrate collagens of the extracellular matrix and is released and activated by the collagen analog (GPO)10. The aim of this study was to elucidate cell specific events initiated by (GPO)10 induced proMMP-2 activation, such as proliferation, migration and expression of the HSC activation marker N-cadherin. Methods: (GPO)10 induced proMMP-2 activation was shown by gel zymography and substrate turnover. The hepatic stellate cell line CFSC was treated with nanomolar concentrations of zymogens and activated MMP-2 with and without specific MMP-inhibition (ilomastat) and (GPO)10. DNA neosynthesis was measured by [3H]thymidine incorporation. Additionally, a MMP-2 dependent migration assay was performed. N-cadherin expression was assessed by western-blotting. Results: A 10fold molar excess of (GPO)10 caused a complete activation of proMMP-2, whereas a control peptide showed no effect. Active MMP-2 and (GPO)10 stimulated CFSC proliferation up to 35 and 20% compared to maximum stimulated cells, respectively. The increase of proliferation induced by (GPO)10 activated endogenous MMP-2 was completely blocked with ilomastat. MMP-2 and (GPO)10 activated proMMP-2 specifically induced strong cell migration and upregulation of N-cadherin in HSC which was blocked by the addition of ilomastat. Conclusion: (GPO)10 activates proMMP-2 leading to enhanced proliferation, migration and activation of mesenchymal and hepatic stellate cells. Thus, (GPO)10 excerts
fibrolytic as well as fibrogenic effects. Animal studies will elucidate the role of this synthetic collagen peptide in liver fibrosis and regeneration.

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CD95 Ligand Induces Proliferation and CD95-Tyrosine Nitration in Quiescent Rat Hepatic Stellate Cells

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Introduction: Quiescent hepatic stellate cells (HSC) are fairly resistant to CD95 ligand (CD95L)-induced apoptotic cell death, while apoptosis upon CD95L-treatment has been observed in activated HSC/myofibroblasts when cycloheximide (CHX) was co-administered (Cariers et al., Cell Physiol Biochem 2002). Recently, quiescent HSC have been reported to express CD133 and to represent a hepatic progenitor cell compartment (Kordes et al., BBRC 2007). Methods: Rat hepatic stellate cells were either cultivated for 1-2 days (quiescent HSC) or 10-14 days (activated HSC) on plastic culture dishes. Activation of Src, EGFR, Erks and JNks were measured by W. blot using respective phospho-specific antibodies. CD95 was immunoprecipitated and detected for CD95/EGFR-association, CD95-tyrosine phosphorylation, CD95-tyrosine nitration and DISC-formation using W. blot technique. HSC proliferation and apoptosis were analyzed by BrdU-incorporation measurements and TUNEL-staining, respectively. Results: In quiescent HSC, CD95L leads to an activation of c-Src, EGFR and Erks which was sensitive to inhibitors of Src-kinases (PP-2), sheddases (GM6001) and to EGF-neutralizing antibodies, while no JNK-activation occurred. Furthermore, an increased bromodesoxyuridine incorporation was observed in quiescent HSC after CD95L-Addition, while no pro-apoptotic CD95-activation became detectable with respect to CD95/EGFR-association, CD95-tyrosine phosphorylation, DISC-formation, TUNEL-staining and PARP-cleavage. In addition, in quiescent HSC CD95L induced CD95-tyrosine nitration in a time- and dose-dependent manner, which was previously shown to inhibit CD95-tyrosine phosphorylation and thereby exhibits anti-apoptotic properties. In contrast, in activated HSC no CD95-tyrosine nitration upon CD95L administration was observed, whereas there was still activation of the EGFR. When CD95L and CHX were co-administered, a sustained JNK-activation occurred which allowed for CD95/EGFR-association, subsequent CD95-tyrosine phosphorylation, DISC formation and apoptotic cell death. Conclusions: In quiescent HSC, CD95L is a mitogen through a ligand-dependent activation of the EGFR which requires Src-kinase- and sheddase-activity. Simultaneously, apoptotic signaling is prevented by CD95L-induced CD95-tyrosine nitration, which may contribute to the recently described progenitor cell properties of quiescent HSC and which is lost after differentiation to activated HSC/myofibroblasts.

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Inhibition and Identification of Nonmuscle Myosin II Isoforms in Hepatic Stellate Cells

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Regulation of liver microcirculation is a complex system where blood flow is under systemic and sinusoidal control. Under normal conditions, quiescent hepatic stellate cells (HSC) wrap around sinusoids to regulate diameter, and thus blood flow, by contracting and dilating in response to local vasconstrictors and vasodilators. However, activated HSCs exert a sustained contractile force, resulting in hyper-constricted vessels. This leads to hepatic microcirculation dysregulation, which contributes to the progression of diseases such as fibrosis, hepatocellular carcinoma, or viral hepatitis. Nonmuscle myosin II (NMMy II) has been shown to be the motor protein involved in the contraction of HSCs; however, the expression levels and cellular localization of the specific isoforms (NMMy II-A, II-B and II-C) have not been characterized, nor has NMMy II been specifically blocked in activated HSCs. Blebbistatin is a novel chemical inhibitor which specifically blocks NMMy II activity. HSCs treated with blebbistatin in a collagen contraction assay inhibited vasconstrictor-induced contraction, as well as basal contraction. Cellular localization in activated HSCs visualized by immunocytochemistry demonstrated that NMMy II-A and II-B were localized to the periphery and actin stress fibers, while NMMy II-C protein expression was dispersed. The data are consistent with previous studies that suggest NMMy II-A and II-B function in cell migration and contraction, respectively. Using RT-PCR and Western blotting, in vitro data revealed robust mRNA and protein expression of NMMy II-B with lower expression levels of NMMy II-A and II-C in culture-activated HSCs. Additionally, protein expression of all three isoforms was undetectable in freshly isolated quiescent HSCs; however, minimal levels of mRNA were detected. As compared to normal liver, fibrotic tissue as visualized by immunohistochemistry showed increased staining of NMMy II-B in activated HSCs; however, NMMy II-A and II-C did not colocalize in HSCs even though expression of these isoforms was visualized in other cell types. In conclusion, our study demonstrates that NMMy II-B is strongly upregulated in activated HSCs. Since this isoform is known to spend a larger period of its ATPase cycle bound to actin than NMMy II-A and II-C, its enhanced expression may be responsible for inducing the hyper-contractile activity of HSCs. Determining the expression profile of NMMy II isoforms and their functional role in HSCs may lead to specific therapeutic targets in the treatment of liver diseases. Supported by NIH Grant AA14891.

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Different Regulation of the Connective Tissue Growth Factor in Hepatic Stellate Cells and Hepatocytes

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Background: The connective tissue growth factor (CTGF), a CCN family protein is described as a down-stream modulator of proliferative TGF-β effects, e.g. in activating hepatic stellate cells (HSC) and promoting the deposition of extracellular matrix (ECM). CTGF has been demonstrated in experimental fibrosis, in which its expression appears to correlate with the degree of
fibrosis. The activation of HSC and the proximate ECM production are triggered by TGF-β. Active TGF-β binds and phosphorylates transmembrane receptors, which in turn propagate the signal via Smads into the nucleus in order to bind the appropriate promoter sequence of a target gene. CTGF expression could be induced by TGF-β as well as by glucocorticoids. Previous studies showed that glucocorticoids decrease TGF-β-sensitive reporter signalling in primary HSC. The aim of this study was to characterize the molecular effects of glucocorticoids and TGF-β on CTGF expression in primary HSC and hepatocytes and to ascertain the impact of glucocorticoid-TGF-β interaction. Results: Therefore, we analysed CTGF expression by a combined stimulation with dexamethasone and TGF-β in hepatocytes and HSC. We determined by western blots that CTGF was significantly induced in both cell types after co-stimulation with TGF-β and dexamethasone. It seems that different pathways for CTGF regulation were used in HSC and hepatocytes: Whereas TGF-β and dexamethasone induced CTGF is decreased when JNK function is inhibited in HSC, CTGF increased expression was repressed by Erk-inhibitor in hepatocytes. Interestingly, we observed a different CTGF secretion: It was directly secreted by activated HSC but selectively released into the culture medium by TGF-β-stimulated hepatocytes. Moreover, TGF-β and dexamethasone have antagonistic effects on Erk phosphorylation: TGF-β induce it, dexamethasone abrogate it. Our data suggest that the cell-type specific regulation seems to be dependent on MEK, which decide between a downstream signalling via Erk or via JNK. Conclusions: CTGF expression control is accomplished by the use of the TGF-β-Smad and the TGF-β-Ras-Mek pathway in a cell-type specific manner and our data indicate that a complex signalling network regulate the fine tuning of expression and secretion in HSC and hepatocytes.

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1418 RELAXIN INCREASES THE EXPRESSION AND ACTIVITY OF PPARγ IN ACTIVATED HEPATIC STELLATE CELLS
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Peroxisome proliferator-activated receptor gamma (PPARγ) is a transcription factor expressed in quiescent hepatic stellate cells (HSC). With conversion to the activated HSC phenotype, PPARγ expression is markedly reduced. Restoration of PPARγ expression or activity results in a reversal of many of the profibrotic properties of HSC. The polypeptide hormone relaxin has antifibrotic effects on HSC, and through activation of its receptor RXFP1 induces elevated levels of cAMP. Because inducers of the cAMP/Protein kinase-A pathway have been implicated in the activation of PPARγ in other fibroblastic cells, we sought to determine the effect of relaxin treatment on PPARγ expression and activity in HSC. Primary rat HSC were culture-activated for 12 days before treatment with relaxin or a PPARγ agonist. As determined by Western blotting, PPARγ protein was present at a low level in untreated HSC. Relaxin caused a dose-dependent increase in the PPARγ protein level. To determine the effect of relaxin on PPARγ gene expression, HSC were treated with relaxin (1 nM), the PPARγ activator troglitazone (1 μM), or both, for 24 hours. Total RNA was then extracted from the cells, and expression of PPARγ was determined by real-time TaqMan RT-PCR. The expression level was normalized to that of 18S ribosomal RNA in the same samples. Troglitazone treatment resulted in a 60% increase in the expression of PPARγ, while relaxin had a more modest effect (43% increase in PPARγ expression). The combination of troglitazone and relaxin induced a 135% increase (p<.01 by ANOVA) in PPARγ expression, an effect greater than the additive effect of each agent alone. Finally, to examine the effect of relaxin and troglitazone on a fibrotic property of HSC, the cells were treated as above for 48 hours, and expression of α2(I) procollagen was determined by real-time RT-PCR. Troglitazone decreased in procollagen expression 65%, while relaxin alone had a lesser effect (55% decrease). The combination of relaxin and troglitazone was more effective than either treatment alone (75% decrease).

In summary, relaxin treatment of HSC increased PPARγ gene expression and protein levels. The combined action of relaxin and troglitazone on the expression of PPARγ and procollagen was greater than either agent alone. These data suggest that relaxin treatment may sensitize HSC to PPARγ agonist treatment by increasing the amount of cellular PPARγ. Through this mechanism, relaxin might be used to enhance the antifibrotic effects of these drugs and improve the treatment of hepatic fibrosis.

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1419 HSC EXPRESS NALP3 AND ASC, AND UNDERGO DIFFERENTIATION IN RESPONSE TO URIC ACID CRYSTALS
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Background: Hepatocyte apoptosis is known to induce hepatic stellate cell (HSC) differentiation but the signals mediating this are not known. Uric acid is produced during hepatocyte apoptosis, and has been identified as an important differentiation signal for dendritic cells and macrophages via the NALP3 pattern recognition receptor and the adapter molecule ASC. We hypothesized that uric acid also communicates hepatocyte apoptosis by inducing differentiation of HSC. Methods: RTPCR was performed using cDNA from primary murine HSC with primers for NALP3 and the adaptor protein ASC. Uric acid crystals (100 μg/ml) were added to LX-2 cells, and HSC from wild-type and ASC KO mice. 24 hours later cells were stained with α-smooth muscle actin and visualized by confocal microscopy, and cDNA was prepared for quantification of TGF-β and collagen1 mRNA expression by real-time PCR. LX-2 cells were cultured with or without uric acid crystals for 24 hours in a trans-well assay with PDGF as the chemo-attractant. We also examined inhibition of calcium signaling in LX-2 cells treated with or without uric acid crystals using by caged IP3. Finally, we confirmed the role of ASC in in-vivo liver fibrosis by the injection of carbon tetrachloride (CCL4) (850 μg/kg) in wild-type and ASC KO mice for 8 weeks. Liver tissue was stained with Sirius Red. Results: NALP3 and ASC KO mice were treated with Sirius Red Stain- ing, and up-regulated TGF-β and procollagen1 mRNA in HSC from wild-type but not ASC KO mice. Uric acid crystals inhibited the release of calcium via IP3 in LX-2 cells, and also inhibited PDGF induced chemotaxis. ASC KO mice injected with CCL4 had less fibrosis than wild-type mice on Sirius Red Staining. Conclusion: Uric acid inhibits HSC chemotaxis, induces actin reorganization and HSC differentiation via NALP3 and ASC.

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1420
L-TYROXINE (T4) INDUCES PROLIFERATION OF HEPATIC STELLATE CELLS VIA CELL SURFACE INTEGRIN \( \alpha V \beta 3 \) AND ENHANCES EXPRESSION OF \( \alpha \)-SMOOTH MUSCLE ACTIN AND COLLAGEN I TRANSCRIPTION
Isabel Zvibel\(^1\), Ella Barlev\(^1\), Adam Phillips\(^2\), Dikla Atias\(^1\), Shirley Abramovitch\(^1\), Zamir Halpern\(^1\), Ran Oren\(^1\);
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Background/aims: We have previously shown that hypothyroidism is beneficial both for preventing and for treating liver fibrosis in animal models, while hyperthyroidism enhanced liver fibrosis. The aim of these studies was to determine the effect of thyroid hormone L-Tyroxine (T4) on the proliferation and activation of hepatic stellate cells via its newly described cell surface receptor, integrin \( \alpha V \beta 3 \) and to determine regulation of integrin \( \alpha V \) by T4 and extracellular matrix in hepatic stellate cells.

Methods: We have used primary rat hepatic stellate cells and the human hepatic stellate cell line LX-2. Total expression of integrin \( \alpha V \), \( \alpha \)-smooth muscle actin (\( \alpha \)-SMA) and signal transduction was assessed by Western blot analysis and cell surface integrin \( \alpha V \beta 3 \) expression was measured by fluorescence activated cell sorter. Proliferation was measured by cell counting and by a cell proliferation kit. Collagen I \( \alpha 1 \) and \( \alpha 2 \) promoter activity was assessed by transient transfection. Results: Expression of integrin \( \alpha V \) was low in quiescent cells and increased as cells became activated and trans-differentiated into myofibroblasts. LX-2 cells expressed \( \alpha V \beta 3 \) integrin and expression of this integrin was up-regulated by fibronectin, vitronectin and also by T4. T4, but not triiodothyronine, was a mitogen for primary hepatic stellate cells and for LX-2 cells. Proliferation of LX-2 cells was also enhanced when the cells were plated on fibronectin and vitronectin, ligands of \( \alpha V \beta 3 \). T4 induced nuclear translocation of phosphorylated erk1/erk2 and STAT3 in LX-2 cells. This effect was mediated via \( \alpha V \beta 3 \) integrin and was abolished in the presence of RGD peptide and tetrac, which compete with \( \alpha V \beta 3 \) cell surface receptor, integrin \( \alpha V \beta 3 \). Primary hepatic stellate cells cultured in thyroid hormone-depleted serum expressed very low levels of \( \alpha \)-SMA after 14 days in culture, but culturing the cells in the presence of 10-7M T4 resulted in strong expression of \( \alpha \)-SMA. Lastly, we have found that T4 enhanced transcription of collagen I \( \alpha 1 \) and \( \alpha 2 \) promoters in primary hepatic stellate cells both after 7 days and after 14 days in culture. Conclusion: We have shown that T4 is a mitogen for hepatic stellate cells via cell surface integrin \( \alpha V \beta 3 \), and is pro-fibrogenic by inducing \( \alpha \)-SMA expression and transcription of collagen I. These results suggest for the first time the mechanism responsible for the deleterious effect of hyperthyroidism on liver fibrosis in vivo.

Disclosures:
The following people have nothing to disclose: Isabel Zvibel, Ella Barlev, Adam Phillips, Dikla Atias, Shirley Abramovitch, Zamir Halpern, Ran Oren

1421
GLUTATHIONE, BUT NOT CATALASE, IS IMPORTANT IN THE PROTECTION OF HEPATIC STELLATE CELLS AGAINST OXIDATIVE STRESS
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Background: We have previously shown that activated HSCs are sensitive to both superoxide anion and hydrogen peroxide induced cell death. HSC have different mechanism to detoxify oxidative stress such as peroxisomal catalase, superoxide dismutases and anti-oxidants like glutathione. Aim: 1) to evaluate the importance of catalase and glutathione in the protection against oxidative stress-induced cell death. 2) to evaluate the effect of oxidative stress on mRNA levels of antioxidant enzymes and activation markers of HSCs.

Methods: Primary rat hepatocytes and serum-starved culture-activated rat HSCs were exposed to oxidative stress induced by menadione (5-50\( \mu \)M), superoxide anion donor or hydrogen peroxide (0.2-5mM). Apoptosis and necrosis were determined by Acridine Orange and Sytox Green nuclear staining, respectively. mRNA expression of genes was determined by quantitative RT-PCR. Catalase was inhibited using 3-aminoatrazole (20mM). Glutathione levels were depleted by treatment with buthionine sulfoximine (BSO) at 200\( \mu \)M. Results: Menadione dose-dependently induced apoptotic but not necrotic death of HSCs. Glutathione depletion did not modulate menadione-induced cell death. Hydrogen peroxide did not induce cell death at concentrations up to 1mM hydrogen peroxide, but induced >90% necrosis at concentrations of 5mM. However, glutathione depletion dramatically increased the sensitivity of HSCs to hydrogen peroxide, resulting in >80% necrosis at 1mM hydrogen peroxide. Blocking catalase did not sensitize HSCs to hydrogen peroxide induced necrosis. In contrast, blocking catalase increased sensitivity of hepatocytes to hydrogen peroxide induced necrosis. In accordance, the relative expression of catalase mRNA was 13-fold higher in hepatocytes compared to HSCs. Menadione and hydrogen peroxide both induced the oxidative stress responsive gene HO-1 and this induction was further increased by GSH-depletion. The mRNA levels of the anti-oxidant enzymes catalase, Mn-SOD, Cu/Zn SOD and the activation markers TGF-\( \beta \), \( \alpha \)-SMA and collagen type 1 mRNA expression were not substantially altered by oxidative stress. Conclusion: In contrast to hepatocytes, catalase is not important in the protection of HSCs against hydrogen peroxide toxicity. Depletion of glutathione increases oxidative stress in activated HSCs as demonstrated by further induction of HO-1 and increases the sensitivity of activated HSC to hydrogen peroxide induced necrosis. Our results indicate that other hydrogen peroxide detoxifying enzymes, e.g. GSH-peroxidase, are important in HSCs. Targeting these enzymes may be a strategy to deplete activated HSCs as anti-fibrotic therapy, without affecting hepatocytes.

