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Editorial introductions

vii Editorial introductions.

Antimicrobial agents

bacterial/fungal: Edited by Thomas Patterson

561 Management of Gram-positive bacteraemia.
Ilker Uçkay; Stephan Harbarth; Didier Pittet

568 Approaches to serious methicillin-resistant Staphylococcus aureus infections with decreased susceptibility to vancomycin: clinical significance and options for management.
James S Lewis II; Michael W Ellis

574 Attenuation of echinocandin activity at elevated concentrations: a review of the paradoxical effect.
Nathan P Wiederhold

579 Mould-active azoles: pharmacokinetics, drug interactions in neutropenic patients.
Paul O Gubbins

587 Impact of pharmacodynamics and pharmacokinetics on echinocandin dosing strategies.
Tawanda Gumbo

592 Antifungal therapy for neonatal candidiasis.
Theoklis Zaoutis; Thomas J Walsh

Antimicrobial agents

parasitic/viral: Edited by Simon Croft and Deenan Pillay

598 Strategies to reverse drug resistance in malaria.
Timothy J Egan; Catherine H Kaschula

605 Artemisinins and synthetic trioxolanes in the treatment of helminth infections.
Jennifer Keiser; Jürg Utzinger

613 Intermittent preventive therapy for malaria: progress and future directions.
Martin P Grobusch; Andrea Egan; Roly D Gosling; Robert D Newman

621 The implications of antiviral drugs with activity against hepatitis B virus and HIV.
Marcelle Bottecchia; Javier Garcia-Samaniego; Vincent Soriano.
Current World Literature
Bibliography

629 Current World Literature.

Erratum

656 Erratum.
Editorial introductions

*Current Opinion in Infectious Diseases* was launched in 1988. It is part of a successful series of review journals whose unique format is designed to provide a systematic and critical assessment of the literature as presented in the many primary journals. The field of infectious diseases is divided into 12 sections that are reviewed once a year. Each section is assigned a Section Editor, a leading authority in the area, who identifies the most important topics at that time. Here we are pleased to introduce the Section Editors for this issue.

**Section Editors**

**Thomas F. Patterson**

Dr Thomas F. Patterson received his Bachelor of Arts degree from Baylor University, in Waco, Texas and his Medical Doctor from the University of Texas Medical School at Houston, Texas. He completed his internship and residency at Vanderbilt University Medical School, in Nashville, Tennessee and Yale-New Haven Hospital, and a fellowship in infectious diseases at Yale University School of Medicine, New Haven, Connecticut where he also served as an Assistant Professor of Medicine.

Dr Patterson currently is a Professor of Medicine in Infectious Diseases and Director of the Infectious Diseases Fellowship Training Program at the University of Texas Health Science Center in San Antonio, Texas. He is also an Attending Physician at the South Texas Veterans Healthcare System, Audie Murphy Division, San Antonio and Director of the San Antonio Center for Medical Mycology.

He has extensive experience in opportunistic fungal infections. His clinical and research interests focus on the diagnosis and treatment of fungal diseases particularly in immunocompromised hosts. He has been involved in developing new antifungal drugs and in clinical trials of new antifungal compounds.

Dr Patterson has published and lectured extensively on fungal infections. He is a member of the American Board of Internal Medicine Subspecialty on Infectious Diseases and is co-Editor-in-Chief of the popular mycology website www.doctorfungus.org. He is a Fellow of the American College of Physicians and Past-President of the Texas Infectious Disease Society.

**Simon L. Croft**

Simon Croft is Professor of Parasitology in the Department of Infectious and Tropical Diseases at the London School of Hygiene & Tropical Medicine, London, UK. He trained as a parasitologist at the Liverpool School of Tropical Medicine and, after post-doctoral periods working in the laboratory on parasite ultrastructure and biochemistry and overseas on the transmission of African trypanosomiasis, he moved to research on anti-protozoal chemotherapy. His expertise and knowledge on anti-protozoal chemotherapy was developed while working with the Wellcome Research Laboratories, Beckenham, UK for 5 years in the 1980s. Following his return to academia, Simon focused his research on the identification and evaluation of novel drugs and formulations for the treatment of leishmaniasis, malaria, human African trypanosomiasis (sleeping sickness) and South American trypanosomiasis (Chagas disease). This work included projects on miltefosine, AmBisome and topical paromomycin, all of which reached clinical trials for the treatment of leishmaniasis. Other current research interests include the drug – immune response interaction and drug resistance in leishmaniasis and malaria. From 2004 to 2007 he was R & D Director at the Drugs for Neglected Diseases Initiative (DNDi), Geneva.

**Deenan Pillay**

Following a PhD in Biochemistry, Dr Pillay studied Medicine in Newcastle upon Tyne and undertook postgraduate training in Medical Virology at St Thomas’s Hospital and Royal Free Hospital, London. He was then a visiting NIH Fellow in the laboratory of
Dr Doug Richman, University of California, San Diego, where he developed an interest in HIV Drug Resistance. On return to the UK Dr Pillay took up the post of Consultant Medical Virologist, Birmingham Public Health Laboratory and in 1996 became Director of the National PHLS Reference Laboratory for antiviral drug resistance. In 2003 he moved to University College London, with a joint appointment at the Central Public Health Laboratory, Colindale, where he is pursuing academic and epidemiological studies on drug resistance, with particular reference to HIV. Dr Pillay’s main areas of interest are the treatment of viral infections and laboratory monitoring of such treatment. His current research is focused on clinical correlates of antiviral drug resistance, viral informatic approaches to antiviral drug resistance data, and epidemiology of drug resistance.
Management of Gram-positive bacteraemia
Ilker Uçkay\textsuperscript{a,b}, Stephan Harbarth\textsuperscript{a,b} and Didier Pittet\textsuperscript{a,b}

**Purpose of review**
Gram-positive bacteraemic infections are frequent and associated with high morbidity and mortality. This paper reviews publications focusing exclusively on new findings related to Gram-positive bacteraemia in the published literature from July 2006 to June 2007.

**Recent findings**
Ninety-eight articles have been reviewed. Of the 66 incorporated in this review, 21 focused on epidemiology or prevention. Thirty-two concerned staphylococcal bacteraemia, while 11 addressed other Gram-positive pathogens. There were seven articles on daptomycin, nine on endocarditis, seven on diagnostic issues, five on haemodialysis-related bacteraemia, and four on antibiotic lock techniques.

**Summary**
In contrast to the large amount of articles dealing with epidemiological issues, the past year did not reveal any new fundamental insights into the treatment of Gram-positive bacteraemia. The rise in the minimal inhibitory concentrations of *Staphylococcus aureus* to vancomycin may become a threat. Several publications underlined the in-vivo efficacy of daptomycin, the new kid on the block against Gram-positive bacteraemia and endocarditis. The antibiotic lock technique showed some promising potential for secondary prevention or treatment of catheter-related infection, while rapid molecular techniques for early species identification may become a valuable diagnostic tool. Most evidence was not based on large, randomized trials and needs future confirmation.