Disclosures:
The following people have nothing to disclose: Sandra Dunning, Rebekka Hannivoort, Laura Conde de la Rosa, Manon Buist-Homan, Klaas Nico Faber, Han Moshage

1422
THE ADENINE RECEPTOR IS PRESENT IN RAT HEPATIC STELLATE CELLS (HSC), INDUCES HSC DIFFERENTIATION AND INHIBITS HSC CHEMOTAXIS
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Background: Adenine was recently identified as the endogenous ligand of an orphan rat G protein-coupled, but the function of the adenine receptor is poorly characterized. We hypothesized that adenine, a uric acid pathway metabolite downstream of adenosine, promotes HSC differentiation. Methods: RTPCR was performed for adenine receptor expression in primary rats HSCs. Primary rats HSC were cultured with and without adenine (500\( \mu \)M). Twenty-four hours later, cDNA was prepared for quantification of collagen1 and TGF-\( \beta \) mRNA using Real Time RT-PCR. HSCs were also stained for \( \alpha \)-smooth muscle actin to visualize actin reorganization. To test if adenine inhibits HSC chemotaxis LX2 cells and rats HSCs were cultured with or without adenine (500\( \mu \)M) for 24 hours in a transwell
and integrin αV subunit co-localized on the membrane of primary rats HSCs. In LX-2 cells and primary rats HSCs, adenine induces actin reorganization. Adenine also up-regulates collagen 1 mRNA in rats HSCs, and inhibits chemotaxis in LX-2 cells and rats HSCs. Adenine also inhibits IP3 mediated increase in cytosolic Ca2+. Conclusions: Adenine receptor is present in primary rats HSCs. Adenine induces morphological change, and up-regulates collagen 1 mRNA in HSCs. Adenine inhibits chemotaxis and cytosolic calcium signaling in HSCs.

**Adenine increase collagen1 mRNA in Rats HSCs (P < 0.01).**

![Collagen1 Graph]

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1424

OSTEOPONTIN, AN OXIDANT STRESS-SENSOR IN HEPATOCYTES AND KUPFFER CELLS, TRIGGERS A FIBROGENIC RESPONSE IN STELLATE CELLS

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Background: Activation of hepatic stellate cells (HSC) is the most critical feature in the pathogenesis of liver fibrosis and it appears to be mediated, among others, by factors released from hepatocytes and from Kupffer cells (KC). We have previously shown an essential role for hepatocyte- and KC-derived reactive oxygen species (ROS) on HSC activation and collagen I deposition. Microarray analysis identified osteopontin (OPN), an extracellular glycoprotein and a cell adhesion molecule implicated in tissue remodeling, as an ROS-sensitive protein. The Aim of this work was to address whether OPN, secreted by both hepatocytes and KC, could modulate the fibrogenic response in HSC. Results: Experiments were first carried out with either hepatocytes or with a HepG2 cell line overexpressing cytomechrome P450 2E1 (CYP2E1) to generate a state of oxidant stress. CYP2E1-expressing cells showed a 5-fold induction of OPN when compared to control cells. Arachidonic acid, a representative polyunsaturated fatty acid, further increased OPN levels 2-fold. These effects were prevented by addition of vitamin E, which blocks lipid peroxidation, and by diallylsulfide and diethyldithiocarbamate, CYP2E1 inhibitors. L-buthionine sulfoxime, a GSH-depleting agent, increased OPN mRNA levels by 2-fold. Similar results were found in primary hepatocytes from chronic ethanol-fed rats (with high CYP2E1) when compared to control hepatocytes. Likewise, KC isolated from chronic ethanol-fed rats (KCchal) displayed a 6-fold increase in OPN protein and mRNA when compared to controls. To understand the potential pro-fibrogenic actions of OPN, primary HSC were incubated with recombinant OPN (1-20nM) at different time points (6-48h). A significant increase in the expression of both intra- and extracellular collagen type I as well as α-smooth muscle actin (α-SMA) was observed at 24h post-treatment. Moreover, collagen type I up-regulation was accompanied by a decrease in matrix metalloproteinase 13 (MMP13), the MMP that specifically degrades collagen I. In contrast, MMP2 and MMP9 activities remained similar to those of non-treated cells. HSCcontrol and HSCethanol incubated with 1nM recombinant OPN showed a time-dependent increase in collagen I protein which was more
apparent in HSC_{ethanol}. Co-culture of HSC_{ethanol} with KC_{optical} or KC_{ethanol} also promoted HSC proliferation. OPN was found only in the extracellular medium of the ethanol co-culture. Conclusion: these results unveil that ROS trigger an increase in OPN which behaves as a profibrogenic factor playing a major role in extracellular matrix remodeling.

Disclosures:
The following people have nothing to disclose: Raquel Urtasun, Natalia Nieto

1425
UPREGULATION OF SMOOTH MUSCLE MARKER GENES IN ACTIVATED HEPATIC STELLATE CELLS IS MEDIATED BY TRANSFORMING GROWTH FACTOR-β INDUCED EXPRESSION OF SERUM RESPONSE FACTOR

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Background: Hepatic stellate cells (HSC) are prominent cellular effectors of liver fibrosis, which become activated during liver injury and transdifferentiate from quiescent, fat storing cells into a proliferative myofibroblast-like cell type (MBF). A common feature of activated HSC is upregulation of genes encoding known markers of smooth muscle cells (SMC) like α-smooth muscle actin (α-SMA) or SM22α. In SMC these genes are regulated by the serum response factor (SRF) in cooperation with specific co-factors. Aims: The aim of this study was to investigate the expression profiles of SRF and important co-factors in activated HSC and its role in the upregulation of SMC marker genes. Material/Methods: Primary rat HSC were culture-activated by seeding onto plastic surfaces and lysed at different days of cultivation. The expression levels of SRF, myocardin, and MRTF-A were detected by Western blotting and immunofluorescent cytochemistry. Transcriptional activity of SRF was examined by EMSAs. Results: During HSC transdifferentiation the SRF content increased in parallel to its known target genes α-SMA or SM22α associated with a nuclear accumulation and an enhanced DNA binding capacity of SRF in activated HSC. We also found an upregulation of the SRF co-factors myocardin and MRTF-A. Inhibition of SRF activity by specific siRNA or the Rho kinase inhibitor Y27632 resulted in decreased expression of SMC marker genes. SRF expression in HSC is depending on transforming growth factor-β (TGF-β) as demonstrated by direct induction or by inhibition of TGF-β signalling via a soluble TGF-β type II receptor or the ALK5 inhibitor SB431542 resulting in a decrease of SRF contents in activated HSC. Conclusions: We could demonstrate that SRF as well as its important SMC marker gene-specific co-factors myocardin and MRTF-A are upregulated during HSC activation and that SRF is functionally active. As TGF-β is a key effector in HSC activation and transdifferentiation, it is important that this cytokine also mediates SRF expression in these cells resulting in the well known upregulation of α-SMA and other SMC marker genes during this relevant process of liver fibrogenesis. To date it is still unknown which factors are involved in TGF-β induced upregulation of proteins that are important for cytoskeletal reorganization in activated HSC. We could show that the transcription factor SRF, which is known to be essential for SMC marker gene expression, is also a potent mediator of TGF-β signals during HSC transdifferentiation. Our findings suggest that SMC marker gene expression is similarly regulated in SMC and activated HSC, thereby representing their high plasticity.

Disclosures:
The following people have nothing to disclose: Jens Herrmann, Ute Haas, Axel M. Gressner, Ralf Weiskirchen

1426
HBV INFECT HEPATIC STELLATE CELLS AND AFFECT THEIR PROLIFERATION AND EXPRESSION OF EXTRACELLULAR MATRIX

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BACKGROUND AND AIMS: The activation of hepatic stellate cell (HSC) plays an important role in liver fibrosis. But the exact mechanism of fibrogenesis induced by chronic hepatitis B has not been fully understood till now. The aim of this study was to observe whether HBV can infect HSC and its effect on proliferation of HSC and expression of extracellular matrix (ECM) in vitro. MATERIALS AND METHODS: Firstly, LX-2 cells (human activated HSC cell line) were incubated with the supernatant of HepG2.2.15 cells with the MOI 0.1-10 HBVDNA copies/cell and harvested after 24, 48 and 72 hours. HBV particles in LX-2 cells were observed by Electron microscopy (EM) and immunogold EM. HBVDNA and cccDNA were evaluated by real-time polymerase chain reaction (PCR). HBV replicative intermediates and HBV mRNA were detected by Southern blot/Northern blot and Dot blot. Secondly, various concentrations of HBVDNA, HBsAg or HBeAg were added into LX-2 cell culture for 48 hours. Cells survival was tested by MTT assay. And then, flow cytometric analysis was used to evaluate proliferation of HSC. Finally, the mRNA level of Collagen-I, Collagen-III were quantified by real-time polymerase chain reaction; and their protein levels were measured by enzyme-linked immunosorbent assay (ELISA). RESULTS: Some viral-like particles, diameter from 20 to 40nm, were found in endocytic vesicles but not in the cytoplasm in some of LX-2 cells. In these LX-2 cells, HBVDNA level was less than 0.05 copies /cell without finding of the replicative intermediates. However, HBVDNA, HBsAg and HBeAg can increase the cell cycle's [S+G2M]% and S% (P<0.05). Compare to control group, the mRNA level of collagen-I was significant increased by HBV(0.658±0.108 vs. 0.513±0.063, P<0.01) and HBeAg (0.689±0.182 vs. 0.45±0.078, 0.05); collagen-III mRNA level was increased by HBV(0.196±0.016 vs. 0.121±0.035, P<0.05) and HBeAg (0.171±0.019 vs. 0.118±0.082, P<0.05). HBV and HBeAg can also significantly increase the collagen-I protein expression (P<0.05). CONCLUSIONS: HBV might enter LX-2 cells through cytaphagy in vitro. No evidence supports HBV can replicate and express antigens in LX-2 cells. HBV and HBeAg have the effect of both promoting the proliferation of HSC and increasing the synthesis of major ECM.

Disclosures:
The following people have nothing to disclose: Yan Cui, Yu Wang, Jidong Jia

1427
REACTIVE NITROGEN SPECIES MODULATE EARLY EXTRACELLULAR MATRIX REMODELING VIA INDUCTION OF MATRIX METALLOPROTEINASE-1 AND TUMOR NECROSIS FACTOR α

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To investigate whether reactive nitrogen species such as peroxynitrite (ONOO−) modulate the fibrogenic response and extracellular matrix remodeling, hepatic stellate cells (HSC) were incubated in the presence of either pure ONOO− or 3-morpholininosydnonimine (SIN-1), a ONOO− donor. Loss of cell viability, apoptosis, and cell proliferation did not occur as assessed by the MTT assay, Annexin V/propidium iodide staining, and methyl[3H]Thymidine incorporation, respectively.

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The following people have nothing to disclose: Natalia Nieto, Francisco J. Cubero, Maria Vera, Raquel Urtasun.
Nuclear in vitro transcription run-on assays, Northern blot analysis, and quantitative RT-PCR indicated similar COL1A1 and COL1A2 mRNA expression under both treatments, suggesting a likely post-translational regulation of collagen I expression. There was a dose-dependent decline in intra- and extracellular collagen I protein along with an increase in matrix metalloproteinase 1 (MMP1), the metalloproteinase that specifically degrades fibrillar collagen I, and in tumor necrosis factor α (TNFα), which were blocked by the ONOO- scavengers glutathione ethyl ester (GSH-EE), uric acid, and ebselen. Tissue inhibitor of metalloproteinase 1 (TIMP1) protein expression remained similar to that of non-treated HSC, and α-smooth muscle cell actin expression suggested decreased HSC activation. ONOO- triggered nitration of MMP1 and MMP13 likely promoting collagenolytic activity as shown by the lower active molecular weight bands, and it also led to transactivation of the MMP1, MMP13, and TNFα promoters. A TNFα neutralizing antibody and GSH-EE, a permeable and soluble form of GSH, blocked the transactivation of the MMP1 promoter, while treatment with L-buthionine sulfoximine, which depletes GSH stores, or with TNFα further transactivated the MMP1 promoter. Incubation of HSC with TGFβ after SIN-1 or ONOO- pre-treatment, did not lead to a pro-fibrogenic response. Similar results were obtained in rats injected with CCl4 and co-treated with SIN-1, validating a protective role for ONOO in the early fibrogenic response. However, highly activated HSC and HSC from chronic alcohol-fed rats with higher levels of GSH, an efficient ONOO- scavenger, were insensitive to the antifibrogenic actions of ONOO- due to overproduction of TGFβ and of ROS rather than of TNFα, as well as to high GSH levels, a powerful ONOO- chelating agent. The data suggest that although protective, the antifibrogenic actions of ONOO- are likely to occur only in the early stages of HSC activation.

Disclosures:
The following people have nothing to disclose: Natalia Nieto, Francisco J. Cubero, Maria Vera, Raquel Urtasun

1428 THY-1 DISTINGUISHES BETWEEN HEPATIC STELLATE CELLS AND LIVER MYOFIBROBLASTS IN VITRO AND IN VIVO
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Background/Aims: Several fibroblastic cell types have been described in the liver. So far, there is no reliable way to distinguish between activated hepatic stellate cells (HSC) and liver myofibroblasts in vivo. Thy-1, a recently described membrane-anchored myofibroblast marker was tested as a candidate for this function. Methods: The expression of Thy-1 was studied at mRNA and protein levels in vivo: in normal rat liver, in CCl4-induced acute and chronic rat liver injury, in partial hepatectomy (PH)-induced rat liver regeneration and in the rat liver regeneration model of 2-acetylaminofluorene treatment and PH (AAF/PH), in vitro: in isolated rat liver cells. Results: Thy-1+ cells were detected in the periportal area of rat liver specimens in normal-, injured- and regenerative-conditions. In chronic liver injury weak Thy-1+ reaction was found in the sinusoids of the parenchyma. Portal and septal myofibroblasts were Thy-1+ cells. In liver regeneration Thy-1, smooth-muscle alpha-actin (SMA) and desmin were located mainly in the portal area, but all of them showed perisinusoidal reaction as well. While desmin and SMA were relative homogenously distributed in the sinusoids, Thy-1 was only present in the sinusoids adjacent to the portal tract. The pericentral sinusoids were Thy-1+. SMA in the pericentral sinusoids also co-localised with Fas-ligand (Fasl).

Comparative immunohistochemical analysis revealed a co-localisation of Thy-1 and SMA, but not of Thy-1 and cytokertatin-19 or Thy-1 and alpha-fetoprotein in the AAF/PH model. Investigation of isolated rat liver cell populations confirmed that liver myofibroblasts are Thy-1-positive cells, while hepatocytes, HSC and liver macrophages are not, moreover, Thy-1 was not inducible by several cytokines in HSC. Conclusion: Thy-1 is the first cell surface marker to identify liver myofibroblasts in vivo and in vitro, and is not expressed in "oval cells".

Disclosures:
The following people have nothing to disclose: Jozsef Dudas, Tuemen Mansuroglu, Danko Batusic, Federico Moriconi, Bernhard Saile, Giuliano Ramadori

1429 T3 ENHANCES EXPRESSION OF α-SMOOTH MUSCLE ACTIN AND OF CYTOKINES TNFα AND SDF1α IN PRIMARY STELLATE CELLS, YET INHIBITS COLLAGEN I TRANSCRIPTION
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Background/Aims: We have previously shown that hyperthyroidism caused more rapid appearance of liver fibrosis in a thioacetamide rat model of liver fibrosis. The purpose of the present studies was to determine whether expression of thyroid hormone receptors changes in quiescent and activated primary hepatic stellate cells and hepatic stellate cells that trans-differentiated into myofibroblasts. Moreover, we have investigated the effect of T3 on proliferation and activation of primary hepatic stellate cells, determined by expression of α-smooth muscle actin, various cytokines and activity of collagen I α1 and α2 promoters. Methods: Primary hepatic stellate cells were isolated from rat livers by pronase/collagenase digestion and Nycodenz gradient. Expression of thyroid hormone receptor α1 (TRα1), PDGF receptors and α-smooth muscle actin (α-SMA) was determined by Western blot analysis. Expression of cytokines MCP-1, TGFβ1, TIMP1 and 2, TNFα, SDF1α was determined by RT-PCR. Activity of collagen I α1 and α2 promoters was assayed by transient transfection. Results: Expression of TRα1 dramatically decreased as primary hepatic stellate cells progressed from quiescence to transdifferentiation into myofibroblasts, which was the opposite of the expression of PDGF receptors. T3 had no effect on the proliferation of activated hepatic stellate cells (7 days in culture) or of myofibroblasts (14 days in culture). Activated hepatic stellate cells grown in thyroid hormone-depleted serum showed low expression of TNFα and SDF1α, but in the presence of 10-7M T3, there was induction of expression of these two cytokines. Haptic stellate cells grown in thyroid hormone-depleted serum showed low α-SMA expression, but culturing the cells in this serum, supplemented with 10-7M T3, caused a two-fold induction of α-SMA expression. T3, in a dose responsive manner, inhibited transcription of collagen I α1 promoter in hepatic stellate cells after 7 days in culture, and less so in hepatic stellate cells after 14 days in culture, cells which have reduced expression of TRα1. Conclusion: The results suggest a pro-inflammatory effect of T3 and a role in activation of hepatic stellate cells. However, there is an anti-fibrogenic effect in early cultures of hepatic stellate cells, an effect which is less apparent in myofibroblasts, indicating that T3 may enhance appearance of liver fibrosis by increasing inflammation.

Disclosures:
1430
PRIMARY MOUSE HEPATIC STELLATE CELLS ARE LEPTIN AND ADIPONECTIN RESISTANT

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Background & aims: Obesity is a well-known risk factor for the development of NAFLD. Therefore, we studied the relation between adipocytokines and the main fibrogenic effector cells of the liver: the stellate cells. We investigated the expression of leptin and adiponectin receptor isoforms, and studied the effects of both adipocytokines on quiescent and activated mouse hepatic stellate cells (mHSC). Methods: Activated stellate cells were obtained by culturing on polystyrene in the presence of 10% fetal bovine serum. The expression of the different receptor isoforms was examined by RTQ-PCR and Western blotting. Quiescent and activated mHSC cultures were exposed for different periods of time to leptin and adiponectin. Results: Leptin receptor isoform A (Ob-Ra) was expressed at the RNA level by both quiescent and activated mHSC. The long isoform Ob-Rb and short isoforms Ob-Rd and Re were only expressed by quiescent mHSC. Ob-Rc mRNA was not present. By Western blotting, we found 100 kDa immunoreactive protein, i.e. one or more of the short receptors. The 125 kDa variant (Ob-Rb) was absent. Long-term incubation with recombinant mouse leptin did not result in any morphological changes, neither in changes of α-SMA, GFAP, SREBP-1α/c and PAI-1 gene expression. We observed only marginal phosphorylation of ERK1/2, AKT and STAT3 following short-term incubation. Adiponectin receptor isoform 1 (Adipo-R1) mRNA was expressed by quiescent and activated mHSC at approximately the same level. Adipo-R2 mRNA was low in quiescent stellate cells and decreased further during activation. Using our own antibody to Adipo-R1, the 35 kDa naked receptor, as well as the posttranslationally modified receptor of 66 kDa was recognized in transfected HEC293T cells. In hepatocytes, we found an immunoreactive band of 55 kDa; in stellate cells a band of 72 kDa. At present, we examine whether these variations in molecular weight are due to glycosylation or other posttranslational modifications. By Western blotting with a commercially available antibody to mouse Adipo-R2, we detected a clear immunoreactive band (53 kDa) in hepatocytes. In contrast, we found no significant band in mHSC, irrespective of their stage of activation. Following incubation with recombinant globular mouse adiponectin, we found no changes in overall morphology of mHSC. Neither did we find measurable phosphorylation of AMPKα. Conclusions: Quiescent and activated mHSC carry the short leptin receptor(s) and a variant form of Adipo-R1. The nature of the posttranslational modification of Adipo-R1 is currently under investigation. Neither leptin, nor globular adiponectin have measurable effects on mHSC.

Disclosures:
The following people have nothing to disclose: Ben Schroyen, Inge Mannaeerts, Leo A. van Grunsven, Albert Geerts

1431
NOTCH SIGNALING IN NODULAR REGENERATIVE HYPERPLASIA AND HEPATIC MICROCIRCULATION

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Background/Aims: Nodular regenerative hyperplasia (NRH) is a liver disease characterized by transformation of the liver parenchyma into small nodules with little to no fibrosis. The etiology of NRH is incompletely understood and probably related to vascular injury of the hepatic microcirculation. We have previously reported that knockout of Notch1 leads to NRH in mice (Croquefois et al. Hepatology 2005). The Notch signaling pathway is a signal transduction pathway which is essential for cellular proliferation, specification and differentiation. Notch signaling mediates intercellular communication through membrane-bound receptors. Notch1 regulates vascular development as well as angiogenesis and is expressed in liver sinusoidal endothelial cells. The aim of our study is to investigate the role of Notch1 in the pathogenesis of NRH. Methods: Isolated human primary liver sinusoidal endothelial cells (LSEC) were exposed to the Notch inhibitogamma-secretase inhibitor 1 (GSI, 0.1-10 uM). In vitro effects of Notch inhibition on LSEC were assessed by vascular tube formation on Matrigel, MTS proliferation assay and detection of apoptosis after nuclear staining with Hoechst 33342. In vivo proliferation rate of LSEC was assessed after BrdU incorporation on liver sections from control and conditional Notch1 knockout mice (inducible Notch1 disruption using an interferon-inducible Cre-recombinase transgene in combination with the loxP flanked Notch1 gene). Results: Inhibition of Notch signaling by GSI reduced vascular tube formation in vitro (100% DMSO vs. 32+/−31% at 1 uM GSI, n=3, p=0.018). GSI significantly inhibited proliferation in a dose-dependent manner and induced LSEC apoptosis (4.8+/−1.8% DMSO vs. 16.3+/−7.6% at 1 uM, n=4, p=0.025). Besides marked hepatocyte proliferation, Notch1 knockout mice with NRH (n=4) displayed increased BrdU incorporation in hepatic endothelial cells in comparison to controls (n=4) (54+/−13 vs. 19+/−14 positive endothelial cells/5 visual fields at 100x, p=0.011) in the absence of vascular thrombi. Conclusion: Pharmacological inhibition of Notch signaling inhibits vascular tube formation as well as proliferation and induces LSEC apoptosis in vitro. Knockout of Notch1 in mice leads to NRH and activation of hepatic angiogenesis. Further studies are ongoing to elucidate the molecular mechanisms in our model.

Disclosures:
The following people have nothing to disclose: Mona Y. Ali, Michael Dill, Luigi Terracciano, Markus H. Heim, David Semela

1432
EFFECT OF ACUTE AND CHRONIC LIVER INJURY ON HEPATOTROPIC TARGETING PROPERTIES OF ERYTHROCYTE GHOSTS

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Background: Acute and chronic liver injury are accompanied by an enlargement of the macrophage pool due to proliferation of Kupffer cells (KC) and invasion of monocytes. Multiple mediators secreted by these cell types play a prominent role in the pathogenesis of liver diseases. Vesicular carriers, e.g. liposomes or erythrocyte ghosts (EG) with encapsulated appropriate drugs might be a promising tool to modulate their paracrine influ-
ences on pathogenetic reactions, such as cell damage and fibrogenesis, if efficient and highly specific uptake is guaranteed under disease conditions. Aim: Comparison of organotrophic, in particular KC targeting properties of xenogenic (human) and isogenic (rat) erythrocyte ghosts in rats with healthy, acutely and chronically damaged livers and evaluation of their utility in hepatotropic drug targeting. Methods: EG from humans and rats were prepared by controlled hemolysis with the hypotonic dilution technique of Wood at 0°C applying agarose A size exclusion column chromatography. Hemoglobin-free EG (residual haemoglobin < 1%, MCV average 61 fl) were loaded with model drugs for detection (FITC-dextran, 3H-inulin, 3H-doxorubicin), resuspended and injected intravenously into healthy, acute with thioacetamide (TAA) or D-galactosamine (GalN) injured livers and in TAA long-term treated (1-4 months) rats. Elimination kinetics from circulation, organ distribution and liver cell-specific uptakes were analyzed. For the latter purpose, hepatocytes, KC and other non-parenchymal liver cell types were isolated by collagenase digestion. Results: Healthy rats eliminated efficiently EG [t1/2 < 2 min], about 90% was found in the liver, selective uptake by KC. Only a minor fraction was localized in the spleen (liver/spleen ratio 15.4). The elimination rate decreased with the number of ghosts injected. In acute liver damage elimination rate decreased and correlated with the severity of injury [t1/2 < 14 min], about 30% was found in the liver with an increased fraction in spleen [liver/spleen ratio 6.4 (TAA) to 1.8 (GalN)]. Strongly reduced uptake by KC. If isolated KC from these livers were exposed with EG, a reduction of phagocytosis index by about 80% was measured. In chronic injury (1, 2, 4 months) leading to fibrosis (4 months) all the parameters of ghost elimination and uptake were similar to those in healthy rats. Conclusion: In acute damage KC targeting is hampered depending on the degree of injury. In chronic injury KC targeting is feasible with EG as a well tolerated isogenic vesicle carrier.

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1433 ACTIVATION OF KUPFFER CELLS IS INVOLVED IN ALCOHOL-INDUCED DOWN-REGULATION OF HEPcidin EXPRESSION IN THE LIVER

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Hepcidin plays a key role in iron homeostasis. We have demonstrated a role for alcohol in the regulation of iron metabolism, via down-regulation of liver hepcidin mRNA and up-regulation of duodenal iron transporter protein expression. This effect was abolished by antioxidant treatment. Moreover, a more prominent down-regulation of hepcidin expression was observed by alcohol in vivo than in vitro in alcohol-metabolizing HepG2 cells. These findings suggest that the non-parenchymal cells of the liver may play a role in alcohol-mediated regulation of hepcidin expression. Kupffer cells play an important role in alcholic liver injury. Of note, hepcidin is also regulated by inflammatory signals. However, the role of Kupffer cells in inflammation-mediated regulation of hepcidin expression is controversial. Furthermore, the role of Kupffer cells in alcohol-mediated regulation of hepcidin expression is unknown. The aim of this study was to investigate the role of Kupffer cells in the regulation of hepcidin expression by alcohol in vivo. For these studies, 129/Sv male mice were treated with gadolinium chloride (GdCl3), which is known to inactivate Kupffer cells. One day after the GdCl3 injection (IP, 400 µg/mouse), the mice were exposed to 20% ethanol in the drinking water for 7 days. The gadolinium injection was repeated on days 2 and 5 of alcohol treatment. Control mice were injected with 0.9 % NaCl. Mouse hepcidin 1 gene expression was determined by real-time quantitative PCR using Taqman fluorescent probes. As we have reported previously, mice exposed to ethanol displayed a significant decrease in liver hepcidin expression compared to mice fed with plain water. GdCl3 itself did not have an effect on hepcidin expression in the liver. However, the down-regulation of hepcidin expression was abolished in ethanol-treated mice injected with gadolinium chloride compared to mice injected with 0.9 % NaCl. Conclusions: Our findings suggest that Kupffer cell activation is required for the alcohol-mediated in vivo regulation of hepcidin expression in the liver. Both parenchymal and non-parenchymal cells may play a role in the regulation of iron metabolism by alcohol. These findings enhance our understanding of the mechanisms underlying hepatic injury in alcoholic liver disease.

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1434 DIFFERENTIAL MODULATION OF PRO- AND ANTI-REGENERATIVE CYTOKINES BY TLR STIMULATION IN NON-PARENCHYMAL LIVER CELLS

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BACKGROUND: Recently, it has been suggested that the Toll-like receptor (TLR) system may play an important role in regulating liver regeneration after partial hepatectomy. To date, only little is known, however, how this effect is mediated and if the local innate immune system of the liver is involved. Therefore, we have studied the capacity of the most abundant innate immune cells of the liver, Kupffer cells (KC) and sinusoidal endothelial cells (LSEC), to produce mediators after TLR-stimulation that have pro- or anti-regenerative properties. METHODS: Isolation of murine KC was performed by counterflow elutriation while LSEC were isolated by anti-LSEC microbeads from C57BL6 mice. These freshly isolated KC and LSEC were stimulated by KC and LSEC. However, the pattern of pro- and anti-regenerative mediators varied significantly between the individual TLR ligands. Stimulation of TLR1, -2, -4, -7 and -8 induced pro-inflammatory (IL-6, TNF-α, HGF, IFN-α and IFN-β) cytokines on the transcriptional (quantitative RT-PCR) and protein level (ELISA). Supernatants from TLR-stimulated KCs and LSEC were used to stimulate freshly isolated hepatocytes. RESULTS: TLR1-9 agonists could induce the production of all mediators including HGF by KC and LSEC. However, the pattern of pro- and anti-regenerative mediators varied significantly between the individual TLR ligands. Stimulation of TLR1, -2, -4, -7 and -8 induced the expression of a pro-regenerative pattern (IL-6, TGF-α, HGF, TNF-α and anti-regenerative (IFN-α, IFN-β, TGF-β) cytokines on the transcriptional (quantitative RT-PCR) and protein level (ELISA). Supernatants from TLR-stimulated KCs and LSEC were used to stimulate freshly isolated hepatocytes. RESULTS: TLR1-9 agonists could induce the production of all mediators including HGF by KC and LSEC. However, the pattern of pro- and anti-regenerative mediators varied significantly between the individual TLR ligands. Stimulation of TLR1, -2, -4, -7 and -8 induced the expression of a pro-regenerative pattern (IL-6, TGF-α, HGF, TNF-α and IFN-β) in KC and LSEC, respectively. TLR3 ligands only were able to stimulate the production of IFN-α and IFN-β in both cell types. Stimulation of TLR5, -6 and -9 induced both pro- and anti-regenerative mediators. CONCLUSIONS: Our data show that the stimulation of non-parenchymal liver cells by TLR agonists leads to induction of mediators that are known to have pro- and anti-regenerative properties. Of note, this includes the production of HGF which was thought to be produced by stellate cells only. Therefore, the administration of TLR agonists with favourable ratios of pro- and anti-regenerative mediators before liver resection (e.g. TLR1, -2, -4, -7 and...
-8) may be used for optimizing liver regeneration postoperatively as has been previously shown for LPS (TLR4 agonist).

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1435 CAUSAL KUPFFER CELL ACTIVATION BY FREE FATTY ACIDS SUPPRESSED THROUGH PEROXISOME PROLIFERATOR-ACTIVATED RECEPTORS (PPAR) δ

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Backgrounds: Kupffer cells (KCs) play a central role in the development of non-alcoholic steatohepatitis (NASH). In terms of therapy, several options are under clinical trials, including ligands for PPAR as therapeutic agents. For establishment of the therapeutic basis, we postulated that there are mutual interactions among PPARδ, Toll-like receptor4 (TLR4) and saturated fatty acids (FA) regarding the activation of KCs. Materials and Methods: KCs isolated freshly from Balb-c mice were pre-incubated in a culture dish under the combination of or either of GW0742. PPARδ agonist, 9-cis retinoic acid (RA): retinoïd X receptor (RXR) agonist, resveratrol, 5-aminoimidazole-4-carboxamide-β-D-ribofuranoside (AICAR): AMPK activator, full length adiponectin (AD), C12, C14, C16, C18 saturated FA, cis- or trans-C18 FA for 24 hr in triplicate. After incubation with an additional supplement of S-form lipopolysaccharide (LPS) and LPS binding protein (LBP) as TLR4 ligands for 6hrs, the supernatant was measured for the measurement of concentration of TNFα and IL6 by enzyme-linked immunosorbent assay. Results: LPS activated KCs with a significant increase in the concentration of TNFα (100%; mean). GW0742 and RA suppressed the secretion of these cytokines slightly (97.5% and 94.9%, respectively). In contrast, combination of two agonists synergistically decreased the concentration of TNFα to 49.4%. AD, which alone reduced the TNFα concentration to 20.3%, did not enhance the suppression when in combination with GW0742 and RA (33.5%). C12, C14 and C16 saturated FA enhanced LPS/LBP inducible TNFα production in proportion to their carbon chain lengths (139.8%, 134.7% and 102.5%, respectively). Conversely, C18 FA suppressed it to 69.1%. PPARδ alone notably decreased TNFα production by the combination of FAs and LPS/LBP (C14: 94.9%, C16: 82.7%, C18: 53.1%), which was in contrast to the minimal suppressive effect of PPARδ ligand on TNFα secretion caused by LPS alone. IL6 secretion was affected by stimuli in the same manner as TNFα except that AD that increased the IL6 concentration to 119.5% and decreased the TNFα concentration to 20.3%. Trans-C18 FA alone, resveratrol and AICAR in combination with LPS did not appear to affect the cytokine production by KC. Conclusions: Activation of KCs was attenuated by PPARδ and RXR ligands, especially in combination. PPARδ alone suppressed the synergistic cytokine production by medium chain FA and LPS, but only minimally the part induced by LPS alone. These agonists and AD would be potent candidates as therapeutic options for NASH, along with ubiquitous expression of PPARδ and redundant free FAs in the circulation.

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1436 VARIANT ERYTHROPOIETIC PROTOPORPHYRIA (EPP): A DISORDER WITH SEVERE EPP PHENOTYPE, NO MUTATIONS IN FERROCHELATASE DNA, AND ABNORMAL MITOFERRIN EXPRESSION.

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EPP is a genetic disorder in which excess production of protoporphyrin causes photosensitivity and in some cases liver disease that may necessitate transplantation. The enzyme defect is deficient activity of ferrochelatase (FECH), a mitochondrial enzyme that catalyzes insertion of Fe into protoporphyrin to form heme. Studies from this and other laboratories showed that most symptomatic patients have a mutation in one FECH allele that causes altered enzyme structure/function, along with a polymorphism (IVS3-48c) in the non-mutant FECH allele that lowers gene expression. However, we identified 8 individuals with EPP phenotype in 6 families, who have neither FECH mutations nor the polymorphism. The gender distribution, age range, and red cell protoporphyrin levels in the patients with variant EPP were similar to those in 32 symptomatic patients with usual EPP. All had photosensitivity; 5 (63%) had liver transplantation, compared to 31% in usual EPP. This suggests that genetic abnormalities outside the FECH locus can cause severe EPP, and led us to examine the possibility of abnormal mitoferrin (MFRN) expression. MFRN was recently shown by Paw and coworkers to be the major transporter for Fe in mitochondria for heme and Fe/S formation in vertebrate erythroblasts (Nature 2006: 440; 96-100), both of which could impact FECH activity since the enzyme contains a [2Fe-2S] cluster critical for activity. Specific RT-PCR amplification/sequencing of MFRN cDNA from RNA extracted from blood leukocytes in all 8 patients revealed both a normal and abnormal cDNA species. The abnormal species, which was not detected in a group of normal individuals or patients with usual EPP, had insertion of 477 bases of intron 2 between exon 2 and 3, causing a stop codon at triplet position 156 and resulting in a truncated protein. The abnormal species did not rescue anemia in zebrafish MFRN mutants, demonstrating loss of function in the transcript/protein. Studies using cultured lymphoblasts from 5 patients with variant EPP showed that formation of the abnormal MFRN mRNA was attended by a decrease in formation of normal MFRN mRNA as measured by quantitative real time PCR, when compared to 5 normal lymphoblast lines (1473±154 versus 2039±79 copies/ug total RNA, p=0.02). There was also a significant reduction in FECH activity in variant EPP lines (9.0±3.7 versus 22.1±3.2 v, p=0.01). Conclusions: A subpopulation with severe EPP has neither mutations nor the polymorphism IVS3-48c in FECH DNA, unlike patients with usual EPP. This is associated with abnormal expression of MFRN, the gene for the major transporter of Fe in vertebrate mitochondria.

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The following people have nothing to disclose: Joseph R. Bloomer, Yongming Wang, George Shaw, Nathaniel B. Langer, Barry Paw
DONOR HEPATOCYTES HAVE A SELECTIVE REPOLAPATION ADVANTAGE OVER HOST HEPATOCYTES IN ALPHA 1-ANTITRYPSIN DEFICIENCY

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Introduction: Alpha-1-antitrypsin (AAT) deficiency is a common genetic disorder where the mutant AT-Z protein is retained in the endoplasmic reticulum of liver cells rather than secreted into the blood. In the liver, accumulation of the aggregated mutant protein produces cirrhosis and increases the risk of cancer by a gain-of-toxic function mechanism. In the lung, AAT deficiency results in premature development of pulmonary emphysema by a loss-of-function mechanism. Hepatocyte transplantation is a potentially effective therapy for liver based metabolic disorders, but expansion of donor cells appears to be necessary for maximum efficacy. In theory, increased apoptosis of native liver cells in patients with AAT should allow selective repopulation of the liver by donor hepatocytes following transplantation. We examined this issue in human AAT (hAAT) transgenic mice. Methods: hAAT transgenic mice (PiZ mouse model) were used as transplant recipients. Expression of the mutant human AAT protein in native liver cells generates liver cell injury that mimics that associated with AAT deficiency in man. Five-hundred thousand to one million hepatocytes derived from beta-galactosidase (ROSA26) transgenic mice were transplanted in the engraftment in the liver by intrasplenic injection. For some experiments, ROSA26 hepatocytes were transduced, at an MOI of 30, with a recombinant lentivirus expressing firefly luciferase. The efficiency of donor hepatocyte transduction was 30%. Engraftment and repopulation was assessed non-invasively by bioluminescence imaging, and, histologically, by lacZ staining. Results: After transplantation, the liver was progressively repopulated with donor hepatocytes, as assessed by non-invasive IVIS imaging, and two-to-three months after transplantation, approximately 20% of the native liver was replaced by ROSA26 hepatocytes, as determined, histologically, by lacZ staining. Conclusion: Hepatocyte transplantation should be effective therapy in patients with AAT deficiency that don’t have cirrhosis-induced liver failure, such as those with emphysema, because transplanted donor hepatocytes have a natural selective repopulation advantage over host hepatocytes following transplantation. Even with low transduction efficiency, non-invasive imaging is an important tool for cell transplantation that allows independent assessment of graft status, avoiding sampling error and the risks associated with biopsy.

Functional Loss of Transferrin Receptor 2 Decreases Basal Hepcidin Expression, But Does Not Abolish Regulation by Iron or Erythropoietin

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Hepcidin is a peptide hormone produced by the liver that negatively regulates dietary iron absorption and reticuloendothelial iron release. Hepcidin expression is downregulated by iron deficiency and erythropoietin, and is upregulated by inflammation. Loss of function mutations in transferrin receptor 2 (TfR2) lead to inappropriately low levels of hepcidin and iron overload, suggesting that hepatocellular TfR2 regulates hepcidin by sensing circulating iron. Aim: The aim of this study was to test the hypothesis that dietary iron or erythropoietin would effect changes in hepcidin expression in wild-type but not TfR2 mutant mice. Methods: Homozygous male TfR2 mutant (Y245X) mice and wild-type FVB mice were used. Some mice (n = 5-7) were placed on diets containing either 15, 250 or 25,000 ppm iron ad lib for 2 weeks after weaning. Additional mice (n = 5-6) were treated with erythropoietin (50 U/mouse/day s.c. for 3 days) or an equivalent volume of saline. After sacrifice, iron parameters (tissue iron concentration, transferrin saturation) were measured, and liver hepcidin mRNA expression was quantified by RNA blot analysis. Results: For each diet, hepatic iron concentrations were significantly higher and splenic iron concentrations were lower in TfR2 mutant mice compared with wild-type mice. Liver hepcidin mRNA expression was decreased by at least 75% in TfR2 mutant mice compared with wild-type mice at each level of dietary iron. However, both wild-type and TfR2 mutant mice demonstrated increased hepcidin expression in response to increased dietary iron (see Table). Treatment with erythropoietin stimulated erythropoiesis in both types of mice, as evidenced by an increase in hematocrit. Erythropoietin treatment of wild-type mice decreased hepcidin mRNA levels by 73%, and in TfR2 mutant mice, erythropoietin further suppressed the already low basal hepcidin mRNA levels. Conclusions: Loss of functional TfR2 results in a substantial decrease in basal expression of hepcidin mRNA in the liver. However, relative to basal expression, hepcidin remains responsive to changes in dietary iron levels and to erythropoietin, indicating that TfR2 is not essential for these responses.