**Keywords**
bacteraemia, daptomycin, Gram-positive, MRSA, *Staphylococcus aureus*

**Introduction**
Gram-positive bacteraemic infections are frequent [1\textsuperscript{--4}]. Clinicians therefore have to face a large amount of epidemiological, clinical and chemotherapeutical articles related to these potentially life-threatening infections. This review focuses exclusively on new findings related to Gram-positive bacteraemia in English, French and German-language medical literature from July 2006 to June 2007. Exceptional case reports were included while experimental trials, animal studies and information concerning the development of new antimicrobial agents, the subject of a previous review [5], were avoided. Ninety-eight articles have been reviewed. Of the 66 incorporated in this review, 21 focused on epidemiology or prevention. Thirty-two concerned staphylococcal bacteraemia, while 11 addressed other Gram-positive pathogens. Seven articles focused on daptomycin, nine on endocarditis, seven on diagnostic issues, five on haemodialysis-related bacteraemia, and four on antibiotic lock techniques for secondary prevention or treatment of catheter-related infection. The review is subdivided by main groups of retrieved publications.

**Epidemiology and risk factors**
Medical progress in the last decades has been undeniable [3\textsuperscript{*},4\textsuperscript{*}], however, the last 12 months have reconfirmed the high prevalence, incidence and mortality related to Gram-positive pathogens for adults [2\textsuperscript{*},6\textsuperscript{*},7\textsuperscript{*},8\textsuperscript{*},9\textsuperscript{*}] and nursing home residents [10], and for children and adolescents, where mortality is lower [4\textsuperscript{*},11\textsuperscript{*}]. In the adult US population, the incidence density for Gram-positive coccal bacteraemia is currently reported as 133 cases per 100 000 person-years for men (probably due to the burden of nosocomial infections), and about half as much for women [12\textsuperscript{*}]. In Denmark, the incidence rate is also increasing, albeit to a lesser extent (30.5 cases/100 000 person-years) [3\textsuperscript{*}]. Bacteraemia due to currently prevalent methicillin-resistant *Staphylococcus aureus* (MRSA) strains still harbours a 1.5 to 2-fold increased mortality and additional costs [2\textsuperscript{*},13,14], compared with its methicillin-sensitive
counterparts and controlled for the severity of underlying illnesses [15]. Methicillin resistance does not seem to be a risk factor for embolism in endocarditis [16], although resistance does matter in enterococcal bacteraemia [17].

Group B streptococcal skin and soft tissue infections with bacteraemia are an emerging disease worldwide and are found to have a mortality of 7.8% in an elderly population [18]. Gram-positive pathogens predominate among the 18.5% bacteraemia associated with limb cellulitis [19], and the presence of severe atopic dermatitis may be a potential source of recurrent endocarditis by *S. aureus* [20]. Complicating infectious foci resulting from haematogenous or local spread of microorganisms are observed frequently in patients with *S. aureus* or nonpneumococcal streptococcal bacteraemia. In a recent study [21], the rate of infectious foci was higher in patients with *S. aureus* than in streptococcal bacteraemia (39% vs. 25%). Conversely, endocarditis and cerebral involvement were more common in the streptococcal group [21].

**Prevention**

Antimicrobial treatment aside, prevention remains the most beneficial course for the patient. Numerous articles demonstrated a positive effect of surveillance and implementation of precautionary programmes. Huang *et al.* [22] reported a 40–75% decrease of MRSA bacteraemia rates in an 800-bed hospital following maximal sterile barrier precautions, hand hygiene campaigning and admission screening for MRSA. Similar successes were observed in prospective trials in intensive care units [23–24]. Bacteraemia was a complication in up to 1% of patients after peripheral arterial bypass surgery and half of the episodes were due to *S. aureus* [25]. Obesity, smoking [26] and hyperglycaemia [27] were independent risk factors associated with mortality due to Gram-positive bacteraemia. The protective effect of antipneumococcal vaccination in adults against bacteraemic invasive infection (54%) (or against invasive disease in general) has been proven once again [28], while an age over 65 years was a mortality predictor (odds ratio 2.6) [29].

As in-vitro trials suggested, haemodialysis patients under acetylsalicylic acid therapy developed a significantly lower rate of catheter-associated *S. aureus* bacteraemia than their adjusted counterparts not receiving acetylsalicylic acid therapy [30]. This difference was dose-dependent, seen mostly with 325 mg, and could not be found for other bacteria, suggesting that the hypothesized direct antistaphylococcal effects of acetylsalicylic acid might be true.

**Daptomycin**

Daptomycin is a bactericidal antibiotic and in 2003 was approved in the USA for the treatment of skin and soft tissue infections caused by Gram-positive bacteria, including MRSA and vancomycin-resistant enterococci, at a dose of 4 mg/kg/day. Last year, the unblinded, randomized trial conducted by Fowler *et al.* [31] established daptomycin as an alternative in the treatment of Gram-positive bacteraemia and right-sided endocarditis. When administered as a single daily intravenous infusion at a dose of 6 mg/kg, daptomycin was not inferior (and neither superior) to standard antibiotic therapy with vancomycin or antistaphylococcal penicillins (with or without gentamicin), in terms of clinical efficacy, but was significantly less associated with renal dysfunction [31] (93% of all patients received gentamicin in the control group vs. 0.8% in the daptomycin group). In turn, daptomycin provoked more creatine kinase elevations and was associated with a tendency to higher microbiological failure rates (19 vs. 11 patients). Moreover, in six of the 19 patients in the daptomycin group, isolates with reduced susceptibility to daptomycin emerged after a median administration time of only 14 days. The clinical significance remains unknown and it would be wise to further investigate the effectiveness of daptomycin for the indications cited above. Further details of the study by Fowler are summarized in Fig. 1.

Meanwhile, several other articles described the role of daptomycin as a new agent for Gram-positive infections, including bacteraemia and right-sided endocarditis [32–33]. Sufficient data concerning the treatment of left-side endocarditis or prostheses-associated bacteraemic infections are currently lacking. As with every new antimicrobial agent, resistance and clinical failures, notably with *Enterococcus* spp., have been rapidly published [34,35], although successful outcomes do exist [31,32,36,37].

Besides daptomycin, tigecycline, a new glycycline licensed in June 2005 in the USA, showed to be a promising alternative for the treatment of MRSA bacteraemia [38]. It remains unclear, however, if tigecycline is superior for MRSA treatment compared with older drugs such as minocycline or cotrimoxazole.