Effect of Dietary Iron (15, 250 or 25K ppm) on Liver Hepcidin mRNA Expression in Wild-Type (wt) and TfR2 Mutant Mice

<table>
<thead>
<tr>
<th>Dietary Iron</th>
<th>wt 15 ppm</th>
<th>wt 250 ppm</th>
<th>wt 25K ppm</th>
<th>TfR2 15 ppm</th>
<th>TfR2 250 ppm</th>
<th>TfR2 25K ppm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hepcidin mRNA (vs wt 250 ppm)</td>
<td>0.4 ± 0.05</td>
<td>1 ± 0.1</td>
<td>3.9 ± 0.66</td>
<td>0.05 ± 0.02</td>
<td>0.2 ± 0.05</td>
<td>0.65 ± 0.2</td>
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*P<0.05 for 25K ppm vs 15 ppm within genotype; mean ± SEM (n = 5-7)

Disclosures:
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1439
HEPATOESPLENOMEGALY AND BLOOD PARAMETERS IN GAUCHER DISEASE - ANALYSIS OF DOSE-RESPONSE RELATIONSHIPS IN ENZYME REPLACEMENT THERAPY
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Background: Gaucher disease is based on a deficiency of glucocerebrosidase and is the most frequent lysosomal storage disease, leading to hepatosplenomegaly, pancytopenia and bone destruction. Elevated transaminases are frequent, cirrhosis occurs and hepatocellular carcinoma without preexisting cirrhosis has been described. Aims: The purpose of the present study was to analyze if enzyme replacement therapy (ERT) with imiglucerase demonstrates dose-response relationships across disease parameters in patients with type 1 Gaucher disease (GD) within the range of doses used in routine clinical practice. Methods: The analysis included all patients with type 1 GD enrolled in the International Gaucher Registry (ICGG) diagnosed after 1991, who received ERT with imiglucerase and had an intact spleen. ERT dose was defined as the average dose over the first 3 years of treatment. Propensity score matching was used to control for differences in baseline disease severity between ERT dose groups categorized as 15U/kg (5 ≤ <29 U/kg/2 wks.), 30 U/kg (29 ≤ <48 U/kg/2 wks.), 60 U/kg (48 ≤ <75 U/kg/2 wks.). Liver and spleen volumes, hemoglobin and platelet count were assessed during follow-up (0-60 months) through non-linear mixed effects models. The rate (T50) and extent (Emax) of ERT treatment effect for each parameter analyzed: liver and spleen volumes, hemoglobin, platelet count were compared across groups. Results: Propensity score matching resulted in 3 comparable groups of 122 patients each. ERT with imiglucerase at 15, 30, 60 U/kg/2 wks., respectively. Statistically significant dose-response relationships were found across groups for each parameter analyzed: liver and spleen volumes, hemoglobin, platelet count, in regard to rate and extent of improvement over 60 months. Conclusions: ERT with imiglucerase results in statistically significant dose-dependent improvement in organomegaly and hematological parameters in patients with type 1 GD. Propensity score matching and non-linear mixed effects models can be used to assess outcomes based on observational data from an international rare disease registry.

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1440
DIVERGENT PATHOGENESIS FOR HEPATOCYSTIN AND SEC63P ASSOCIATED POLYCYSTIC LIVER DISEASE?
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Polycystic liver disease (PCLD) is an inherited disorder characterized by the presence of numerous hepatic cysts. Cysts are thought to arise from malformation of the ductal plate. PCLD is associated with mutations in PRKCSH or SEC63, encoding hepatocystin and Sec63p respectively, but expression patterns in diseased and normal liver are unknown. To gain insight in the pathogenesis of PCLD, we assessed the immunohistochemical expression of hepatocystin and Sec63p in fetal liver (n=17, range 11 weeks after gestation to 15 months of age), PCLD (n=21) and normal adult liver (n=13). Cytokeratin 18 and 19 staining was used to ascertain the biliary origin of cysts. In fetal tissue, there was intense expression of hepatocystin in ductal plate, bile ducts and hepatocytes. After 26 weeks of gestation the intensity dropped to levels seen in adult liver tissue. In contrast, Sec63p staining was weak in bile ducts throughout development but prominent in early hepatocytes. In adult PCLD tissue, hepatocystin was expressed in hepatocytes, bile ducts, and in cyst epithelium of PRKCSH mutation negative patients. In contrast the majority of cysts (90%) from PRKCSH mutation carriers did not express hepatocystin. We detected an on-off mechanism: hepatocystin expression was either absent or present in all cuboid cells lining the cyst. Sec63p expression was observed in all cyst epithelia regardless of mutational state. Bile duct origin of the cyst epithelia was confirmed by cytokeratin staining patterns.

In conclusion: Hepatocystin is probably required for the development of bile ducts in fetal liver, and is lost from cyst lining in the large majority of germline PRKCSH mutation carriers. This observation corroborated with the hypothesis that cyst formation in PCLD results from a cellular recessive mechanism involving loss of hepatocystin. In contrast, Sec63p was present in all samples, regardless of mutational status, suggesting that SEC63 associated PCLD occurs via another mechanism.

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1441
EX VIVO GENE THERAPY REVISITED: COMBINATION OF LENTIVIRUS-MEDIATED GENE TRANSFER AND PREPARATIVE IRRADIATION OF HOST LIVER RESULTED IN LONG-TERM AMELIORATION OF JAUNDICE IN A RAT MODEL OF CRIGLER-NAJJAR SYNDROME TYPE 1
Jinlan Jiang, Xia Wang, Laibin Liu, Yong Chen, Chandan Guha, Namita Roy-Chowdhury, Jayanta Roy-Chowdhury; Albert Einstein College of Medicine, Bronx, NY

Background and aim: Ex vivo gene therapy for inherited metabolic liver diseases offers the advantages of not requiring livers from live or deceased donors and not needing immunosuppression. However, heretofore, the benefits of ex vivo gene therapy for liver diseases have been minimal because of the relative inefficiency of retroviral vectors to transduce primary hepatocytes and the small number of phenotypically modified hepatocytes that can be transplanted in one sitting. In the present study, we have overcome these hurdles using highly effec-
tive third generation lentiviral vectors and preparative hepatic irradiation to induce preferential proliferation of the transplanted hepatocytes. Methods: Adult UGT1A1-deficient jaundiced Gunn rats (model of Crigler-Najjar syndrome type 1) underwent 66% hepatectomy followed by hepatic irradiation (50 Gy). Primary hepatocytes were isolated by collagenase perfusion of the resected lobes. The isolated cells were incubated with a lentiviral vector expressing human UGT1A1 from a hepatocyte-specific albumin promoter (MOI = 5) at 40°C for 4 hr. After plating an aliquot for subsequent determination of gene transfer efficiency, 106 hepatocytes were transplanted back into the Gunn rat by intrasplenic injection through a flank incision. The recipients were followed for serum bilirubin levels. Recipients were sacrificed at various intervals, up to 6 months, for evaluation of liver repopulation by phenotypically corrected cells by immunohistochemical staining of liver sections, western blot of liver homogenates and determination of hepatic UGT1A1 activity. Results: Transduction efficiency of the isolated primary hepatocytes was 20-30%. After transplantation, there was progressive repopulation of the liver by engrafted hepatocytes over 8-12 weeks, at which time point 8-12% of the hepatocytes stained positive for human UGT1A1. Western blot and hepatic UGT1A1 activity exhibited commensurate increase. Serum bilirubin levels declined by 50-70% in 8 weeks and remained at that level for the 6-month observation period. Liver histology and other liver functions tests remained normal, and there were no detectable serum antibodies against human UGT1A1. Conclusions: Efficient transduction of the primary hepatocytes and preferential proliferation of the transplanted hepatocytes resulting from preparative hepatic irradiation and partial hepatectomy led to successful long-term amelioration of jaundice. Although UGT1A1 is a foreign protein to Gunn rats, there was no immune response against the transgene product, probably because its expression was limited to hepatocytes.

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1442
PULSATILE, PRO-AUTOPHAGIC THERAPY WITH RAPAMYCIN REDUCES THE INTRAHEPATIC ACCUMULATION OF ALPHA-1-ANTITRYPSIN MUTANT Z PROTEIN AND REDUCES LIVER INJURY IN AN IN VIVO MODEL
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Homozygous, PIZZ, alpha-1-antitrypsin (a1AT) deficiency occurs in 1 in 2,000 Americans, and can cause liver disease and hepatocellular carcinoma in children and adults. Studies in human tissue and model systems have shown that the a1AT mutant Z gene encodes a mutant protein which accumulates within hepatocytes leading to chronic hepatocellular death, and a low-grade hepatic regenerative response. Several proteolytic mechanisms are activated within hepatocytes to cope with the intracellular burden of the accumulated mutant protein, including autophagy. Autophagy is thought to be especially important in disposal of the unique polymerized conformation of the a1AT mutant Z protein, which forms insoluble intracellular aggregates and is thought to be especially injurious to the cell. Autophagy is a vacuolar system of intracellular disposal involved in the management of aggregated proteins, organelle recycling, cell stress, and differentiation, and which is known to be upregulated by the agent, rapamycin. Recent data from our laboratories indicates that rapamycin most efficiently upregulates autophagy in murine systems when given in weekly dose pulses, as compared to daily doses. This scheme causes a cyclical induction of autophagy through specific molecular binding mechanisms. Therefore, we evaluated the effect of rapamycin administration on PiZ mice, a well characterized model which recapitulates many features of human a1AT deficiency liver disease. As expected, daily dosing had no effect on autophagic activity, on the intracellular accumulation of a1AT mutant Z protein, or on the progression of liver injury, even when therapeutic blood levels were confirmed. Weekly dosing of rapamycin however, did increase autophagic activity, as shown by increased numbers of autophagic vacuoles and by quantitative LC3 immunoreactivity. The increased autophagy was also associated with a reduction in the intracellular accumulation of a1AT mutant Z protein in the polymerized conformation. Markers of hepatocellular injury, including cleavage of ER stress-related caspase 12, and lobular infiltration with inflammatory cells, were also decreased. This is the first report of a successful in vivo drug method for reduction of intracellular accumulation a1AT mutant Z protein and by reducing the progression of liver injury.

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ALCOHOL CONSUMPTION FURTHER MODULATES HEPATIC HEPCIDIN GENE EXPRESSION IN AN ANIMAL MODEL OF HEMOCHROMATOSIS
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Background: Co-toxicity is an important factor in determining the pathogenesis of progressive liver disease. The mRNA expression of the hepatic iron sensor peptide hepcidin, is decreased in human subjects and in animal models of HFE-related hemochromatosis and also following chronic alcohol consumption. We aimed to determine i) whether the inhibition of hepcidin expression is an early event following alcohol consumption, ii) whether alcohol further suppresses the decreased hepcidin mRNA expression seen in Hfe-/- mice and iii) whether the co-toxic effect of alcohol and iron exacerbates liver injury.

Methods: Hfe-/- knockout and C57BL/6 (wild type; WT) mice were pair-fed an alcoholic liquid diet 8 weeks. A time course experiment (1-4 weeks alcohol feeding) was also conducted in WT mice to determine whether the effect of alcohol on hepcidin gene transcription was immediate, or effective only following long term alcohol exposure. mRNA expression of hepatic hepcidin, duodenal ferroportin and DMT-1 were measured by RT-PCR. Liver injury was estimated by histopathology, ALT and hepatic lipid peroxidation. Results: Hepcidin mRNA expression was significantly decreased (p<0.003), and duodenal ferroportin and DMT-1 expression were increased (p<0.0003, p<0.0001 respectively) in alcohol-fed WT mice (n=7) following 8 weeks of alcohol feeding. Consistent with the genetic detect,
haptocorrin mRNA was decreased [2.6 fold; p<0.0001] in Hfe-/ mice (n=9) fed the control diet compared to WT and this was further suppressed [3.6 fold; p<0.0001] in alcohol-fed Hfe-/ mice. The earliest time point at which alcohol significantly decreased haptocorrin expression was 4 weeks [p<0.036]. ALT was increased in all alcohol-fed mice at 8 weeks of feeding, but this only reached significance in Hfe-/ mice [p<0.03], implying the 5 fold increased hepatic iron in these animals may have compounded the liver injury, even though lipid peroxidation was not significantly increased. Conclusion: Hepcidin inhibition due to excess alcohol consumption occurs after 2 - 4 weeks of alcohol consumption. The fact that alcohol further suppresses haptocorrin mRNA expression in Hfe-/ mice, suggests that alcohol-specific pathways, independent of iron signalling, modulate hepcidin gene expression. The co-toxic effect of moderate iron overload and alcohol is present, but subtle, at 8 weeks of chronic alcohol consumption.

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REVISITATION OF A COHORT OF 482 ITALIAN PATIENTS WITH HEREDITARY HEMOCROMATOSIS: CHANGES IN THE LAST THREE DECADES

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Background: Significant improvement in diagnosis and management of hereditary hemochromatosis (HH) has occurred in the last years. Whether this has led to changes in severity of the disease at presentation and to a different importance of genetic and environmental factors has not been assessed. The aim of this study was to revisit our series of Italian patients with HH and compare their clinical and genetic characteristics along 3 consecutive decades of surveillance (1975-84 group A, 1985-94 group B, 1995-05 group C). Patients: A cohort of 482 (369 male, 112 female), outpatients with HH diagnosed according to standard criteria, prospectively followed for 367 months (range, 1-367) in three liver units. Methods: demographie, anthropometric, clinical, biochemical and virological data were collected at diagnosis, HFE gene mutations were detected in 433 patients, 317 underwent liver biopsy. All cirrhotic patients underwent a surveillance program for early detection of hepatocellular carcinoma (HCC) with ultrasonography every 6 months. Results: A significant lower prevalence of cirrhosis (75%; 57%, 28%, p<0.001), HCC (41%, 27%, 5%, p=0.0001), HBV infection [12%, 7%, 1.3% group A and B vs C p<0.03], HCV infection [26%, 16%, 3%, group A and B vs C p=0.001], alcohol intake [g/day] (56±65, 48±50, 23±32, p=0.001) and ALT levels [61±80, 76±117, 46±38 U/l, group A and B vs C p=0.01] was observed in the last decade. Patients in the three decades had similar prevalence of C282Y homozygosity (61%) and of transferrin saturation, and increased prevalence of compound heterozygosity during the 3 decades. Significant lower serum ferritin [ 2269±1388, 2174±2095, 1217±1171 ng/ml, group A and B vs C p<0.001], and iron removed to reach depletion [16±11, 10±6, 7±10 g, p=0.001] but significant higher total cholesterol [155±43, 163±53, 189±42, mg/dl, p=0.001], and BMI [23±2.3, 23±2.8, 24±2.9, Kg/m2, p 0.001] and lower fasting glucose [119±4.5, 104±33, 98±27mg/dl, p=0.01] were observed in the last decade. A significant decrease in the prevalence of diabetes, hypogonadism and cardiac alterations but not of arthropathy was observed. Conclusion Patients with HH diagnosed during the last 10 years have less severe liver disease likely for an improved medical attention to this disease and for a better prevention of environmental risk factors. The higher prevalence of metabolic alterations in patients diagnosed more recently reflects findings in general population and implies modification of HH clinical presentation.

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UPREGULATION OF FERROPORTIN AND DMT1 EXPRESSION IN CIRRHOTIC HUMAN LIVERS: A LINK TO HEMOSIDEROSIS?

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Iron deposition unrelated to hereditary hemochromatosis (hemosiderosis) occurs commonly in cirrhotic livers and has been associated with more advanced disease and accelerated decompensation, but the pathophysiology of this condition is poorly understood. The aim of this study was to compare the expression of proteins involved in the regulation of iron metabolism in control and cirrhotic human livers to gain insight into the possible causes of hemosiderosis. Methods: Cirrhotic human livers (n=23) were collected at liver transplantation, donor livers (n=6) were obtained from a tissue bank, and normal liver tissue was obtained at the time of resection of liver tumors (n=5). Hepatic levels of mRNA for DMT1 (all isoforms), ferritin (H chain), ferroportin, hemosudulin, hepcidin, transferrin, transferrin receptor (TFRC) and transferrin receptor 2 (TFR2) were quantified by real-time PCR. Expression of DMT1 isoforms was assessed by semiquantitative RT-PCR. Western analysis was performed for ferroportin, DMT1, TRFC and ferritin. Hepatic iron concentrations (HIC) were measured using a spectrophotometric method. Results: Levels of ferroportin and DMT1 mRNA were significantly higher in cirrhotic livers than in normals (p=0.031 and 0.008, respectively). Levels of ferroportin and DMT1 transcripts were strongly correlated in cirrhotic livers (r=0.75; p<0.001), but neither correlated with hepcidin mRNA or with HIC. Both normal and cirrhotic livers expressed the 1B, 1E and non-IRE isoforms of DMT1, but the 1A isoform was detected in only a few cirrhotic livers; however, there was no relationship apparent between expression of the isoforms and HIC in the cirrhotic livers. Hepcidin transcripts were significantly higher in the donor livers than in the cirrhotics (p=0.003); nonetheless, hepcidin mRNA correlated significantly with HIC in the latter group (r=0.57; p=0.005) but not the former. No differences in mRNA levels for ferritin, hemosudulin, TFRC or TFR2 were seen. By Western blot, neither DMT1 nor ferroportin varied with HIC in the cirrhotic livers, while ferroportin was dramatically reduced in donor livers. In contrast, TRFC decreased while ferritin increased with increasing HIC in the cirrhotic livers. Conclusions: Despite apparently normal regulation of hep-
cidin expression, DMT1 and ferroportin are increased at both the level of mRNA and protein in cirrhotic liver. Similar findings have been reported in the duodenum of cirrhotics, suggesting these alterations may play a role in aberrant regulation of iron metabolism in cirrhosis. Donor livers show evidence of an acute phase response with elevated hepcidin mRNA and decreased ferroportin.

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13C-METHACETIN BREATHE TEST: A NEW TOOL TO PREDICT THE PRESENCE OF LIVER VASCULAR MALFORMATIONS IN PATIENTS WITH HEREDITARY HAEMORRHAGIC TELEANGIECTASIA

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Hereditary Hemorrhagic Teleangiectasia (HHT) is a systemic disease with arteriovenous malformations (AVMs) due to ENG or ALK1 gene mutations. The liver is involved in about 70% of patients; the diagnostic gold standard is multislice computed tomography (MSCT). Breath tests based on 13C-methacetin (MBT) and aminopyrine (ABT) have been used to explore liver functions; the former permits the evaluation of hepatic blood flow as this drug has a high hepatic extraction ratio altered by vascular shunts in the liver, while the latter is an indirect measurement of hepatocellular function. The aim of our study was to evaluate the effect of AVMs on results of MBT and ABT in HHT patients and the accuracy of MBT in depicting AVMs compared to MSCT. Methods: forty-five patients (22 males; mean age 52 ± 15 years) with HHT were enrolled along with 45 healthy controls (19 male; mean age 52 ± 15 years). Thirty patients with liver cirrhosis were excluded from the study. All subjects performed MSCT to evaluate HHT liver involvement. MBT (39 pts) and ABT (16 pts) were performed as follows: expired air samples were collected before administration of 75 mg of 13C-methacetin or 2 mg/Kg of 13C-aminopyrine. Breath samples were collected at 15-minute intervals for 2 hours. The 13C enrichment was measured by an isotope ratio mass spectrometer. The results were expressed as cumulative percentage of 13C recovered in breath after 2 hours (CPD2). Statistical analysis was performed with Student’s T test and ROC curve analysis. Results: MBT: The CPD2 of healthy controls and patients was 39.05% ± 5.1 and 24.1% ± 8.8, respectively (p=0.00001). Among HHT patients, MSCT indicated vascular shunts (arterio- systemic and/or arterio-portal) in 34 cases (75%). The CPD2 in controls was significantly higher than that in HHT patients with (22.5% ± 7.3 [p<0.00001]) and without (28.4% ± 6.2) p <0.05 liver involvement. The CPD2 in HHT patients with AVMs was lower than that in those without AVMs (p=0.05). Patients with a CPD2 >22% showed a 93.8% positive predictive value (PPV) to show AVMs at MSCT (specificity 90%). Regarding ABT, no difference in CPD2 values were found between controls (15.7% ± 3.1), HHT patients (16.2% ± 2.7), HHT patients with (15% ± 1.8) and without AMs (18.3% ± 3.2). Conclusions: MBT showed a high positive predictive value to identify hepatic shunts in HHT patients and could be proposed as a screening tool to select patients to be studied with hCT. By contrast, ABT, which is an indirect index of hepatocellular function, is not altered in HHT patients. Further studies are needed to correlate the MBT with alteration of the first pass effect in the liver of the HHT patients.

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HEPATIC COPPER, IRON AND ZINC CONCENTRATIONS IN A NEAR HISTOLOGICAL RESOLUTION IN WILSON DISEASE DETERMINED BY MICROSCOPIC SYNCHROTRON RADIATION X-RAY FLUORESCENCE

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Hepatic copper (Cu) is only indirectly stainable in histological slices. Therefore a fivefold increase of hepatic Cu is considered as the best available test for diagnosis of hepatic Wilson disease (WD). However Cu values from single biopsy specimen may be misleading and can not quantify regional differences. The aim of this study was to establish a method for Cu quantification (along with zinc (Zn) and iron (Fe)) using microscopic synchrotron radiation X-ray fluorescence technique (µ-SR XR). Methods: Paraffin embedded liver tissue from 7 explanted livers of WD patients and of normal liver tissue obtained at post-mortem examination of 7 subjects dying from myocardial infarction was investigated. µ-SR XR measurements were performed at beam line L of DORIS III storage ring at HASYLAB/DESY (Hamburg, Germany). A monochromatic X-ray beam was focused by a polycapillary half lens to a cross section of 15 µm for two dimensional scanning of liver slices. Position of measurements was controlled by a long-distance light microscope with a CCD camera for controlling areas of interest in comparison with histochemically prepared slices. A total of 72,000 spectra were analyzed using the AXIL program package. For quantification a Ge standard foil before and after each measurement was used as external standard. Results: As expected, Cu was about 10-fold increased in WD (263±190 ppm/g [±SD]; normal: 23; ± 5; p<0.001). Cu measured by flame atomic ab- sorption spectroscopy was 1176±737 µg/g dry weight (normal: - 50 µg/g). Cu was 7 times higher in hepatocytes (562±136) than in portal tracts (82±21). Within cirrhotic nodules copper was inhomogeneously distributed. Zn was lower in WD (59±18 vs. 89±19; p<0.05), and iron was increased (246±132 vs. 152±54; p<0.05). Fe in portal tracts was higher than in hepatocytes. Additionally, it turned out that associations between Cu, Zn and Fe were different for portal tracts and hepatocytes. Conclusion: µ-SR XR analysis is a valuable tool to measure simultaneously Cu and other metal elements in WD such as e.g. Fe and Zn. This technique (continuous scanning mode) allows a near light microscopic resolution (20x) of hepatic metal distribution. The decrease of hepatic zinc and the increase of hepatic iron content in WD reflect the interplay of copper with these metals.

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Background - AAV serotypes 2 (AAV2) and 8 (AAV8) are widely used for hepatic gene transfer applications in animal models. Due to the single-stranded nature of AAV, gene targeting to correct mutations through homologous recombination (HR) has proven successful in vitro and in vivo. It is superior to episomal gene expression for site specificity and stability. Aims - To examine time course and dose responses for both serotypes to examine time course and dose responses for both serotypes

To show that AAV2 and AAV8-mediated HR can correct the metabolic liver disease hereditary tyrosinemia 1 (HT1) in vivo.

- To show that AAV2 and AAV8-mediated HR can correct the episomal gene expression for site specificity and stability.

Aims - To examine time course and dose responses for both serotypes as well as the integration stability after induction of hepatocyte (HC) turnover. Methods - We designed a vector carrying 4520 nucleotides homologous to the mouse fumarylacetoacetate hydrolase (Fah) genomic sequence with a central point mutation leading to a premature stop codon (pAAV-4k). AAV2 and AAV8 were intravenously injected into Fah5961SB mice, a model for HT1. Targeted recombination events were quantified by Fah immunohistochemistry. Results - Neonatal AAV8-4k administration resulted in faster and higher gene targeting efficiency compared to AAV2. Up to 0.3% Fah positive (Fah+) HC could be detected as early as 3 days after AAV8-4k injection. With AAV2-4k Fah+ HC could only be detected after one week at a lower frequency of about 20 / 100,000 HC. These were stable up to 4 weeks without selection. Doses from 3x108 to 2x1011 of each serotype were neonatally injected and liver tissue analyzed after 2 (AAV8) or 3 weeks (AAV2) without selection. For AAV8, 95 Fah+ HC / 100,000 HC were counted at the lowest dose (< 10 / 100,000 at the highest dose). For AAV2, 40 – 50 Fah+ cell clusters / 100,000 HC could be identified at concentrations < 4x1010. The administration of lower doses resulted in lower gene targeting efficiency. Neonatal injection of AAV2-4k, similar to AAV8-4k, led to HT1 correction after selecting for Fah+ HC for >4 weeks as measured by weight gain. After partial hepatectomy the mice exhibited clinical recovery similar to untreated controls. Recipient Fah5961SB mice undergoing serial transplantation with > 300,000 HC from clinically restored mice show Fah+ HC engraftment after 5-6 weeks of selection. This is indicative of stable HR. Conclusions - Liver directed AAV-mediated HR is feasible and stable with AAV2 and AAV8. AAV8 leads to earlier HR events than AAV2. Recombination frequencies are higher at lower vector doses. The metabolic function in a model for HT1 can be fully and stably restored by both serotypes. Studies to enhance the rate of gene targeting are currently under investigation.

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BENCHMARK ANALYSIS OF THE ACHIEVEMENT OF THERAPEUTIC GOALS FOR PATIENTS WITH TYPE 1 GAUCHER DISEASE

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Background: Gaucher disease, the most frequent lysosomal storage disease, is based on a deficiency of glucocerebrosidase and leads to hepatosplenomegaly, pancytopenia and disabling bone disease. Hepatic complications are elevated transaminases, cirrhosis and hepatocellular carcinoma. Enzyme therapy with intravenous infusions of macrophage-targeted recombinant glucocerebrosidase (imiglucerase) is the standard therapy with intravenous infusions of macrophage-targeted recombinant glucocerebrosidase (imiglucerase) is the standard of care for patients with symptomatic GD. Treatment requires an individualized approach to therapy and disease management. Aims: To report the frequency with which the therapeutic goals for several key clinical aspects of type 1 GD are met 4 years after starting imiglucerase. Methods: The data source was the (ICCG) Gaucher Registry, with currently more than 4.700 patients enrolled. Included were patients with type 1 GD on imiglucerase therapy for minimum 4 years, with data available at minimum 4 ± 1 years for 6 parameters: hemoglobin, platelets, liver and spleen volume, bone pain and bone crises. Goals were: Hb > 11 g/dl (for males: 12 g/dl); platelets 120.000/µl or ≥ 2x baseline value in those with baseline platelets < 60.000/µl; liver volume < 1.5 x normal, spleen volume < 8.0 x normal; bone crises absent and bone pain mild to absent. Patients were compared for the total number of therapeutic goals met of the 6 parameters. The response groups were compared by genotype group. The 6 parameters were also compared by the proportion of patients achieving the relevant therapeutic goal for each parameter. Results: 337 of a potential 1,473 patients met the inclusion criteria. Overall, 37.4% (126) had achieved all 6 therapeutic goals at the 4-year observation point. 72.1% (243) achieved at least 5 goals; 89.3% (301) achieved at least 4 goals; 98.2% (331) achieved at least 3 goals; 99.4% (335) achieved at least 2 goals. All 337 patients achieved at least one therapeutic goal. The achievement of goals varied among organ systems with 98.8% (bone crises), 91.0% (haemoglobin level), 90.0% (liver volume), 74.8% (bone pain), 76.7% (platelet count) and 69.4% (spleen volume) achieving the goal. Genotype at the glucocerebrosidase locus was not a significant determinant of treatment goal attainment. Conclusions: The therapeutic goals for anemia, thrombocytopenia, hepatomegaly, splenomegaly, bone pain and crises in type 1 GD are variably attained within the predicted intervals. The variability is not related to genotype. Definition of clearly defined clinical goals is a prerequisite of successful therapy and benchmark analysis may be used as a tool to assess whether a structured disease management approach improves outcomes in patients with type 1 GD.

FUNCTIONAL ANALYSIS OF ATP7B VARIANTS AS AN AID TO DIAGNOSIS OF WILSON DISEASE

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We are testing the function of mutations in ATP7B, the defective gene in Wilson disease, a disorder of copper transport and accumulation. Age of onset of symptoms varies from 3 to almost 70 years, and diagnosis for this treatable disorder is challenging. Mutation analysis is a feasible and effective adjunct to clinical and biochemical features for diagnosis. We have documented all reported mutations in ATP7B in the Wilson Disease Database, that includes more than 500 variants (probable disease-causing and the remainder possible normal variants) from populations worldwide (www.medicalgenetics.med.ualberta.ca/wilson/index.php). Of 380 mutations in the database, 199 are missense, 114 insertions and deletions, 34 nonsense and 33 splice site mutations. An important aspect for diagnosis is discrimination between disease-causing variants and rare normal variants, highlighting the need for functional studies, carried out to date on only 26 of 199 missense variants. ATP7B has two major functions: first, the intracellular transport of copper to the Golgi for incorporation into proteins that require copper for normal function including ceruloplasmin; second, elimination of copper from the liver. This function requires the trafficking of ATP7B in copper-loaded vesicles to the plasma membrane of hepatocytes, to allow export of excess copper into bile. To test for the functional defects in potential ATP7B mutants, we exploit a highly conserved copper transport pathway in yeast. Human ATP7B can replace the yeast homologue, Ccc2p, and allows for normal copper transport in ccc2p yeast strains. Copper is then available for incorporation into Fet3p (ceruloplasmin homologue) that imports iron into yeast. Normal transport function is assessed by growth on low iron media. Variants that do not support normal copper transport function, do not grow on low iron media, and are considered ATP7B mutations. Variants that display a specific trafficking defect, and are tested in mammalian copper cytotoxicity and trafficking assays, using CHO cells stably transfected with ATP7B or variants. 3D-protein modeling allows an assessment of the potential impact of amino acid changes upon ATP7B protein structure and function. We studied ten additional mutants in ATP7B in potential ATP7B mutants, we exploit a highly conserved copper transport pathway in yeast. Human ATP7B can replace the yeast homologue, Ccc2p, and allows for normal copper transport in ccc2p yeast strains. Copper is then available for incorporation into Fet3p (ceruloplasmin homologue) that imports iron into yeast. Normal transport function is assessed by growth on low iron media. Variants that do not support normal copper transport function, do not grow on low iron media, and are considered ATP7B mutations. Variants that display a specific trafficking defect, and are tested in mammalian copper cytotoxicity and trafficking assays, using CHO cells stably transfected with ATP7B or variants. 3D-protein modeling allows an assessment of the potential impact of amino acid changes upon ATP7B protein structure and function. We studied ten additional mutants in ATP7B in potential ATP7B mutants, we exploit a highly conserved copper transport pathway in yeast. Human ATP7B can replace the yeast homologue, Ccc2p, and allows for normal copper transport in ccc2p yeast strains. Copper is then available for incorporation into Fet3p (ceruloplasmin homologue) that imports iron into yeast. Normal transport function is assessed by growth on low iron media. Variants that do not support normal copper transport function, do not grow on low iron media, and are considered ATP7B mutations. Variants that display a specific trafficking defect, and are tested in mammalian copper cytotoxicity and trafficking assays, using CHO cells stably transfected with ATP7B or variants. 3D-protein modeling allows an assessment of the potential impact of amino acid changes upon ATP7B protein structure and function.

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Details of the intracellular site at which replication of HEV occurs is unknown. This study attempts to explore the intracellular site of HEV replication by localizing HEV Replicase in transfected HepG2 cells. Methods: In-frame fusion construct of replicase-enhanced green fluorescent protein (EGFP) gene was made in eukaryotic expression vector. Stable transfected cells expressing Replicase-EGFP fusion protein were generated. The functionality of this protein was determined by its ability to synthesize negative sense HEV RNA. Positive sense HEV transcript was used as a template to demonstrate synthesis of negative sense HEV RNA in the replicase-EGFP expressing stable transfected cells, by strand specific anchored RT-PCR. It was also confirmed with the use of anti-sense strand specific molecular beacon. Sequence analysis and structure prediction of HEV replicase identified possible membrane binding helices. Subcellular co-localization was investigated with several organelle specific fluorescent protein expressing plasmids and fluorophores using confocal microscope. Transfection of alexa 548 UTP labeled 3’ end of HEV RNA in Replicase-EGFP expressing stable transfected cells, was used to detect the in vivo interaction between Replicase and the 3’ cis-acting elements by Fluorescence Resonance Energy Transfer (FRET) analysis. Deletion constructs of predicted membrane binding helices of Replicase, fused with EGFP were used for their role in subcellular localization analysis. immuno-electron microscopy was used to further confirm localization of HEV replicase. Results: The results show that EGFP fused Replicase was functionally active and could synthesize negative sense HEV RNA as demonstrated by strand specific anchored RT-PCR and binding to anti sense specific sense beacon emitting the Fluorescence. HEV replicase was found to localize on the membrane of endoplasmic reticulum (ER) and loss of localization was observed when the region with predicted membrane binding domain at the carboxy terminal end of replicase was deleted. FRET analysis showed subcellular interaction of Replicase with the 3’ cis-acting elements on HEV RNA in live cells. Conclusions: Based on these observations it is believed that in Hepatitis E virus, endoplasmic reticulum is the site of Replicase localization and possible replication as in many other positive sense RNA genome containing Viruses.

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2005-2006. The patient population consisted of Asian/Pacific Islanders (26.1%), Blacks (12.7%), Hispanics (42.3%), Native Americans (0.2%), and Whites (7.0%). 69.6% were foreign born. Among the 18,457 patients, 42.4% (7,826) were screened for HBsAg. 1026 (71.8%) of 1429 persons with elevated ALT were screened for HBsAg compared to 5826 (49.6%) of 11749 with normal ALTs, p<0.01. The screening rate was greatest for those 19-24 years of age (47.7%) and 25-29 years of age (48.5%), decreased among age group 30-34 (44.4%), 35-39 (41.3%), 40-44 (37.0%) and least among the eldest group, 45-49 (34.4%), p for trend<0.01. Women were slightly more likely to be tested than men (43.4% vs. 41.3%, p<0.01). A higher rate of APIs were screened (65.6%) than Blacks (38.8%), Hispanics (33.4%), Whites (32.1%) and Native Americans (28.3%), p<0.01. Among patients born in regions with high rates of HBV infection (>8%), those born in East Asia countries had the highest screening rate (692/1088, 63.6%) followed by Southeast Asia (153/304, 50.3%), and Africa (230/521, 44.1%). Patients born in regions of intermediate HBV endemicity (2.7%) including Eastern Europe, South Asia, Middle East, Central America, South America, and the Caribbean had screening rates of 35.5-40.1%, comparable to rates for patients born in the U.S. (39.5%). The screening rates varied greatly among individual clinics, highest (1040/1810, 57.5%) at community clinics which served large numbers of APIs and lowest (25/212, 11.8%) at a community clinic which served large numbers of Hispanics. This pilot study revealed that screening for HBsAg was correlated with elevated ALT, younger age, Asian race and region of birth with HBV epidemic. However, nearly 50% of patients from countries where HBV infection is endemic, and for whom screening is recommended by the CDC, had not been tested. More rigorous efforts will be necessary to increase compliance for HBV screening in this group of patients in order to decrease long-term morbidity and mortality.

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1455 INSULIN RESISTANCE AND SEVERITY OF FIBROSIS IN HCV GENOTYPE 1 CHRONIC HEPATITIS
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BACKGROUND AND AIMs Patients with HCV G1 chronic hepatitis often have insulin resistance (IR), either due to metabolic causes and associated to visceral obesity or linked to virus-induced phenomena and non-metabolic. IR is central in the pathogenesis of steatosis, but contrasting data exist on its direct role in the progression of fibrosis. We aimed to assess whether the presence and degree of IR are linked to the stage of liver fibrosis in chronic hepatitis C genotype 1 patients. Methods Two hundred and one consecutive patients (11.04±12.06) with HCV G1 chronic hepatitis, all naive to antiviral therapy and abstinent from alcohol, were evaluated by biopsy and by anthropometric and metabolic measurements including IR assessed by the homeostasis model assessment (HOMA) score. Non-diabetic patients were defined as insulin resistant if HOMA-IR>2.7. A single pathologist graded all liver biopsies (>15 mm) by Scheuer’s score. Steatosis was graded as absent (<5% of hepatocytes) or present. Results Twenty-nine patients were diabetic (Group 1), 76 insulin resistant, non diabetic (Group 2), and 96 non diabetic, non insulin resistant (Group 3). Mean waist circumference was comparable among the three groups (Group 1, 96.5±14.6 cm; Group 2, 94.2±11.7 cm; Group 3, 90.8±10.6 cm; p=0.08), while a significant difference was found in the mean age (Group 1, 60.2±6 yrs; Group 2, 54±11 yrs; Group 3, 49±13 yrs; p=0.0003). Steatosis was present in 62% of patients in group 1, 63% in group 2 and 34% in group 3 (p=0.0006), and severe necroinflammatory activity (>1 by Scheuer) in 90%, 75% and 65% (p=0.034). Severe fibrosis (>2 by Scheuer) was found in 59%, 30% and 15% of patients (p=0.0001) in group 1, 2 and 3. At multivariate analysis severe fibrosis was independently linked to the degree of IR (OR 2.692; 95%CI 1.463-4.954), to a high necroinflammatory activity (OR 2.944; 95%CI 1.422-6.098), to platelet counts <140.000 mln (OR 7.170 95%CI 3.000-16.700), to low cholesterol (OR 0.987; 95%CI 0.976-0.998) and to high ferritin (OR 1.002; 95%CI 1.000-1.003). Conclusions In subjects infected by HCV genotype 1, a state of metabolic insulin resistance, regardless of the presence of clinically overt diabetes, and a high necroinflammatory activity are strongly linked to advanced fibrosis. Severity of IR may thus be used in nonfibrotic or mildly fibrotic HCV G1 patients as a predictor of a high risk of progression into more severe stages of fibrosis.