**Staphylococcus aureus**

Literature on *S. aureus* bacteraemia was dominated by three main topics: epidemiology, haemodialysis-related infections [13,39–41] and development of resistance to vancomycin. Despite the increased prevalence of skin and soft tissue infections due to community-acquired MRSA in US outpatients [42,43], a parallel increase of community-acquired MRSA-associated bacteraemia or endocarditis has not yet been reported. Indeed, the Panton-Valentine leukocidin (PVL) gene was absent in bacteraemic patients according to several articles. In community-acquired MRSA endocarditis among intravenous drug users, PVL-negative strains
clearly predominated [44*], suggesting that PVL-positive strains rather infect skin and soft tissues. This has also been witnessed in paediatric patients from the USA [43*] and Israel [45*]. Another survey from the UK and Ireland found only 1.6% PVL-positive strains of *S. aureus* among bacteraemic patients, again suggesting that PVL has no particular significance as a risk factor for bacteraemia [46*]. Moise-Broder *et al.* [48*] demonstrated a significantly higher clearance time of MRSA bacteraemia with vancomycin MIC of 2 μg/ml compared with less than 1 μg/ml. In another study among 50 haemodialysis patients with MRSA bacteraemia, six-fold increased nursing costs and a longer hospital stay were witnessed in multivariate analysis when the vancomycin MIC was 2 μg/ml compared with less than 0.5 μg/ml. Unfortunately, prior vancomycin use was not reported [41*]. In the study by Howden *et al.* [49*] concerning persisting bacteraemia due to MRSA, all five isolates were initially fully vancomycin-susceptible. After 8–32 days of vancomycin treatment, isolates developed heterogenous or low-level vancomycin resistance (MIC 4 μg/ml). All five pairs appeared to be isogenic and genomic DNA microarray comparison suggested that major genetic changes were not required for the development of resistant phenotype.

### Vancomycin and *Staphylococcus aureus*

An emerging topic of last year’s medical literature was the creep of the minimal inhibitory concentration (MIC) in *S. aureus* against vancomycin, a problem that may become associated with clinical and microbiological treatment difficulties [47*]. This fear is enhanced by the high prevalence and incidence of healthcare-associated infection caused by multiresistant *S. aureus* in most parts of the world where glycopeptide use is frequent.

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Standard therapy better</th>
<th>Daptomycin better</th>
<th>Daptomycin standard therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Success 42 days after the end of therapy</td>
<td>-20%</td>
<td>53/124 (42.7)</td>
<td>48/122 (39.3)</td>
</tr>
<tr>
<td>Intention to treat</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Modified intention to treat</td>
<td></td>
<td>53/120 (44.2)</td>
<td>48/115 (41.7)</td>
</tr>
<tr>
<td>Per protocol</td>
<td></td>
<td>43/79 (54.4)</td>
<td>32/60 (53.3)</td>
</tr>
<tr>
<td>Success including patients with failure owing to lack of efficacy only (modified intention to treat)</td>
<td></td>
<td>84/120 (70.0)</td>
<td>79/115 (68.7)</td>
</tr>
<tr>
<td>Success at end of therapy</td>
<td></td>
<td>74/120 (61.7)</td>
<td>70/115 (60.9)</td>
</tr>
<tr>
<td>Modified intention to treat</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Per protocol</td>
<td></td>
<td>53/79 (67.1)</td>
<td>40/60 (66.7)</td>
</tr>
<tr>
<td>Success in prespecified subgroups 42 days after the end of therapy</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MRSA (modified intention to treat)</td>
<td></td>
<td>20/45 (44.4)</td>
<td>14/44 (31.8)</td>
</tr>
<tr>
<td>MSSA (modified intention to treat)</td>
<td></td>
<td>33/74 (44.6)</td>
<td>34/70 (48.6)</td>
</tr>
<tr>
<td>According to the final diagnosis (modified intention to treat)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Uncomplicated bacteraemia</td>
<td></td>
<td>18/32 (56.3)</td>
<td>16/29 (55.2)</td>
</tr>
<tr>
<td>Complicated bacteraemia</td>
<td></td>
<td>26/60 (43.3)</td>
<td>23/61 (37.7)</td>
</tr>
<tr>
<td>Right-sided endocarditis</td>
<td></td>
<td>8/1 (82.1)</td>
<td>7/6 (39.8)</td>
</tr>
<tr>
<td>Left-sided endocarditis</td>
<td></td>
<td>1/9 (11.1)</td>
<td>2/9 (22.2)</td>
</tr>
<tr>
<td>According to entry diagnosis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Definite and possible endocarditis</td>
<td></td>
<td>41/90 (45.6)</td>
<td>37/91 (40.7)</td>
</tr>
<tr>
<td>Not endocarditis</td>
<td></td>
<td>12/30 (40.0)</td>
<td>11/24 (45.8)</td>
</tr>
</tbody>
</table>

Horizontal bars represent 95% confidence intervals (Reprinted with permission from [31**]).

Figure 1 Comparison of the rates of success of daptomycin and standard therapy for *Staphylococcus aureus* bacteraemia and endocarditis
Meanwhile, a new debate echoes in the literature regarding whether vancomycin has become obsolete [50\*] or still keeps its place in the treatment of antibiotic-resistant Gram-positive organisms [51\*]. Arguments against vancomycin include its inferiority to some β-lactams for the treatment of bacteraemia and endocarditis due to methicillin-sensitive _S. aureus_. For instance, a retrospective study of 123 patients undergoing haemodialysis who developed methicillin-sensitive _S. aureus_ bacteraemia found that vancomycin treatment was associated with a significantly greater risk of failure vs. treatment with cefazolin (31% vs. 13%, _P_ = 0.02) [40\*]. Owing, at least in part, to the recognition of the poor therapeutic performance of vancomycin, in 2006 the Clinical and Laboratory Standards Institute implemented a downward revision in vancomycin susceptibility breakpoints from 4 μg/ml to less than 2 μg/ml [50\*,51\*]. As a counterpoint, proof of the existence of a superior antibiotic is currently lacking, and vancomycin is already established as a relatively safe drug, despite occasional reports of potential severe adverse effects [52]; it also remains inexpensive [51\*].

**Antibiotic lock technique**

Prevention of haemodialysis-catheter infections with cefotaxime and heparin was reported as significant better in a double-blind randomized trial in terms of thrombosis and infection-free survival than with heparin alone [53\*]. A meta-analysis of seven other prospective, randomized studies regrouping 463 patients by Saďar and Maki [54\*] showed the same result for vancomycin. Potential detrimental effects on acquired antibiotic resistance, however, mean that further studies, including cost-effectiveness and antibiotic-resistance analyses, are clearly warranted before antibiotic locks should be used in primary or secondary prophylaxis.

As for therapeutic purposes, Fortun _et al._ [55\*] compared the outcome of 92 episodes of catheter-related bacteraemia in chemotherapy patients with systemic antibiotics and locks, used for a minimum of 8–12 h/day for 5–14 days vs. a historical control group of patients treated with systemic antibiotics alone. The antibiotic lock group had a tendency to higher clinical and microbiological success rates. The difference (84% vs. 65%) was not statistically significant, however, due to the small sample size.