Disclosures:
The following people have nothing to disclose: Salvatore Petta, Calogero Cammà, Vito Di Marco, Nicola Alessi, Francesco Barbaria, Daniela Cabibi, Rosalia Caldarella, Giuseppe Tarantino, Francesco Vitale, Antonio Craxi

1456 INTERLEUKIN 12B GENE POLYMORPHISM AND APPARENT RESISTANCE TO HCV INFECTION
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Background: Identifying mechanisms of protection from hepatitis C virus (HCV) infection remains a key research goal. We have identified a cohort of cases at very high risk of HCV infection through injection drug use but who remain both HCV antibody and HCV RNA negative. We have termed these ‘exposed uninfected’ (EU) cases. Interleukin 12 (IL-12) is a key cytokine that promotes anti-viral T helper 1 (Th1) responses. A functional IL-12p40 polymorphism has been associated with spontaneous clearance of HCV infection and we hypothesised that a genetic background able to promote cellular responses may be associated with apparent protection from infection. Methods: We identified 76 EU cases from long-term injection drug users who tested both HCV antibody and HCV RNA negative despite reported risk behaviour. The IL-12 p40 polymorphism was studied using restriction fragment length genotyping and findings compared to 105 healthy controls. Results: The high IL-12 producing ‘C’ allele was found in 27.6% of exposed uninfected cases compared to 16.7% of healthy controls (χ² = 6.3, p = 0.01, RR= 1.7). Conclusions: Individuals at high risk of HCV infection yet who remain uninfected may be resistant to infection. In our cohort of exposed uninfected cases a genetic background of enhanced IL-12 production was associated with apparent resistance to HCV infection. This lends support to a central role for cellular immune responses in protecting from infection.

Disclosures:
GBV-C CO-INFECTION IN HIV PATIENTS IS ASSOCIATED WITH LOW CCR5 AND CXCR4 SURFACE EXPRESSION ON CD4+ CELLS

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Background: The flavivirus GBV-C, not known to cause any disease, has been associated with delayed progression of HIV disease. Recently, in vitro studies demonstrated down-regulation of CCR5 as a potential mechanism of GBV-C to modulate HIV disease. We therefore studied surface expression of the two major HIV co-receptors, CCR5 and CXCR4, on CD4+/CD8+ T-cells in 128 HIV patients stratified with respect to GBV-C status and immune function. Methods: GBV-C infection was studied in 128 HIV patients by RT-PCR. CCR5Δ32 mutation was analyzed by real-time PCR. FACS analysis was used to measure CCR5 and CXCR4 surface expression on CD4+/CD8+ T-cells. Results: GBV-C RNA replication was detected in 30% (38/128) of patients. Fourteen patients were excluded from the analysis because of reduced CCR5 surface expression due to a heterozygous CCR5Δ32 mutation. In the remaining HIV/GBV-C co-infected patients with CD4 <200/µl CCR5 and CXCR4 expression on CD4+ T-cells was significantly reduced to 77% and 80%, respectively, of levels measured in HIV mono-infected patients (p<0.05). In contrast, HIV-monoinfected and HIV/GBV-C co-infected patients did not differ with respect to CCR5 and CXCR4 expression on CD4+ T-cells in the subgroup with preserved immune function (CD4>200/µl) and on CD8+ T cells independent of immune function. Conclusions: GBV-C co-infection is common in HIV infected patients and is associated with reduced expression of both major HIV co-receptors on CD4+ T-cells in the subset of HIV patients with advanced immunodeficiency. Therefore, the molecular and cellular mechanisms underlying down-regulation of HIV co-receptors in GBV-C co-infection merit further investigation.

Disclosures: The following people have nothing to disclose: Carolynne Schwarze-Zander, Markus Neibecker, Sabrina Othman, Carolin Welzel, Monika Schulz, Esther Voigt, Martin Vogel, Jan-Christian Wasmuth, Guido Luechters, Tilman Sauерbruch, Juergen Rockstroh, Ulrich Spengler.
23 patients IP-10 values were significantly lower than in patients without anti-HCV activity in their plasma (250.1 pg/ml and 651.3 pg/ml respectively, p<0.01). This is of importance, since recent studies identify a low pretreatment IP-10 as predictive of sustained viral response to Peg-IFNα-2a/Ribavirin combination therapy. CONCLUSIONS: The HCV-replicon based bioassay allows us to identify host-derived anti-HCV activity in heparinized plasma of CHC patients prior to treatment. This could be of great importance in a better understanding of the host response to antiviral treatment. A next step will be to investigate plasma samples of these patients during treatment.

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1460
DIFFERENTIAL ROLE OF LIVER-TARGETED REGULATORY T CELL TO HEPATITIS B AND C VIRUS IN CHRONICALLY INFECTED PATIENTS
Hisamitsu Miyaoaki, Tatsuki Ichikawa, Kazuhiko Nakao, Hidetaka Shibata, Motohisa Akiyama, Satoshi Miuma, Yasuhide Motoyoshi, Masumi Fujimoto, Katsumi Eguchi; The First Department of Internal Medicine, Nagasaki University School of Medicine, Nagasaki, Japan

Regulatory T cells play a critical role in chronic virus infection. The role of regulatory T cell in chronic hepatitis B and chronic hepatitis C is unknown. We examined the distribution and frequency of Foxp3 positive regulatory T cell in the liver tissues and compared the clinicopathological characters of HBV and HCV. Liver needle biopsies were collected from 26 patients with hepatitis B surface antigen positive (mean age:33.6±10.4, male:female=23:3) and 27 patients with hepatitis C virus antibody positive (mean age:57.8±10.6, male:female=15:12). In each liver tissues, T cells were examined with anti-CD3 antibody (Novocastra, Newcastle, UK) and regulatory T cells were examined with anti-Foxp3 antibody (eBioscience, San Diego, CA, USA). The average proportions of Foxp3+ Tregs among the total number of CD3+ T cells in each portal tracts was determined. The ratio of Foxp3 in CD3 was similar in HBV and in HCV cases (HBV: HCV = 0.98±0.04:0.90±0.05). In HBV cases, there was significant correlation between the ratio of Foxp3 in CD3 and serum ALT level (P=0.025, r=0.402). The ratio of Foxp3 in CD3 was significantly increase in severe activity group than in mild activity group (mild:severe=0.08±0.03:0.11±0.04, P=0.04). There were no significant differences in the ratio of Foxp3 in CD3 in HBV cases among the other clinical factors (age, gender, platelet, fibrosis, HBVDNA viral load). In HCV cases, the variables that were significantly associated with the ratio of Foxp3 in CD3 were:

- Gender: Male (male:female=0.07±0.03:0.11±0.05, P=0.002)
- Serotype 1 (serotype1:serotype2=0.07±0.04:0.15±0.05, P=0.0002)
- Age (male:female=0.07±0.10.03:0.11±0.05, P=0.04)

These factors were easy sensitivity factors for interferon therapy. There were no significant differences in the ratio of Foxp3 in CD3 in HCV cases among the other clinical factors (age, ALT level, platelet, activity, fibrosis, HCV viral load). In conclusions, these data provided the impaired of regulatory T cell function cause acute exacerbation in chronic hepatitis B. On the other hand, regulatory T cell may be related with action of interferon in chronic hepatitis C.

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1461
SUCCESSFUL USE OF HEPATITIS C POSITIVE KIDNEYS FOR TRANSPLANTATION: CAUTION ON THE USE OF OLDER DONORS
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Recent data is scarce regarding the use of HCV+ donor allografts in HCV+ kidney recipients. Review of the literature demonstrates there is benefit to proceeding with transplant. However, outcome analysis suggests patient and graft survival to be lower in this population. The natural history of HCV and liver disease progression is unclear. HCV+ donors account for 5% of all donors in the United States. A few small studies suggest outcomes to be similar to those HCV+ recipients receiving HCV- organs. There is little or no data on the selection criteria of HCV+ donors. The present study reports our experience and provides suggestions for donor selection. Patients & methods: Since 1996, 41 HCV+ patients underwent deceased donor kidney transplant with an HCV+ donor organ. Only well-compensated patients with genotype 1 and a measurable viral load were offered these organs. Two patients were excluded due to graft loss within the first week, leaving 39 patients to be evaluated for longterm (3 year) follow-up. Mean age of the recipients was 49 years, with the majority being male (79%) and African-American (85%). HTN was present in 97% and DM in 28%. Mean time of cold ischemia was 18 hours. Perioperative biopsy of the donor kidney was available in 29 patients. Standard, triple drug immunosuppression was used without induction. Statistical analyses were performed using SAS 9.1.3. Results: The overall incidence of acute rejection during the study period was 11/100 person year. Patient and graft survival at 1 year was 100% and 94.6% and at 3 years, 84.4% and 79.3%, respectively. This is comparable to our overall patient and graft survival. Outcome analysis was performed in recipients who received a donor organ < 49 years as compared to older donors. Overall incidence of graft loss and death was higher in the older donor group. While not powered to be statistically significant, the trend is clear. In donors ≥49 years, the overall incidence of graft loss and death was 27% and 18% as compared to 11% and 7% in younger donors. Biopsy review in donors > 49 years found 4/6 (67%) to have >15% fibrosis as compared to 2/16 (12.5%) in younger donors. Conclusion: Use of HCV+ kidney donors remains a viable option for HCV+ recipients but requires careful donor selection. We advocate that HCV+ donors > 49 years should not be considered for transplant as the kidneys seem to have worse outcomes and more advanced fibrosis. Furthermore, HCV+ recipients are frequently transplanted with short waiting times obviating the need to use older donors.

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The following people have nothing to disclose: Mary T. Killackey, Kyle Fargen, Tareq Islam, Anil Paramesh, Rubin Zhang, A Brent Alper, Shobha N. Joshi, Fredric G. Regenstein, Douglas Slakey, Sander Florman

1462
BILE DUCT DILATION IN HEPATITIS PATIENTS ON CHRONIC METHADONE THERAPY
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Methadone maintenance therapy is commonly used in the treatment of intravenous drug users (IVDUs). Zybelberg et al. showed that methadone may lead to dilated common bile duct...
(CBD). We tried to assess this hypothesis in a large group of asymptomatic hepatitis B (HBV) and/or hepatitis C (HCV), ex-IVDU patients on chronic methadone therapy with age- and gender-matched control group of patients with HBV and/or HCV of similar severity by using abdominal imaging tests. Methods: Charts of all adult patients with HBV and/or HCV with and without methadone between January 2002 and 2007 at GI and ID clinics at our center were reviewed. The data collected were age, gender, size of CBD (with ultrasound and MRI), cirrhosis, AST, ALT, ALP, bilirubin (Tbi), dose and duration of methadone. Patients with history of pancreatitis, cholelithiasis and cholecystectomy were excluded from the study. Data were analyzed using t-test, chi-square test and Fisher’s exact test. Results: There were a total of 215 HCV and/or HBV patients (70% males, mean age 47.3 ± 7.3 years) in the methadone study group and 108 patients (71.3% males, mean age 49.6 ± 8.7 years) in the control group. The size of CBD in the study group was statistically significantly more dilated than the control group (5.87 mm vs. 3.79 mm, p<0.0001). Dilated CBD (CBD ≥ 8mm) were seen in 26.1% and 2.78% of study and control group, respectively (p < 0.0001). Normal size bile duct were seen in 55.3% and 89.8% of the study and control group, respectively (p <0.0001). AST, ALP, Tbi, cirrhosis and history of alcohol were significantly higher in the study group than the control group.

Conclusion: Chronic methadone therapy may cause dilated CBD in this population. Patients with history of pancreatitis, cholelithiasis and cholecystectomy were excluded from the study. The data collected were age, gender, size of CBD (with ultrasound and MRI), cirrhosis, AST, ALT, ALP, bilirubin (Tbi), dose and duration of methadone. Patients with history of pancreatitis, cholelithiasis and cholecystectomy were excluded from the study. Data were analyzed using t-test, chi-square test and Fisher’s exact test. Results: There were a total of 215 HCV and/or HBV patients (70% males, mean age 47.3 ± 7.3 years) in the methadone study group and 108 patients (71.3% males, mean age 49.6 ± 8.7 years) in the control group. The size of CBD in the study group was statistically significantly more dilated than the control group (5.87 mm vs. 3.79 mm, p<0.0001). Dilated CBD (CBD ≥ 8mm) were seen in 26.1% and 2.78% of study and control group, respectively (p < 0.0001). Normal size bile duct were seen in 55.3% and 89.8% of the study and control group, respectively (p <0.0001). AST, ALP, Tbi, cirrhosis and history of alcohol were significantly higher in the study group than the control group.

Conclusion: Chronic methadone therapy may cause dilated CBD. Dilated CBD was statistically significantly associated with the duration of methadone (p<0.01), but not the dose of methadone (p =0.83). On average, the patients with dilated CBD were on methadone for 33 months versus 23 months in the normal size CBD patients. Multivariate logistic regression of the data showed that patients on chronic methadone are 17.5 times more likely to develop dilated CBD (Odds Ratio=17.5). Conclusion: Chronic methadone therapy may cause dilated CBD. Dilated CBD was significantly correlated with the duration of methadone. Greater awareness is needed of the potential association between bile duct dilation and chronic use of methadone. Therapeutic intervention of biliary dilation in this specific group of patients may be unnecessary.

Disclosures: The following people have nothing to disclose: Trinh Meyer, Denis Kapkov, Michael Lipp, Douglas Meyer, Henry C. Bodenheimer, Albert Min

1463
PRESENCE OF HEPATIC PROGENITOR CELLS IN HUMAN CHRONIC LIVER DISEASES AND RELATION TO HEPATOCELLULAR CARCINOMA DEVELOPMENT

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Aim: This study assesses the potential role of hepatic progenitor cells (HPC) presence and relation to hepatocellular carcinoma (HCC) future development in patients with chronic liver disease (CLD). Methods: The study included 52 liver biopsies obtained from 52 patients with CLD assigned in 2 groups (26 each). Group A: chronic hepatitis B (HBV-A1-n=9), chronic hepatitis C (HCV-A2-n=5), genetic hemochromatosis (GH-A3-n=5), and alcoholic liver disease (ALD-A4-n=7). These 26 patients developed HCC (confirmed by liver biopsy) in a 1-5 (median 3) year period after the time of the initial biopsy. None of these 26 patients had HCC at the first time. Group B: 26 biopsies: HBV (B1-n=9), HCV (B2-n=3), GH (B3-n=5) and ALD (B4-n=7).

Patients of group B did not develop HCC at the same time period. Liver biopsies were matched for inflammation grade and fibrosis stage: subgroup A1 to B1, A2-B2, A3-B3, A4-B4. Paraffin sections were subjected to a) immunohistochemistry using antibodies for CK19, LCA, CD34 and b) in-situ hybridization for alpha fetoprotein mRNA detection. Cells with morphologic features of HPC (Roskams T et al Hepatology 39:1739, 2004) that were CK19+/AFP+ and LCA(-)/CD34(-) were scored. In addition, we performed gene analysis for AFP mRNA, on dissected tissue samples removed from liver sections that included areas with HPC expression, as this was described above. Results: The table lists the results for CK19 and AFP mRNA expression. Similar results were obtained from AFP gene analysis. The numbers of HCC were directly correlated with i) inflammation degree (p<0.01) and ii) fibrosis stage (p<0.01).

Conclusions: This study shows that HPC are frequently present in CLD cases and their numbers are directly related to the severity of the disease. The fact that biopsies of group A (cases that developed HCC in the future) showed significantly higher HPC numbers, supports the hypothesis that HPC proliferation is associated with increased risk for hepatocellular carcinoma. Further studies are needed to determine if the increased expression of HPC in CLD cases predicts the risk for future development of HCC in such cases.

<table>
<thead>
<tr>
<th></th>
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<td>57.3±4.6</td>
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<tr>
<td>B1</td>
<td>33.5±1.6</td>
<td>25.8±2.4</td>
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<tr>
<td>HCV A2</td>
<td>57.5±2.3</td>
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</tr>
</tbody>
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* p<0.01; ** p<0.001; *** p<0.0001

Disclosures: The following people have nothing to disclose: Athanassios C. Tsamandas, Konstantinos Thomopoulos, Dimitra Dimitropoulou, Ioulia Syrrokosta, Dimitrios Siagris, Theodore Petsas, Chrisoula Karatza, Charalambos Gogos

1464
WHAT MOTIVATES PRIMARY CARE PROVIDERS TO TEST FOR HCV?

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Background: HCV testing is uncommon in primary care; risk factor ascertainment is even less frequent (Trooskin, 2007). Little is known about why this behavior exists and whether HCV testing is regarded as a priority in primary care. The aims of this study were to explore motivations for HCV testing in primary care, to survey the perceived priority of HCV testing, and to identify practice characteristics associated with the likelihood of testing. Methods: A survey was mailed to 2,365 primary care providers in the Philadelphia area. The survey ascertained provider and practice characteristics, HCV risk assessment and testing strategies, and the perceived importance of HCV risk assessment and testing. Results: A total of 1,219 surveys were returned (58% response). The mean age of respondents was 50 years, 73% were male
and 83% were non-Hispanic White. Fifty-one percent were interns; 67% were in private practice, 59% were in a suburban area. Ninety-six percent reported routinely asking about drug use and 58% reported routinely asking about blood transfusions. Abnormal ALTs were cited as the most common reason for HCV testing (83%), followed by patient symptoms (60%), positive HCV antibody after blood donation (56%), physician-ascertained HCV risk factor (54%), and patient-requested testing (48%). Providers with predominately Black/Hispanic patient populations were more likely than providers with predominantly White populations to report patient risk factors as a motivation for HCV testing [p<.05]. The likelihood of HCV testing based on patient risk factors and elevated ALTs increased with the number of patients with a history of drug use seen by the provider [p<.05]. Seventy-six percent of the providers reported that risk factors for HCV should be ascertained routinely, and HCV testing should be offered to those at risk, while 13% reported that HCV testing should be offered to all patients.

Conclusion: Although providers recognize the importance of identifying HCV risk factors and risk factor based testing, our data show that most testing is not motivated by physician identified risk factors. Rather, abnormal ALTs serve as the most common motivation for testing. The chasm between providers' perceived importance of HCV risk factor ascertainment and testing and their reported testing strategies may, in part, be attributable to differing practice characteristics. Testing based on lab abnormalities is insensitive and may fail to identify infected persons. To increase detection of patients with HCV, risk factor ascertainment must be integrated into standard practice, irrespective of practice characteristics.