**Pneumococci and other streptococci**

Antibiotic resistance problems and prevention issues marked the pneumococcal literature in the last year. Underdosing of macrolides as a monotherapy, due to decreased susceptibility at time of hospital admission, predicted a higher mortality in the case of pneumococcal bacteraemia [56,57]. Combination therapy of a β-lactam antibiotic together with a macrolide for bacteraemic community-acquired pneumonia due to _S. pneumoniae_ failed to show a significant survival effect in an international, prospective trial involving 340 adult patients from Sweden, Canada, USA, Spain and the UK [29\*]. Unfortunately, underlying conditions for both treatment groups were different in some key parameters such as mechanical ventilation, Acute Physiology Score or APACHE II Score, making case-mix adjustment difficult.

The discussion of the last year of combination vs. monotherapy for pneumococcal bacteraemia bends more and more towards monotherapy, at least in Europe [58**]. The future will show if this return to monotherapy will equally include the empiric antibiotic therapy for community-acquired pneumonia in general.

The incidence of group A streptococcal invasive disease (GAS) or bacteraemia is increasing worldwide. The pathogen remains susceptible to penicillin but still harbours a high mortality, especially in elderly patients [59]. A retrospective survey of 62 intensive care patients with GAS infections in Ontario 1992–2002 revealed an overall mortality of 40% [8\*]. This is not surprising. There was however no association found between survival and the administration of immunoglobulins, clindamycin or surgical intervention. This stands somehow in contrast to common practice and previously published results, while the support in the literature for clinical efficacy of immunoglobulins in severe GAS infection still remains weak at best. The authors included all patients meeting clinical, microbiological and/or histopathologic criteria, avoiding selection bias. The wide confidence intervals, however, suggest a problem of underpowering. Further studies are needed to clarify the unsolved issue of immunoglobulins (and of clindamycin) in GAS.

**Endocarditis**

No new insights can be reported in the antibacterial treatment of endocarditis [60\*] besides the use of daptomycin, as mentioned above [31\*,32**]. Colli _et al._ [61\*] reported success with an early switch (after ~5 days) from vancomycin to oral linezolid 600 mg twice daily in 14 surgically treated patients with left-sided, Gram-positive endocarditis. Only two patients died of noncardiac causes in a follow-up period of up to 1 year without any recurrence; hospital stay averaged 10.5 ± 3.5 days. This survey was retrospective, nonrandomized, and further investigations must clarify the potential of such a therapeutic option. Other studies found a 4.3% risk of secondary osteoarticular infections in the case of endocarditis due to _S. aureus_ [62\*] or a very poor prognosis in haemodialysis patients, where surgical intervention was the only independent factor predicting survival [39\*].

**Diagnostic tools**

Errors in interpretation of Gram stains from positive blood cultures may occur [63]. Moreover, the notification of ‘Gram-positive cocci’ in a blood culture drawn from a seriously ill patient usually triggers empiric vancomycin...
prescribing in institutions where MRSA is endemic. Thomas et al. [64**] developed a duplex real-time polymerase chain reaction (PCR) technique targeting the species-specific nuc gene and the mecA gene encoding methicillin resistance. The assay quickly and reliably identified S. aureus in mixed infections, and methicillin resistance in both S. epidermidis and S. aureus strains. Similar reports, with excellent sensitivity, specificity, negative and positive predictive values, have emerged [65*,66*], opening a field for a more rapid identification of potentially virulent pathogens. Further (randomized) trials will show if this technique is reliable enough to exclude pseudobacteraemia and contamination of the bloodstream by skin commensals, and finally to spare antibiotics.

Conclusion
In contrast to the large amount of epidemiological papers, the past year did not reveal any new fundamental insights in the treatment of Gram-positive bacteraemia. The rise in the MICs of MRSA against vancomycin present a potential threat. Some publications underlined the in-vivo efficacy of daptomycin against Gram-positive bacteraemia and right-side endocarditis. The antibiotic lock technique showed some promising potential, and rapid PCR techniques for early species identification in positive blood cultures may become a new diagnostic tool. Most evidence was not based on large, randomized trials and warrants further investi- gation in future trials.

Acknowledgements
We are indebted to Ms Rosemary Sudan for editorial assistance.

Disclosures: There is no grant, sponsoring, business interest or consultancy that could lead to a conflict of interest.

References and recommended reading
Papers of particular interest, published within the annual period of review, have been highlighted as:
• of special interest
•• of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (pp. 629–630).


A prospective analysis highlighting the value of surveillance programmes and barrier precautions to decrease MRSA bacteraemia rates.
Antimicrobial agents: bacterial/fungal


A prospective trial with reduction of staphylococcal bacteraemia and vancomycin use in an intensive care unit setting by surveillance, barrier precautions and topical antimicrobials.


Good example of reduction of MRSA bacteraemia rates by simple measures.


A review of nosocomial infections after peripheral arterial bypass, including bacteraemia. Some 607 episodes are included.


An pneumococcal vaccine decreases the risk for invasive pneumococcal disease in adults. The incidence of bacteraemia is reduced by 54%.


International prospective trial with 340 patients, assessing the combination therapy of beta-lactam antibiotics with macrolides vs. beta-lactams alone in the treatment of bacteraemic pneumococcal community-acquired pneumonia. No significant differences found.


Retrospective analysis of 4722 blood cultures in 872 haemodialysis patients. Acetylsalicylic acid reduced the rate of catheter-related bacteraemia. Four of nine patients achieved cure according to the definition of the paper. Two patients of the failure group died within 3 days of initiation of daptomycin.


Three case reports about the treatment of MRSA bacteraemia with tigecycline.


Review about the severity of staphylococcal endocarditis in patients undergoing haemodialysis. Highlights the role of surgery.


Vancomycin is inferior to cephalosporins in the treatment of S. aureus bacteraemia.


Case-control study reporting a longer hospital stay and higher nursing costs in haemodialysis patients with MRSA bacteraemia, when the vancomycin MIC is >2 µg/ml compared with ≤0.5 µg/ml. Small sample size.


Interesting analysis indicating that Panton-Valentijn leucocidin-positive community-acquired MRSA strains do not cause bacteraemic disease, in contrast to skin and soft tissue infections.


The Panton-Valentijn leucocidin toxin does not predispose to bacteraemic infections by community-acquired MRSA.


Report about the prevalence of Panton-Valentijn leucocidin toxin in S. aureus bacteraemia in the UK.


Case report illustrating the difficulties in the treatment of MRSA that is not fully susceptible to vancomycin.


A higher MIC for vancomycin leads to a longer clearance time of bacteraemia due to MRSA.


Report showing an increase of the vancomycin MIC in MRSA under current therapy. Carefully analysed.


Debate: Arguments against the use of vancomycin in modern times.


Debate: Arguments in favour of the use of vancomycin.


Trial showing the success of primary prophylaxis against haemodialysis-catheter-associated infections by the use of the antibiotic lock technique.


A meta-analysis of seven prospective randomised trials concerning the use of vancomycin in the antibiotic locks for primary prevention of catheter-related bloodstream infections.


Prospective randomized trial showing the efficacy of the antibiotic lock technique in the treatment of catheter-related bloodstream infections in oncologic patients.


State-of-the-art review of the evidence of combined antibiotic therapy.


Literature review concerning the treatment of endocarditis with an emphasis on surgery.


Retrospective Italian survey reporting a promising potential for an early switch from vancomycin to peroral linezolid in surgically treated, left-side endocarditis patients.


A study highlighting the potential danger of endocarditis due to Gram-positive pathogens.


PCR technique allows identifying more rapidly, accurately and easily methicillin-sensitive or methicillin-resistant S. aureus in blood cultures bottles than growth in automatic systems.


Article about the potentially beneficial use of the PCR technique for positive automatic blood culture bottles. Rapid identification of S. aureus and resistance profiling by PCR.


Microbiological validation of a real-time PCR assay for identification and differentiation of methicillin-sensitive and methicillin-resistant S. aureus in 295 patients with excellent results.
Approaches to serious methicillin-resistant *Staphylococcus aureus* infections with decreased susceptibility to vancomycin: clinical significance and options for management

James S. Lewis II and Michael W. Ellis

**Purpose of review**

This review addresses therapeutic approaches to *Staphylococcus aureus* infections with diminished susceptibility to vancomycin, focusing on recently published data in English language medical literature between June 2006 and July 2007.

**Recent findings**

Knowledge regarding the potential limitations of vancomycin therapy for *S. aureus* infections continues to emerge. Recent changes include alteration of the Clinical Laboratory and Standards Institute vancomycin breakpoint for *S. aureus* and questions regarding the utility of the lower breakpoint of 2.0 mg/l. Interest continues in the accessory gene regulator (*agr*) locus and its impact on the activity of vancomycin. Newer options for drug therapy progress, with strengths and limitations becoming more apparent for each.

**Summary**

Newer antimicrobial agents active against methicillin-resistant *S. aureus* such as daptomycin and linezolid continue to show value. Older antimicrobial agents may play an important therapeutic role and warrant further examination. Work is needed to evaluate current agents against methicillin-resistant *S. aureus* in the setting of elevated vancomycin minimum inhibitory concentrations or clinical failure. Antimicrobial selection for methicillin-resistant *S. aureus* infections with reduced susceptibility to vancomycin should be governed by disease severity, susceptibility patterns, knowledge of the limitations of current susceptibility testing, and strengths and weaknesses of the agents being considered.

**Keywords**

accessory gene regulator (*agr*), methicillin-resistant *Staphylococcus aureus*, vancomycin-intermediate *Staphylococcus aureus*

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**Abbreviations**

CLSI: Clinical Laboratory and Standards Institute
cSSTI: complicated skin and soft tissue infection
MIC: minimum inhibitory concentration
MRSA: methicillin-resistant *Staphylococcus aureus*
MSSA: methicillin-susceptible *Staphylococcus aureus*
SA-RVS: *Staphylococcus aureus* with reduced susceptibility to vancomycin
TMP–SMX: trimethoprim–sulfamethoxazole
VISA: vancomycin-intermediate *Staphylococcus aureus*
VRSA: vancomycin-resistant *Staphylococcus aureus*

**Introduction**

*Staphylococcus aureus* resistance to vancomycin is one of the greatest concerns in infectious diseases. Over the past 50 years this common pathogen has demonstrated a remarkable ability to overcome many classes of antibiotics; however, vancomycin has largely remained unscathed. Unfortunately, methicillin-resistant *Staphylococcus aureus* (MRSA) rates now approach or exceed 50% in many US healthcare systems, and the emergence of this pathogen also in outpatient infections has resulted in a marked increase in the use of vancomycin. Not surprisingly, worrisome events have followed this increase in vancomycin use. Increasing reports of vancomycin clinical failures in *S. aureus* infections with minimum inhibitory concentrations (MICs) considered susceptible, vancomycin-intermediate *Staphylococcus aureus* (VISA), hetero-VISA, and vancomycin-resistant *Staphylococcus aureus* (VRSA) have emerged over the past several years. These events call into question how best to manage *S. aureus* infections that have clinically failed vancomycin or have MICs that would raise concerns regarding the efficacy of vancomycin. Although the literature on this subject remains incomplete and in many ways generates more questions than it answers, we will review the currently available data in an attempt to provide guidance on the management of infections caused by these problematic organisms.

**Issues with the breakpoints**

Historically, the Clinical Laboratory and Standards Institute (CLSI; formerly the National Committee on Clinical Laboratory Standards) defined *S. aureus* isolates with a MIC of 4 μg/ml or less as susceptible to vancomycin. It has, however, become abundantly clear during the past few years that there are problems with this...
frequently been associated with clinical failures [2]. Fridkin et al. [2] reviewed the epidemiology of *S. aureus* with reduced susceptibility to vancomycin (SA-RVS) in the United States from 1997 to 2001. The authors found attributable mortality rates in patients with SA-RVS infections of 63% compared with only 12% for MRSA controls, and that vancomycin exposure in the month before the isolation of the SA-RVS isolate was the strongest risk factor for the acquisition of these organisms. Importantly, they also found no clinical difference between infections caused by organisms with MICs of 4 µg/ml and those with MICs of 8 µg/ml, again indicating that organisms with MICs of 4 µg/ml are problematic. Howden and colleagues [3] reviewed 25 cases of *S. aureus* defined as having reduced susceptibility to vancomycin. Of these 25 isolates only nine had MICs of 4 µg/ml, with the remainder having MICs of 2 µg/ml. The glycopeptide failure rate in this series was 76%. Whereas the available data on MICs of 4 µg/ml remain very limited because of the scarcity of these organisms, the above data provide compelling evidence that organisms with MICs of 4 µg/ml to vancomycin should not be managed with vancomycin therapy at conventional doses and probably should be treated with alternative therapy. An excellent review of the rationale for the CLSI decision to change the vancomycin breakpoints, which includes 14 cases of vancomycin MICs of 4 µg/ml that resulted in treatment failures, has been recently published [4**].