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1465
CD4-CD8- T CELLS CONTRIBUTE TO THE PERSISTENCE OF MHV-3 INDUCED CHRONIC VIRAL HEPATITIS
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Objective: To study the contribution of T cell subsets to the pathogenesis in MHV-3 induced chronic viral hepatitis in C3H/Hej mouse. Methods: In this work, ninety C3H/Hej mice individually received 10 plaque forming units (PFU) of MHV-3 intraperitoneally. The ratios of T cell subsets including CD3+CD4+CD8-, CD3+CD4-CD8+, CD3+CD4-CD8- (DNT cells), CD3+CD4+CD25+, CD3+CD4+CD25-, CD3+CD4-CD25+, CD3+CD4-CD25+ T lymphocytes in total T lymphocytes in blood, spleen and liver were examined at 0,2,4,6,8,10,12,15,20,25,30,40 days post MHV-3 infection by flow cytosorting. Magnetic bead sorting was applied for the purification of T cell subsets. The cytotoxic effects of DNT cells and CD4+CD25-T cells on both CD8+T cells and hepatocytes were examined by CytoTox96 Non-Radioactive Cytotoxicity Assay at 0, 4, 15, 30, 40 days post MHV-3 infection. Results: The DNT cell and CD4+CD25-T cell ratios raised significantly since 2 days post MHV-3 infection in C3H/Hej mice, and CD3+CD4+CD8-, CD3+CD4+CD8-, CD3+CD4-CD25-, and CD3+CD4-CD25- T cell ratios decreased accordingly. DNT cells showed significant cytotoxic effects on CD8+T cells with a cytotoxic rate of 97.00%±0.01%, while CD4+CD25+T cells only had slight cytotoxic effects on it with a cytotoxic rate of 51.27%±0.01%. Conclusions: Here we first report that DNT cells, rather than CD4+CD25-T cells, have more profound immune modulatory effect on CD8+T cells in MHV-3 induced chronic viral hepatitis in C3H/Hej mice, suggesting their contribution to viral persistence. Further characterizations of DNT cells are under studying. This study was supported by NSFC 3057164, 30672380 and National Key Basic Research Program of China (2005CB522901).

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1466
COMPLETE GENOME SEQUENCES AND PHYLLOGENETIC ANALYSIS OF HEPATITIS D VIRUSES (HDV) ISOLATED FROM TURKISH PATIENTS WITH CHRONIC HDV INFECTION
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Hepatitis Delta virus (HDV) infection is a global health problem with more than 10 million infected people. Phylogenetic relatedness and different genotypes of HDV which vary with respect to geographical distribution may affect the overall clinical outcome of the disease. In this study, we aimed to find out the complete nucleotide sequence of HDV genomes isolated from 5 naive Turkish patients and to perform phylogenetic analysis of these genomes. Five naive HDV-infected Turkish patients (3 male, 2 female; median 52 years old) were included in the study. Twelve pairs of primers were used in order to amplify six overlapping fragments covering the whole HDV genome by RT-nested PCR. The overlapping PCR products were then sequenced using ABI PRISM 310 Genetic Analyzer and the complete HDV genome sequences were determined. Phylogenetic analysis was performed using MEGA 3.1 software by UPGMA (Unweighted Pair Group Method with Arithmetic mean). The reference sequences obtained from GenBank database are from France, USA, China, Japan, Peru, Iran, Lebanon, Italy and 3 sequences from Taiwan. Results of direct sequencing revealed that all 5 HDV genomes were genotype 1 and consisted of 1676 nucleotides encoding the hepatitis delta antigen (HDAg) of 214 amino acid in length. Based on phylogenetic tree, all Turkish patients were dispersed through the same branch with 2 Taiwanese, French, Italian, Chinese, and Iranian sequences with a bootstrap value of 99%. One of the Taiwanese sequences was closest to the Turkish sequences. The American, Japanese, and Peruvian sequences were phylogenetically far away from Turkish sequences with a 68% bootstrap value. Data analysis confirmed genetic heterogeneity of the hepatitis delta virus.

Disclosures:
Filibiz Emir, Mehmet Bektas, Ela Kesen, Ramazan Ildimon, A.Mithat Bozdaiy, Cihan Yurdadaydin

1467
QUANTITATIVE DETECTION OF INTEGRATED HBV SURFACE GENE SEQUENCES IN THE LIVER BY REAL-TIME PCR
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Introduction: Chronic hepatitis B (CHB) patients under long-term nucleos(t)ide analogue treatment may achieve profound...
HBV suppression with non-detectable serum HBV DNA and low intrahepatic cccDNA levels. Continuing surface antigen expression observed in these patients could be attributed to transcribed integrated HBV DNA harbouring intact surface antigen (S) gene sequences. **Aim:** To develop a quantitative method for the detection of integrated HBV DNA harbouring S sequences in the liver of CHB patients under suppressed HBV replication and to evaluate its performance in liver biopsy samples of patients under long-term antiviral therapy. **Methods:** Liver biopsy specimens from 20 CHB patients in profound HBV suppression under nucleos(t)ide analogue treatment were tested for integrated HBV S gene sequences using specifically designed real-time PCR assays that simultaneously measure intrahepatic HBV DNA in the S and the core promoter-precore (CP-preC) regions. HBV integration occurs predominantly in the CP-preC region of the viral genome leaving S sequences sequences frequently intact. Cloned and serum HBV DNA were used as controls. Intrahepatic HBV S gene expression was also measured. HBsAg and HBeAg expression was also assessed in the same biopsy samples by immunohistochemical methods. **Results:** Low HBV cccDNA levels were found in all patients, median 0.31 (range 0.05-0.84) copies/cell. In fact, cccDNA was the predominant form of viral DNA representing on the average 79% of total intrahepatic HBV DNA. These conditions permitted quantitation of low copy integrated HBV sequences. Total intrahepatic HBV DNA levels measured in the S region were significantly higher than in the CP-preC region, 0.70 (0.10-2.65) vs 0.38 (0.05-1.74). In 11/20 patients, as well as in cloned and serum HBV DNA controls, there was no discrepancy between the two measurements. In 9/20 patients S levels were significantly higher (2-fold to 13-fold) than the corresponding CP-preC measurements (0.71 vs 0.20). In these patients, integrated HBV S sequences were detectable in significant levels, 0.51 (0.17-1.4) cp/cell. Consistent with these findings were the results of histochemical demonstration of HBsAg proteins in the same liver samples. **Conclusions:** 1. We have developed a method for quantitative detection of integrated HBV DNA harbouring S gene sequences in liver biopsy samples. 2. This method can only be used in situations of highly suppressed viral replication permitting detection of low copy integrated S sequences. 3. Integrated HBV S sequences were detectable in approximately 50% of treated patients with compatible findings in immunohistochemical observations of HBsAg expression in the liver.

**Disclosures:**
The following people have nothing to disclose: Andreas Laras, Aggeliki Kostamena, Stephanos J. Hadziyannis

### 1469 CHRONIC LIVER DISEASE AMONG ALASKA NATIVE PEOPLE, 2003-2004

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**Purpose:** The proportion of deaths attributable to chronic liver disease (CLD) is four times higher among American Indian (AI)/Alaska Native (AN) people compared to other racial/ethnic groups in the United States, but the epidemiology in this population is not well described. We report the findings of a cross-sectional study of CLD prevalence and its etiologies among AN and AI people. Methods: The study population included AN and AI people ≥18 years old in the Anchorage area who had ≥1 inpatient or outpatient visit at the Alaska Native Medical Center (ANMC) from 2003-2004. ANMC provides primary and specialty care to eligible AI and AN people. CLD was defined as having either: 1) an ICD-9 code indicating CLD; or 2) ≥2 elevated alanine aminotransferase (ALT>40IU/L), aspartate aminotransferase (AST>40IU/L), or gamma-glutamyl transpeptidase (GGT>50IU/L) levels ≥3 months apart or 3) both. CLD etiologies were assigned using ICD-9 codes and clin-
ical test results. Persons could have more than one etiology. Results: The prevalence of CLD was 7.3% (1903/26,166). Among those with CLD, 88% were AN people, 51% were female, 80% were urban residents, and the mean age was 46 years. The most common etiologies were alcohol-related liver disease (42%), non-alcoholic fatty liver disease (NAFLD) (29%), chronic hepatitis C virus (HCV) infection (26%) and chronic hepatitis B virus (HBV) infection (8%). 11% had both alcohol-related liver disease and chronic HCV infection. Males compared to females had a higher prevalence of CLD (82.7 vs. 65.3 per 1000; p=0.001), alcohol-related liver disease (36.4 vs. 24.9 per 1000; p=0.001), chronic HCV infection (21.0 vs. 17.3 per 1000; p=0.03) and NAFLD (21.4 vs. 20.9 per 1000; p=0.01). The death rate from CLD was higher among females than males (2.7 vs. 1.5 per 1000; p=0.05), with females twice as likely to die from alcohol-related liver disease (2.3 vs. 1.1 per 1000; p=0.02). Conclusions: The prevalence of CLD was 7.3% among AI and AN Anchorage area residents receiving care at ANMC. Alcohol-related liver disease, NAFLD and chronic HCV infections were the leading causes. Females were less likely than males to have CLD, but were more likely to die from it. Previous reports have shown females to be more susceptible to alcohol-related liver disease than males. The frequency of complications from HCV and HBV may rise as the infected population ages, but treatments for viral hepatitis available at ANMC may reduce disease burden. Increasing rates of diabetes and obesity in AN people may result in a higher burden of CLD due to NAFLD in the future.

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The following people have nothing to disclose: Gayle Fischer, Stephanie R. Bialek, Chriiss Homan, Stephen Livingston, Brian J. McMahon

1470
TEN YEAR FOLLOW-UP OF 126 CONSECUTIVE ITALIAN PATIENTS WITH HEPATITIS DELTA (HDV)-RELATED COMPENSATED CIRRHOSIS
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Background: Data have been accumulating, showing that chronic infection with HDV is less aggressive than previously reported, often leading to slowly evolving almost inactive compensated cirrhosis. Aim: to give more insights into the evolutionary course of patients with HDV-related compensated cirrhosis. Patients: 126 consecutive patients with HDV compensated liver cirrhosis, diagnosed between 1985 and 2005, were followed for a mean of 121 months (range 10-240). There were 100 males, mean age was 42 yrs (range 23-73); 93 patients (74%) were Child-Pugh A and 33 were Child-Pugh B. 23 (18%) had HCV, 7 (5.5%) had HIV, alcohol intake was > 80 gr in 11 (9%), 12 (9%) had HBsAg and 33 (26%) had IgM anti-Hbc. All patients were either HDV-RNA serum positive or displayed HDV-Ag in the liver on immunofluorescence. All patients were under surveillance with clinical exams and abdominal ultrasound every 6 months. Results: During follow-up, HCC developed in 37 (29%), ascites in 25 (20%), jaundice in 19 (15%), variceal bleeding in 4 (3%) and encephalopathy in 1 (1%), corresponding to a mean yearly incidence of 3.2%, 2.1%, 1.6%, 0.3% and 0.1%, respectively. HCC was a single node in 19 (51%) with a mean diameter of 25mm. During follow-up, 14 (11%) patients progressed from Child-Pugh class A to B and 9 (7%) from Child B to C. 36 patients (28%) died, corresponding to a yearly mortality rate of 2.4%. Causes of death were liver failure in 22 (61%), HIV infection in 4 (11%), HCC in 2 (5%), variceal bleeding in 2 (5%), extrathoracic malignancies in 3 (8%), non liver related causes in 3 (8%). Conclusion:

Hepatitis Delta is a long-lasting compensated disease, whose major cause of death is liver decompensation.

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The following people have nothing to disclose: Raffaella Romeo, Angelo Sangiovanni, Ersilio Del Ninno, Massimo Colombo

1471
INFLUENCE OF LIVER FIBROSIS ON HIGHLY ACTIVE ANTIRETROVIRAL THERAPY-ASSOCIATED EPATOTOXICITY IN HIV POSITIVE PATIENTS
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Background -The effects of hepatic fibrosis on HAART-associated hepatotoxicity are not well understood due to the fact that it is hard to perform liver biopsy to all patients. Transient elastography is a non-invasive method for assessing liver fibrosis by measuring liver stiffness. The aim of this study was to evaluate the relationship between liver fibrosis and the rate of HAART-associated liver toxicity. Methods: Prospective cohort study of 353 consecutive HIV+ patients who underwent Liver Stiffness Measurement between January-December 2006. Liver fibrosis was staged using a scoring system of 0 (no fibrosis) to 4 (cirrhosis): < 7.1 KPa for F0/F1; 7.1 to 9.4 KPa for F2; 9.5 to 12.5 KPa for F3 and >12.5 KPa for F4. Hepatotoxicity was defined as an increase in AST/ALT levels over 5 times the upper limit of normal (ULN), or 5 fold increase if baseline levels were abnormal, with no other known precipitating cause and occurring in the last 3 years. The incidence of hepatotoxicity was compared with liver fibrosis stage and HAART duration plus HAART regimen. Backward stepwise logistic regression was employed for statistical analysis. Results: In the study population mean age was 44 yrs; 262/353 (74%) patients were male, 158/ 353 (45%) IDU, 191/353 (54%) HCV+, 22/353 (6,2)% HBV+ and 151/353 (42,8%) HIV + alone. 81/353 (22,9%) patients showed advanced liver fibrosis (>9,5 KPa). Significant hepatic fibrosis was strongly associated with HCV positivity (p <0.001) and significantly associated with AST level (p 0.010), IDV risk (p 0.23), GGT level (p 0.23). 7/353 (1,9%) patients with HIV+ alone and without risk factor for liver disease showed advanced liver fibrosis (all patients were MSM, with lower CD4 count [p ns], elevated ALT [p 0.01], without metabolic complications). There was no association between liver fibrosis and HAART regimen or cumulative period of ARV. Overall, there were 26 episodes (7,4%) of hepatotoxicity degree 3-4. All the events were asymptomatic, and no therapy was changed. There was no association between liver toxicity and age, sex, risk practice, CD4+ count, or HIV RNA level and a specific drug or regimen of HAART. A significant association was observed with liver fibrosis (p <0.041) and ALT level (p <0.001). Conclusions: In HIV infected patients, stage of liver fibrosis could be the most important factor for the risk of hepatotoxicity: patient with advanced liver fibrosis should be monitored more closely during HAART.

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1472 FIBROSIS PROGRESSION RATE AND PORTAL PRESSURE IN RELATION TO CD4 CELL COUNT IN HIV-HCV COINFECTION

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In the HAART era HCV-related liver disease is a leading cause of morbidity & mortality in HIV infection. Reasons for the higher HCV-RNA load and the more rapid fibrosis progression rate (FPR) in HIV-HCV-coinfected patients are not completely clear, so our aim was to identify risk factors for this accelerated course of liver disease. Methods: We analyzed data of 33 HIV-HCV-coinfected patients. All had liver biopsy, indirect measurement of portal pressure (hepatovenous pressure gradient=HVPG) and routine laboratory tests (including CD4 cell count, HIV & HCV viral load). IVD abuse was the main transmission route, so the estimated time of infection was recorded as the date of initial exposure (IVD injection). Results: Median age was 38 years, median BMI was 20.8 kg/m² and median CD4 count was 545/µL. HCV viral load was undetectable in 17 patients, 16 had a median HCV viral load of 3.98 log/mL. HCV-HIV patients had accelerated FPR (0.176 METAVIR fibrosis units/year [FU/y]), accelerated time to cirrhosis (TTC=25.7 years), high HCV viral loads (3.38x10⁶ IU/ml) and a mean HVPG at the upper limit of normal (5mmHg). In case of advanced immunodeficiency (previous CD4 cell nadir <200/µL) the FPR was even higher compared to patients with a CD4 cell nadir >200/µL (0.231 vs 0.140 FU/y). A highly significant indirect correlation between CD4 cell count and FPR (r=0.662; p<0.001; see figure) could be demonstrated. 19 patients received HAART, but HAART itself had no influence on FPR and HVPG. Conclusion: Progression of HCV disease is accelerated in HIV-HCV-coinfection, which is even more pronounced when CD4 cell count is low. A history of a CD4 cell nadir <200 µL is a risk factor for rapid development of cirrhosis. Thus, HCV treatment should be considered early in HIV-HCV-coinfection, when CD4 cell counts are high. Before initiation of HCV therapy, HAART can probably slow down liver disease progression by prevention of CD4 cell nadir values <200/µL.

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1473 CLINICAL AND LABORATORY CHARACTERISTICS OF PREGNANT WOMEN CHRONICALLY INFECTED WITH HEPATITIS B VIRUS (HBV)

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Most states in the U.S. mandate HBsAg testing of all pregnant women during prenatal care in order to prevent mother-infant transmission. The clinical and laboratory characteristics of hepatitis B virus (HBV) infection in these women are largely unknown. In this study, we reviewed the records of all HBV-infected women who received care in Prenatal Clinics at Bellevue Hospital and Gouverneur Healthcare Services in NYC between 2004 and 2006. Analysis was conducted retrospectively, through electronic medical record review of de-identified data, after IRB-approval. Out of 7354 pregnant women tested for HBV, 352 (4.8%) were HBsAg+. The median age of HBV-infected women was 27.1±5.3 years with a median age of 26.0 years. 82.1% were Asian/Pacific Islanders (APIs). 336 (95.5%) was tested for ALT, 318 (90.3%) for HBeAg, and 187 (53.1%) for HBV DNA. Women with elevated ALT were more likely to be tested for viral load than those with normal ALT (87.5% vs. 53.4%, p<0.01) as were women who were HBeAg+ compared to those who were HBeAg- (65.3% vs. 47.4%, p<0.01). 39.0% (124/318, 95% CI: 33.7, 44.4) of the women were HBeAg+, and 4.8% (16/336, 95% CI: 2.8, 7.4) had elevated ALT (>2 x ULN). ALT levels ranged from 5 to 207, with a mean of 27 IU/mL. The median viral load was 1.0 X 10⁴ copies / ml and mean viral load 1.8 X 10⁸ copies / ml. The median viral load was 1.0 X 10⁴ copies / ml and mean viral load 1.8 X 10⁸ copies / ml. HBeAg was detected in 37.9% (124/318, 95% CI: 33.7, 44.4) of the women who had a viral load measurement. 29 (35.8%, 95% CI: 26.0, 46.5) had a viral load > 5.5 X10⁸ copies/ml and at least 11 (13.6%) had a viral load > 10⁹ copies/ml. In conclusion, among HBsAg+ pregnant women in this study, 80% had active infection, almost 50% were at risk of significant future complications of chronic HBV infection, and as many as 13% may have had viral loads >10⁹ copies/ml, associated with a higher risk of neonatal HBV prophylaxis failure. This analysis should be valuable for estimating the burden of HBV-related disease and projecting the cost of care among pregnant women with chronic HBV infection. Recommendations for the evaluation and care of women identified by prenatal HBV screening would be of great value for this vulnerable population as the diagnosis of hepatitis B during pregnancy is an opportune time to provide proper evaluation of risk for future HBV-related complications and the need for HBV-specific treatment.

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1474 BARRIERS TO VIRAL HEPATITIS TREATMENT FOR AT-RISK GROUPS: IMPACTS OF POLICIES AND PROCESSES

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Background: Hepatitis B and C viruses infect an estimated 4.5 million Americans. We know that much morbidity and mortality can be prevented through awareness, preventive behaviors, and treatment. Unfortunately, population groups most impacted
by hepatitis C (racial and ethnic minorities, people with drug, alcohol, and incarceration histories) are typically least able to negotiate the policy and healthcare systems in order to receive optimum care. This project examined barriers to hepatitis C prevention, diagnosis, and especially treatment for at-risk population groups in the Philadelphia, PA region. Methods: Methods of data collection included key informant interviews, extensive literature review, and focus group and individual interviews. Target groups included current or former substance users, currently or formerly incarcerated individuals, MSM, HIV infected adults, and African American, Hispanic, and Asian men and women ages 18-40. Analysis was iterative and inductive. We generated general themes or categories of barriers, identified consensus in the three sources, examined the context of the barriers for our target populations, and identified potential means to overcome barriers. Results: Three barrier categories were identified: Structural Barriers, Social/Community Barriers, and Individual Barriers. Barriers from one category frequently overlapped, exacerbated or created barriers within other categories. We present actions or changes that could be implemented within medical communities, government agencies, insurance providers, and community agencies to remove or alleviate barriers to hepatitis care. Conclusions: Most barriers can be addressed through policy and procedure changes. Others can be addressed through carefully designed awareness and education programs for medical providers, social service agency personnel, hepatitis patients and the general public.