The question then becomes if 4 µg/ml results in an unacceptable rate of failures then what should be the breakpoint? A reasonable choice seems to be 2 µg/ml, given the aforementioned scarcity of *S. aureus* with higher MICs as well as the clinical data discussed above. Furthermore this was the value decided upon by the CLSI. Recent data examining the increasing incidence of vancomycin MICs of 2 µg/ml and the correlation of this result with outcomes have been disturbing. In what is one of the best studies to date to examine this issue, Hidayat and colleagues [5**] conducted a prospective cohort study of patients with MRSA infections and divided them into high and low vancomycin MIC groups. These groups were defined as an MIC of 2.0 µg/ml or greater and less than 2.0 µg/ml. Patients were aggressively dosed with vancomycin, as the underdosing of vancomycin has been one of the frequent criticisms of previous work examining this issue, to ensure the rapid attainment of trough levels of 15–20 µg/ml. Low trough levels of vancomycin have also been associated with the emergence of VISA in some models [6*]. The authors found that even with aggressive vancomycin dosing and the rapid attainment of desired trough levels that infections caused by MRSA with a vancomycin MIC of 2.0 µg/ml resulted in a higher rate of clinical failures at the end of therapy (85% success versus 62% success, *P* = 0.02) [5**]. Also worrisome was the higher rate of nephrotoxicity that appeared to be associated with the use of vancomycin troughs of 15–20 µg/ml. It is, however, important to note that the vast majority of patients who experienced nephrotoxicity received concurrent potentially nephrotoxic agents. Other authors have also suggested that MRSA isolates with vancomycin MICs of 2.0 µg/ml are less likely to respond to vancomycin therapy, and that alternative agents should be used [7]. These data have, however, been limited mostly to retrospective reviews and analysis of highly selected isolates making their widespread clinical application difficult.

**The role of the accessory gene regulator (agr) operon**

The accessory gene regulator (*agr*) operon plays an important role in the regulation of multiple virulence pathways in *S. aureus*; and isolates belong to one of four major *agr* groups (I–IV). Data have recently emerged suggesting that the *agr* operon of *S. aureus* may also play a significant role in the decreased ability of vancomycin to kill *S. aureus* effectively [8]. An excellent in-depth review of this topic has recently been published [9**]. These data, although mostly generated using in-vitro systems and limited by small numbers, are nevertheless compelling. The focus of recent investigations has been largely on *S. aureus* isolates belonging to the *agr* II group and the impact of decreased or lost *agr* II function. Historically, *agr* II group isolates have represented a minority of *S. aureus* clinical isolates. This group appears particularly prone to the development of hetero-VISA in *vitro* as well as clinical failures and has become the most prevalent *agr* group in some US hospitals [7,10]. It is important to note that although not all glycopeptide-intermediate strains are *agr* group II, the vast majority of VISA and hetero-VISA isolates found in the United States have been part of this group [11]. Interestingly, a recent study reported that *agr* dysfunction within any of the four classes resulted in increased vancomycin MIC when exposed to suboptimal vancomycin concentrations; however, the *agr* II group isolates displayed the largest increase in MIC [6*]. Further evaluation of the clinical relevance of these findings is badly needed. It does appear from much of this work that underdosing of vancomycin creates a milieu prone to generating increased vancomycin MICs and subsequent clinical failures.

**Treatment options for *Staphylococcus aureus* infections with high vancomycin minimum inhibitory concentrations**

Perhaps no currently available antibacterial agent more epitomizes the difficulties of translating in-vitro activity into clinical outcomes than the novel cyclic lipopeptide antibiotic daptomycin.
This compound displays impressive in-vitro activity against VISA and hetero-VISA isolates [12]. Daptomycin’s rapid bactericidal activity as well as the results from a recently published well-designed bacteremia study would seem to make it the ideal replacement candidate for infections caused by MRSA with elevated vancomycin MICs [13]. Nevertheless, it has become apparent that some degree of cross-resistance between vancomycin and daptomycin exists [14,15]. To what degree this decrease in daptomycin susceptibility will confer clinical failure remains unclear. On the basis of data demonstrating clinical failures, it is clear that *S. aureus* isolates with an MIC of 2.0 μg/ml or more to daptomycin, which are considered non-susceptible using the CLSI breakpoint, should not be treated with daptomycin with currently approved doses [17,18]. If daptomycin is used in infections caused by *S. aureus* with elevated vancomycin MICs, then maximal doses should be used. In conjunction with optimizing dosing it is critical that careful baseline MICs and the determination of daptomycin MICs for subsequent clinical isolates is performed.

**Linezolid**

Linezolid is an oxazolidinone antibiotic that exhibits bacteriostatic activity against *S. aureus* by inhibiting bacterial ribosomal protein synthesis through its binding to the 50S ribosome [19]. In-vitro antimicrobial susceptibility testing against over 60 *S. aureus* strains with reduced vancomycin susceptibility, including a half-dozen VRSA strains, has shown no resistance to linezolid [20–22]. Indeed, *S. aureus* resistance to linezolid has rarely been reported [23–25]. In addition, linezolid has been used alone or in combination with other antimicrobial agents for the treatment of VISA infections [26,27].

The available evidence-based literature has led some to suspect that linezolid is superior to vancomycin for the treatment of MRSA complicated skin and soft tissue infections (cSSTIs) and nosocomial pneumonia. Investigators posit that linezolid’s apparent superior clinical activity over vancomycin against MRSA is caused by vancomycin’s low tissue concentrations and low epithelial-lining fluid concentrations [28]. Interestingly, these observations have not been noted in patients infected with methicillin-susceptible *Staphylococcus aureus* (MSSA). In a trial comparing linezolid with vancomycin for patients with cSSTIs caused by MRSA, linezolid exhibited superior microbiological cure: 88.6% (124 of 140) compared with 66.9% (97 of 145; $P < 0.001$) [29]. A prospective multinational trial examining linezolid in the treatment of nosocomial MRSA pneumonia is ongoing, which should help clarify its treatment role (Nosocomial pneumonia with suspected or proven MRSA. ClinicalTrials.gov, Identifier: NCT00084266).

Linezolid has several advantages: it has 100% oral bioavailability and does not require dose adjustment for renal insufficiency. As a result of its cost and the availability of inexpensive oral agents such as trimethoprimsulfamethoxazole (TMP–SMX), doxycycline, and clindamycin, linezolid has little role in the treatment of mild *S. aureus* infections. For VISA or VRSA infections, particularly in the setting of pneumonia or cSSTIs, linezolid should be considered one of the primary therapeutic options.

**Dalbavancin**

Dalbavancin is a novel parenterally administered semisynthetic lipoglycopeptide with potent Gram-positive bactericidal activity. Dalbavancin features a multifaceted mechanism of action that enhances its antimicrobial activity, and its unique pharmacokinetic properties allow once-weekly administration [30,31]. In-vitro assays and animal data have demonstrated that dalbavancin possesses superior bactericidal activity against MSSA and MRSA compared with vancomycin [32,33]. Dalbavancin has been tested against several isolates with reduced susceptibility to vancomycin. Three VISA isolates have been tested with dalbavancin MICs ranging from 1.0 to 4.0 μg/ml [34,35] compared with MICs of 0.06–0.5 for most MSSA and MRSA isolates [33]. One VRSA isolate has been tested against dalbavancin, demonstrating a MIC of less than 0.5 μg/ml [22].