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1475 ANTI-HBV THERAPY CAN REDUCE HEPATITIS DELTA REPlication IN HIV-coinfected Patients
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Background: HDV has a unique replication process using the host cellular polymerase. For this reason together with its high pathogenic potential, chronic hepatitis Delta (CHD) is very challenging. No specific inhibitor of HDV has so far been developed, and treatment is currently limited to intensive interferon-based therapy. However, the role of potent nucleos(t)ide analogues against chronic HDV infection has not been well examined. Methods: A longitudinal study was carried out in all HIV-positive patients with CHD whom attended our hospital. Serum HBV DNA for each patient was longitudinally determined using Cobas HBV Taqman PCR (Roche, LDL 12 IU/ml), and serum HDV RNA was quantified using an in-house real time PCR assay (LLD, 100 cop/ml) at yearly intervals. Treatment regimens, plasma HIV and when pertinent HCV RNA, CD4 counts, ALT/AST levels and liver fibrosis measured by FibroScan® were also assessed. All statistical analyses were made using SPSS v12. Results: A total of 18 HIV-positive patients with CHD were identified, the majority male (83%). Median age was 33 years and baseline ALT, HIV RNA, HBV DNA, and CD4 were 98 IU/ml, 1.69 log10 copies/ml, 1.19 log10 IU/ml, and 344 cells/ml, respectively. Median follow-up was 6 years. All had detectable serum HDV RNA before receiving any anti-HBV treatment (median: 6.5 log10 cop/ml). A statistical significant correlation between HBV and HDV viral load was found (r=0.259; p=0.005). Anti-HBV drugs used were lamivudine (n=17), tenofovir (n=11) and/or emtricitabine (n=5). Overall, 11 patients showed good response to anti-HBV therapy achieving undetectable HBV DNA for a minimum of 2 years. Moreover, all of them showed a statistically significant decrease in plasma HDV RNA (mean: 1.44 log10 cop/ml, p=0.038) and ALT levels (mean: 45 IU/ml, p=0.022). In fact, 3 of these patients (1 received lamivudine and 2 lamivudine + tenofovir) achieved undetectable HDV RNA (<100 cop/ml) after a mean of 5.6 years of HBV antiviral therapy. Conclusion: Patients undergoing successful anti-HBV therapy with potent nucleoside analogs seem to have an indirect benefit for suppressing HDV replication, albeit not very efficient. Hypothetically, a significant and sustained reduction in serum HDV RNA may only be seen when a reduction in HBV cccDNA or HBsAg is achieved, which may require long periods of successful anti-HBV therapy. To our knowledge, this is the first clear evidence of benefit of potent anti-HBV nucleos(t)ide analogue therapy in CHD.

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1476 ACUTE HEPATITIS E: RESULTS OF A FRENCH SURVEY
Christophe Renou1, Xavier Moreau2, Jean-François Cadranel3, Elisabeth Nicand4, Thierry Morin5, Yves Botreau6, Marc Bourniére2, François Combet2, Yves Imbert2, Frédéric Heluaert10, Dominique Louvel1, Valérie Oulès4, Alexandre Païenti12, Jean-Louis Payen13, Eric Poncin14, Hervé Rifflet15, Isabelle Rosa19, Valérie Rossi17, Nicole Pavio18, 1CH d’Hyères, Hyères, France; 2Polyclinique Malartic, Ollioules, France; 3CH de Creil, Creil, France; 4Val de Grâce, Paris, France; 5CH de Tarbes, Tarbes, France; 6CH de Cahors, Cahors, France; 7Hôpital Saint-Joseph, Marseille, France; 8CH de Privas, Privas, France; 9CH d’Agen, Agen, France; 10CH de la Région d’Annecy, Annecy, France; 11CH de Cayenne, Cayenne, France; 12CH de Pau, Pau, France; 13CH de Montauban, Montauban, France; 14CH de Dax, Dax, France; 15CH d’Ajaccio, Ajaccio, France; 16CH de Créteil, Créteil, France; 17CH du Haut Anjou, Chateau-Gontier, France; 18Ecole Nationale Vétérinaire d’Alfort, Maisons-Alfort, France

The incidence of sporadic hepatitis E has been increasing in France, as in other industrialized countries, during the last few years. Several studies have suggested the autochthonous nature of many recent cases of acute hepatitis E, although the source of contamination and the transmission pathways involved have not yet been determined. The aim of this French survey was 1) to draw up an overall picture of recent hepatitis E cases at the participating hospitals since January 2004, 2) to determine whether or not the majority of these cases of hepatitis E are autochthonous and to describe their characteristics, 3) to attempt to determine the sources of contamination possibly responsible for autochthonous cases of hepatitis E. The response rate to an e-mail sent in October 2006 to all French General Hospitals was 34% (65/190). Fourteen of the 65 hospitals contacted reported a total number of 33 cases (10, 8, 3, 2, 1 cases in 1, 1, 1, 1, 10 hospitals, respectively). A North-South (5 cases/27 cases) and a West-East (11 cases/21 cases) gradient were found to exist (plus 1 case in French Guiana). However, the former gradient resulted from a small-scale outbreak in a single department in the South of France (18 cases occurred in the department of the Var). The number of cases increased during the 3 years of retrospective recruitment (3 cases in 2004, 10 cases in 2005, 20 cases in 2006). The presence of HEV was diagnosed by elimination, in addition to elevated ALT levels above 300 IU/ml associated with a strong
reactivity for HEV-specific antibodies with or without detectable HEV-RNA (in the serum and/or stools). Mean age of the 33 patients (23 men and 10 women) was 53±15 years. The first clinical symptom was jaundice (19 cases), either alone or associated with fever, flu-like symptoms and vomiting. The diagnosis was based on the serological results in 29 cases, and on both serological and virological tests in the other four cases. Thirty-one patients had no history of travel outside Europe or any contacts or relationships with persons who had been in an endemic country 3 months before the first symptoms occurred, whereas two patients had travelled to Algeria and Pakistan during this period. Among the group of 31 patients who had not been out of Europe, the most relevant and frequent risk factors were direct or indirect consumption of water from a personal well or a nearby river (13 cases) and eating sea-food (7 cases). This survey confirms the recent increase in the incidence of autochthonous acute hepatitis E in France, for which the consumption of contaminated food or water might be mainly responsible.

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1477
HEPATITIS D (HDV) AND B (HBV) VIRUSES GENOTYPES IN CHRONIC LIVER DISEASE PATIENTS FROM EASTERN AMAZON BASIN

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HDV is a defective hepatotropic virus which infectivity is dependent on HBV. HDV super or co infection is a major health risk in chronic or acute HBV infected patients due to the increased risk of fulminant hepatitis or progression to severe chronic liver disease. In some regions of South America, such as Western Amazon Basin, HDV infection have been associated with hepatitis outbreaks of particularly high morbidity and mortality. HDV is classified in 7 major clades (HDV1 to HDV7) with strong phylogenetic support. The aim of this study was to characterize HBV and HDV genotypes harbored by chronic carriers from the Eastern Amazon Basin. We studied 17 samples from HBV and HDV chronic patients from the Eastern Amazon basin, Brazil, admitted to a large public hospital (Santa Casa de Misericórdia) at Belém, Pará, Brazil, between 1994 and 2002. HDVRNA and HBVDNA were detected by polymerase chain reaction (PCR). For HDV, the partial delta antigen genomic region (403 nucleotide fragment) and for HBV, a 416 nucleotide fragment covering a conserved region of the surface antigen coding gene, were amplified. Both strands of the second PCR products were submitted to cycle sequencing with the ABI Prism BigDye terminator cycle sequencing ready reaction Kit (Applied Biosystems, Foster City, CA, USA). A multiple sequence alignment of the analyzed regions and the related sequences in the GenBank/EMBL database was performed with ClustalX. The substitution model was selected with modeltest 3.06. Maximum-likelihood phylogenetic analyses were performed with PAUP*4.0b10. Robustness of the phylogenetic groups was evaluated using 1000 bootstrap replicates. In this study, we have identified this HDV3 in all cases. Conversely to previous studies from other regions of the Amazon, in the present study, HBV genotype A (subtype adw2) was found co-infecting patients harboring HDV3. The finding of the co-infection HDV3/HBV-A suggest that there is not an specific interaction between HBV and HDV genotypes, but that this co-infection merely reflects the most frequent genotypes found in a particular area. The analysys of the carboxy-terminal region of LHDag, which is related to the interactions to the HBsAg and essential to their binding, showed some diversity between the different isolates from Eastern Amazon. This region was highly conserved among previous South American isolates. These studies have analyzed a limited number of samples what may explain the smaller diversity but we cannot rule out the that HDV3 circulating in Eastern Amazon shows a higher degree of variability than in other regions. ACKNOLEDGEMENT: FAPESP 00/11457-1 AND ALVES DE QUEIROZ FAMILY FUND

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1478
EXPRESSION OF TOLL-LIKE RECEPTOR 3 ON DENDRITIC CELLS IN CHRONIC HEPATITIS B VIRUS INFECTION

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Background and aims: An inadequate immune response of host is thought to play a critical role in chronic hepatitis B virus infection (HBV). Dendritic cells (DCs) are professional antigen presenting cells that are pivotal in immune response. Toll-like receptors (TLRs) function as PRRs principally sense conserved molecule motif. TLR3 recognize dsRNA induce type I interferon. This study is to elucidate the roles of TLR3 on DCs in HBV infection. Methods: Monocytes were isolated from fresh peripheral blood of 22 healthy volunteers (HV) and 28 chronically HBV-infected patients (CH). DCs were induced and proliferated in the culture medium with recombinant human granulocyte-macrophage-colony-stimulating factor (rHGM-CSF) and human interleukin-4 (rhIL-4). The expressions of TLR3, HLA-DR, CD86, and CD1a on DCs were quantified by Flow Cytometry. We stimulated DCs with polyC: and examined TLR3, HLA-DR, CD86, and CD1a expression at 0 hours, 24 hours and 48 hours by Flow Cytometry. Results: TLR3 expression on DCs before and 12h, 24h, 48h after poly-inosinic acid-cytidylic acid (polyC) stimulation are 69%, 75%, 78%, 85% respectively in CH groups, whereas 70%, 67%, 86%, 68% in HV groups. The expressions of TLR3 up regulate significantly (P<0.01) at 24h and recover at 48h after stimulation with poly C in HV groups, whereas, there are no significant increases at 24h but obvious increases were observed at 48h in CH groups. The rate of CD86 expression is 63%, 76%, 91%, 88% in CH groups, and 50%, 81%, 93%, 94% in HV groups at various time points after Polyc stimulation. There was a marked increase of the expression level of CD86 in HV groups (P<0.01). In contrast, only slight increase was found in CH groups (31% vs 13%). The differences (P<0.01) between the groups were maintained at 24h and 48h. No significant differences were detected in HLA-DR and CD1a between two groups. Conclusions: The increase of expression level of TLR3 is slower and delayed in CH groups. A marked increase of the expression level of CD86 is observed in HV groups, whereas, only slight increase was seen in CH
groups. Our results suggest that abnormal expression of TLR3 and CD86 may lead to persistent infection of HBV.

Disclosures:
The following people have nothing to disclose: Baoyan An, Qing Xie, Hui Wang, Nina Jia, Honglian Gui, Xiaqiu Zhou, Qing Guo, Hong Yu

1479 PRIMARY CARE PROVIDER ADHERENCE TO HCV TESTING RECOMMENDATIONS
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Background: Published guidelines regarding HCV testing practices are infrequently followed (Trooskin, 2007). Therefore, the aim of this study was to survey primary care provider adherence to testing recommendations and to identify provider and practice characteristics associated with lack of adherence. Methods: A survey was mailed to 2,365 primary care providers in the Philadelphia area. Analyses investigated the association between provider and practice characteristics with responses to 16 clinical scenarios reflecting HCV testing guidelines. A score from 0-16 was generated for each respondent, with 0 indicating all incorrect responses and 16 indicating all correct responses. Analyses were carried out in SAS 9.1. Results: A total of 1,219 surveys were returned (58% response); 1,205 surveys were included in the analysis. The mean age of respondents was 50 years, 73% were male and 83% self-identified as non-Hispanic white. Fifty-one percent were internists; 17% were assistants; 67% were in private practice, 59% were in a suburban area. The mean adherence score was 9.6. Most providers (74%) reported that they would test patients based on race alone (African American or Hispanic). Most HIV-infected patients will receive anti-HBV as part of ART and were thus considered baseline. ANOVA was used to compare continuous variables across the three groups and chi-squared for dichotomous variables. Results: ESLD and cirrhosis was seen more frequently in those with HIV/HCV/HBV compared to those with HIV/HCV or HIV/HBV (p=0.02 and 0.03, respectively). Log HCV load was similar in those with HIV/HCV (5.08) vs. triple (5.37). Log HBV DNA was lower in those with triple (2.58) compared to those with HIV/HBV (7.25), p=<0.001, however many samples in the triple group were obtained while patients were on ART, often using nucleosides with anti-HBV activity. Baseline samples [HIV/HBV (n=55); HIV/HCV (n=12); triple (n=13)] were examined and showed no difference in HCV viral load between HIV/HCV and triple infection, but log HBV viral load was slightly lower in those with triple infection compared to those with HIV/HBV (5.06 vs. 7.25, p=0.07). A history of alcohol abuse was more frequent in those with HCV/HCV compared to those with HIV/HBV (p=0.06 and 0.03, respectively). Conclusions: HIV-infected patients with both HBV and HCV are more likely to progress to ESLD compared to those with HIV/HBV or HIV/HCV. Cirrhosis is also more likely to be seen in this population. Most HIV-infected patients will receive anti-HBV as part of ART. Triply infected patients should be considered additionally for HCV treatment, given the propensity for disease progression. Supported by the James and Alinda Wikert Fund of the Southwestern Medical Foundation.

Disclosures:
The following people have nothing to disclose: Emmanuel Seremba, Reeti K. Joshi, Nahid Attar, William M. Lee, Mamta K. Jain

1480 LIVER DISEASE PROGRESSION AMONG HIV-INFECTED PATIENTS WITH VIRAL HEPATITIS
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Background: Co-infection with viral hepatitis B (HBV), C (HCV) or both is common in HIV-infected patients due to shared routes of transmission. Little is known about the risk of liver disease progression among those coinfected with HIV/HCV, HIV/HBV or triply infected with HIV/HBV/HCV. Methods: Medical records of 151 HIV-infected patients including 67 HIV/HBV, 43 HIV/HCV, and 41 triply infected patients were reviewed for demographics, CD4 cell count, HIV viral load, liver function tests, hepatitis B, C, and hepatitis delta (HDV) serologies, and radiologic images. Charts were reviewed in 124 for evidence of progression to end stage liver disease (ESLD) and alcohol consumption. HBV DNA was quantified using VERSANT® HBV 3.0 (bDNA) and HCV RNA with VERSANT® HCV RNA 3.0 (Siemens Diagnostics, Tarrytown NY) from stored sera. Eighty samples were obtained prior to start on antiretroviral therapy (ART) and were thus considered baseline. ANOVA was used to compare continuous variables across the three groups and chi-squared for dichotomous variables. Results: ESLD and cirrhosis was seen more frequently in those with HIV/HCV/HBV compared to those with HIV/HCV or HIV/HBV (p=0.02 and 0.03, respectively). Log HCV load was similar in those with HIV/HCV (5.08) vs. triple (5.37). Log HBV DNA was lower in those with triple (2.58) compared to those with HIV/HBV (7.25), p=<0.001, however many samples in the triple group were obtained while patients were on ART, often using nucleosides with anti-HBV activity. Baseline samples [HIV/HBV (n=55); HIV/HCV (n=12); triple (n=13)] were examined and showed no difference in HCV viral load between HIV/HCV and triple infection, but log HBV viral load was slightly lower in those with triple infection compared to those with HIV/HBV (5.06 vs. 7.25, p=0.07). A history of alcohol abuse was more frequent in those with HIV/HCV compared to those with HIV/HBV (p=0.06). Conclusions: HIV-infected patients with both HBV and HCV are more likely to progress to ESLD compared to those with HIV/HBV or HIV/HCV. Cirrhosis is also more likely to be seen in this population. Most HIV-infected patients will receive anti-HBV as part of ART. Triply infected patients should be considered additionally for HCV treatment, given the propensity for disease progression. Supported by the James and Alinda Wikert Fund of the Southwestern Medical Foundation.

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1481 DIAGNOSTIC ACCURACY OF BLOOD TESTS OF LIVER FIBROSIS IN CHRONIC HEPATITIS B: COMPARISON WITH HEPATITIS C
Vincent Leray, Nathalie Sturm, Marie-Noelle Hilleret, Renversez Patrick, Candice Trocmé, Faure Patrice, Jean-Pierre Zarski; CHU de Grenoble, Grenoble, France

Several blood tests are currently used for the non-invasive evaluation of liver fibrosis. They have been mainly described and validated in chronic hepatitis C (CHC). Their diagnostic accuracy is however poorly documented in patients with chronic hepatitis B (CHB). The aim of this study was to describe the diagnostic performance of a panel of blood tests of fibrosis in CHB compared to CHC. Methods: 510 patients seen in our center for a pre-therapeutic liver biopsy between 2000 and
2007 were recruited. They included 255 CHC patients and 255 CHB (monoinfected) patients, matched on the stage of fibrosis. Blood tests (Fibrotest, Hepascore, Fibrometer and MP3) were assessed on frozen serums collected the day of the biopsy. Histological lesions were staged according to the METAVIR system. Perisinusoidal fibrosis was noted. Areas of fibrosis were quantified by morphometry in 100 patients (20 per stage of fibrosis). Results: CHC were significantly older (47 vs 40 years, p<0.01), had lower ALT levels (73 vs 93 UI/ml, p<0.005) and higher GGT levels (91 vs 53 UI/ml, p<0.01) than CHB patients. Other characteristics including alcohol consumption, Metavir activity and biopsy length (median 23 mm) were similar. Fibrosis stages were distributed as follow : F0 n=72, F1 n=192, F2 n=132, F3 n=54, F4 n=56. Diagnostic accuracies of blood tests for significant fibrosis (F0F1 vs F2F3F4) were compared between CHC and CHB and were as follow (AUROCs) : Fibrometer : 0.81 vs 0.82, Fibrotest : 0.81 vs 0.78, MP3 : 0.80 vs 0.76 Hepascore : 0.79 vs 0.77. For the diagnosis of extensive fibrosis the best result was observed for Fibrometer (0.89 vs 0.89). Test performance profiles were also evaluated. The rate of misclassification was significantly higher in CHB compared to CHC in patients with early stages of fibrosis (F0F1F2) (41% vs 30%, p<0.01 for Fibrotest). This was especially true for discriminating F1 vs F2. Morphometric analysis showed a significant correlation between area and stage of fibrosis (r=0.82, p<0.001), with the notable exception of F1 vs F2 (4.1 vs 4.0%, NS). Comparisons between CHC and CHB for F0, F1 and F2 stages showed greater areas of fibrosis in CHC (on average 2-fold, p<0.001), that was explained in part by more pronounced perisinusoidal fibrosis in CHC. No difference was observed between CHC and CHB for F3F4 stages.

Conclusion: Our results show that in CHB blood tests of fibrosis have good global diagnostic accuracies especially for the diagnosis of extensive fibrosis. However, test performance profiles are altered in early stages of fibrosis (F0F1F2), a result explained by a different distribution of liver fibrosis in CHB compared to CHC.

Disclosures: The following people have nothing to disclose: Vincent Leroy, Nathalie Sturm, Marie-Noelle Hilleret, Renversez Patrick, Candice Trocmé, Faure Patrice, Jean-Pierre Zarski
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