The safety and efficacy of dalbavancin has been examined in two phase II and one phase III trial [36–38]. Jauregui *et al.* [38] conducted a phase III multicenter trial comparing dalbavancin with linezolid for the treatment of cSSTIs. The study included 571 patients in the dalbavancin group and 283 in the linezolid group [38]. Fifty-one per cent of infections in each group were attributed to MRSA [38]. MRSA eradication rates at the test-of-cure visit were similar in both groups: 91% in the dalbavancin group and 89% in the linezolid group [38]. This investigation demonstrated that dalbavancin was as effective (non-inferior) to linezolid in the treatment of cSSTIs.

Dalbavancin appears to be safe and well tolerated [36–38]. Drug-related adverse events do not appear to be significantly different than comparator regimens [36–38]. Overall, there is a paucity of data for dalbavancin against *S. aureus* isolates with reduced susceptibility to vancomycin; however, the available data suggest that dalbavancin may be a beneficial addition to the antimicrobial arsenal. Dalbavancin is expected to be available in the United States in late 2007.

**Telavancin**

Telavancin represents a second lipoglycopeptide currently in late phase III development in the United States.
As with dalbavancin, this agent appears to be an improvement over vancomycin against MRSA. It possesses dual mechanisms of action: the inhibition of cell wall synthesis as well as interference with membrane function, which together result in rapid concentration-dependent bactericidal activity [39]. Given that the structure of the compound is similar to vancomycin, the first obvious question is how MRSA isolates with decreased susceptibility to vancomycin will respond to telavancin? A significant increase in telavancin MIC was observed for VRSA strains compared with MSSA or MRSA, but similar increases were not observed for VISA strains [39]. Telavancin has been shown to maintain bactericidal activity against VISA isolates; however, more drug was required to achieve this activity when compared with MSSA and MRSA [39]. Other investigators have also found that telavancin maintains clinically attainable MICs and minimal bactericidal concentrations against VISA, but that VRSA isolates have significantly higher MICs [40]. The clinical relevance of these elevated MICs and minimal bactericidal concentrations for VRSA remain unclear as they remain well below the maximum serum concentrations attained with proposed dosing regimens. Similar to daptomycin and dalbavancin, MICs for telavancin also increase in the presence of serum [40]. Furthermore a rabbit model of endocarditis [41] suggested that telavancin may remain a viable option in difficult-to-treat infections caused by S. aureus with decreased susceptibility to vancomycin. To date, clinical data for telavancin are limited to cSSTIs, which cannot be extrapolated to support telavancin for VISA or VRSA [42].

Ceftobiprole

Ceftobiprole is an investigational parenteral extended-spectrum cephalosporin that exhibits bactericidal activity against many β-lactam-resistant Gram-positive pathogens, including MRSA, VISA, and VRSA. Ceftobiprole owes its bactericidal activity against MRSA to its unique affinity for penicillin-binding protein 2a and its stability against class A β-lactamases [21,43]. Ceftobiprole has been tested against numerous MRSA strains with reduced vancomycin susceptibility [44]. Eighteen VISA strains have been tested with reported MICs of 0.25–4 μg/ml to ceftobiprole [21,22]. Two VRSA strains have been tested against ceftobiprole with a reported MIC of 2 μg/ml [21,22]. These in-vitro findings suggest that reduced susceptibility to vancomycin has no appreciable affect on ceftobiprole’s bactericidal activity.

Numerous animal models of infection have reported in-vivo data that confirm the in-vitro activity of ceftobiprole [45]. In particular, a rabbit endocarditis model comparing ceftobiprole with vancomycin demonstrated that ceftobiprole was significantly more effective against VISA [46]. One phase III trial comparing ceftobiprole with vancomycin for the treatment of cSSTIs has been completed [47]. The study included 397 patients in the ceftobiprole group and 387 in the vancomycin group. Overall cure rates were similar: 93.3% in the ceftobiprole group, and 93.5% in the vancomycin group. In addition, of the 179 patients infected with MRSA, cure rates in each arm were also similar: 91.8% for ceftobiprole and 90% for vancomycin. Although peer-reviewed literature reporting on ceftobiprole’s clinical efficacy is lacking, the bactericidal in-vitro data, animal infection model data, and results from the phase III trial support the belief that ceftobiprole may play a potential role in the treatment of serious S. aureus infections. Currently, several phase III trials of ceftobiprole for the treatment of nosocomial pneumonia are ongoing (http://clinicaltrials.gov/ct/show/NCT00210964?order=3).

Trimethoprim–sulfamethoxazole

At a time when numerous investigational agents are under development, there is also renewed interest in TMP–SMX for the treatment of S. aureus infections [48]. TMP–SMX has numerous merits: it is frequently bactericidal against S. aureus; it is available in both oral and parenteral preparations; it is inexpensive, and has nearly five decades of use and safety experience. In addition, TMP–SMX has been noted to be active against nearly all community-associated MRSA isolates depending on the metropolitan area [25]. TMP–SMX has been tested against numerous MRSA strains with reduced susceptibility to vancomycin [49]. In-vitro susceptibility testing has demonstrated that with few exceptions TMP–SMX remains active against these strains [49]. A community-associated MRSA VISA strain was recently noted to be susceptible to TMP–SMX (MIC < 0.5 μg/ml) [50]. TMP–SMX has also been reported to have in-vitro activity against several VRSA strains [22,51,52]. Effective treatment of VISA infections with TMP–SMX in conjunction with other antimicrobial agents has also been documented [26,53].

There has been one double-blind randomized trial comparing TMP–SMX with vancomycin for the treatment of S. aureus infection (including MRSA) [54]. In that investigation, 37 of 43 TMP–SMX-treated patients were cured compared with 57 of 58 vancomycin-treated patients [54]. The mean duration of bacteremia for the TMP–SMX patients was 6.7 days compared with 4.3 days for vancomycin [54]. Although this investigation showed vancomycin’s superiority, it demonstrated that TMP–SMX could be considered a possible treatment option in selected patients.

As a result of a lack of recent literature supporting the use of TMP–SMX for the treatment of serious S. aureus infections, Lewis and Ellis [57] have considered a possible treatment option in selected patients.
infections, it can only be recommended for less severe skin and soft tissue infections [55]. Despite the shortage of recent evidence-based literature, TMP-SMX should always be tested against S. aureus strains with reduced vancomycin susceptibility and considered as a part of a treatment regimen.

**Conclusion**

Emerging reports of decreasing susceptibility of S. aureus to vancomycin and increasing clinical failures in apparently susceptible MRSA infections raise serious concerns. In spite of the recent reduction in the vancomycin breakpoint for S. aureus to 2.0 mg/l by the CLSI, an increasing amount of clinical literature calls this value into question. Furthermore, S. aureus continues to evolve in the face of increasing vancomycin pressure, and many of these changes may not be readily apparent by MIC. Fortunately, although antibiotic development for Gram-negative infections has come to a standstill, the pharmaceutical industry has continued to develop newer compounds that possess activity against not only MRSA but also VISA isolates. As in the case of daptomycin, however, the newer generation of lipoglycopeptides must overcome concerns regarding cross-resistance with vancomycin. Areas for future research should include the appropriate breakpoint for vancomycin against S. aureus, the importance of the agr locus, the role of new and off-patent anti-infective agents in a variety of MRSA infections, and finally the role of combination therapy for severe MRSA infections.

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**References and recommended reading**

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (pp. 630–631).


Staphylococcus aureus infections Lewis and Ellis 573
Attenuation of echinocandin activity at elevated concentrations: a review of the paradoxical effect
Nathan P. Wiederholda,b

Purpose of review
The echinocandins have been a welcome addition for the treatment of invasive fungal infections caused by Candida and Aspergillus species. Despite their excellent safety profile and clinical efficacy, concerns exist regarding an attenuation of activity at higher concentrations, known as the paradoxical effect. This article will review the literature describing this effect, the potential mechanisms responsible for it, and the clinical implications of this phenomenon.

Recent findings
In-vitro studies have reported a paradoxical effect at higher concentrations against both Candida and Aspergillus species. Recent data have demonstrated this effect in Candida to be species related and echinocandin specific. Although not completely understood, studies have pointed towards involvement of the protein kinase C cell wall integrity and calcineurin pathways as well as increases in cell wall chitin content as potential mechanisms behind the effect. Although some in-vivo studies with echinocandins have reported a paradoxical increase in markers of invasive disease, clinical data are scarce.

Summary
The clinical implications of the paradoxical attenuation of echinocandin activity observed in vitro and in vivo remain unknown. A complete understanding of this effect may further our knowledge of fungal responses to echinocandin cell wall damage and potentially improve treatment strategies.

Keywords
anidulafungin, Aspergillus, Candida, caspofungin, echinocandins, micafungin, paradoxical effect

Introduction
The echinocandins have been a significant advancement in antifungal therapy. Through non-competitive inhibition of β-1,3-glucan synthase, a target unique to the cell wall of fungi, including Candida and Aspergillus species [1,2], these agents avoid harmful toxicities and drug interactions associated with other antifungal classes such as the polyenes and azoles. Each of the clinically available members of this class (caspofungin, micafungin, and anidulafungin) has also been shown to be effective in the treatment of invasive fungal infections caused by Candida species [3–5]. The excellent safety profile of these agents has raised the possibility of dosage escalation in order to enhance their clinical effectiveness further, a concept that appears to be supported by animal studies [6–8]. Despite the improved tolerability and clinical utility of these agents, questions regarding an attenuation of activity at higher concentrations, referred to as the paradoxical effect, have been raised. This effect is analogous to the ‘Eagle effect’ first observed with cell wall active antibacterial agents such as penicillin by Eagle and Musselman [9] in 1948. The purpose of this article is to discuss the paradoxical effect of the echinocandins, including both in-vitro and in-vivo studies in which this effect has been observed, review the mechanisms proposed to be responsible for this effect, and discuss the potential clinical implications.

In-vitro phenotypic observations
A paradoxical attenuation of antifungal activity at higher echinocandin concentrations was first reported by Hall et al. [10] with the investigational agent cilofungin, a semisynthetic derivative of echinocandin B. In that study an inhibitory effect of cilofungin was observed at low concentrations against clinical isolates of Candida species. Unexpectedly, in some C. albicans and C. tropicalis isolates this inhibitory effect was followed by growth at higher concentrations equivalent to that observed in growth control wells, and was referred to by these investigators as a ‘paradoxical effect’. This effect appeared to be media dependent, occurring in Sabouraud dextrose and yeast nitrogen broth, but not antibiotic medium 3, a finding also reported by other investigators [11]. The clinical implications of this finding were never explored because of acute tubular necrosis thought to be associated with the polyethylene glycol formulation of cilofungin [12,13].
The paradoxical effect is not limited to cilofungin. Studies have also reported similar observations in vitro with each of the clinically available members of this class. This effect was observed for caspofungin in an in-vitro study examining the pharmacodynamics of this agent against *C. albicans*-embedded biofilms [14]. Ramage et al. [14] noted decreased caspofungin activity at a caspofungin concentration of 8 µg/ml compared with that of 0.125 and 1 µg/ml. Another study has recently confirmed a paradoxical attenuation of caspofungin activity against *Candida* embedded in biofilms [15].

More thorough investigations into the paradoxical effect have subsequently been conducted. In a detailed examination of the phenotypic characteristics associated with this effect, Stevens et al. [16] reported a paradoxical increase in visual turbidity in three of 21 *C. albicans* clinical isolates at caspofungin concentrations (1.56–12.5 µg/ml) well above the minimum inhibitory concentration (MIC; 0.09 µg/ml) upon exposure of *Candida* species to caspofungin. This phenomenon was quadriphasic, a finding supported by quantitative data from other studies (Fig. 1) [8,17]. Interestingly, the progeny of these cells collected at higher concentrations did not demonstrate total resistance to caspofungin. Instead, the paradoxical effect was reproduced in a similar manner to that of the parent cells. As in the study by Hall et al. [10] this effect appeared to be media specific and was more pronounced in synthetic amino acid medium fungii and RPMI compared with yeast nitrogen broth. When micafungin and anidulafungin were tested the highest echinocandin concentration; phase 4, inhibition of growth at the highest drug concentrations.

recent data have suggested that the paradoxical effect may be both echinocandin and *Candida* species specific. Chamilos et al. [18*] reported that differences in the prevalence of the paradoxical effect were observed for caspofungin, micafungin, and anidulafungin against 60 *Candida* bloodstream isolates. The highest frequency occurred with caspofungin, occurring in 90% of *C. parapsilosis*, 60% of *C. albicans*, 40% of *C. tropicalis*, and in one *C. krusei* isolate. In contrast, a paradoxical effect was not observed for micafungin against any *C. albicans* or *C. parapsilosis* isolates, but did occur in *C. tropicalis* (70%) and *C. krusei* (60%) isolates. For anidulafungin, this effect was reported in 40% of *C. albicans* and 20% of *C. tropicalis* isolates tested. A high prevalence has also been reported in *C. dubliniensis* isolates upon exposure to caspofungin [19]. Of note is the fact that no paradoxical effect has been observed for any echinocandin in *C. glabrata* isolates.

The attenuation of echinocandin activity at higher concentrations is not limited to *Candida* species. Studies have demonstrated that this also occurs in *A. fumigatus* and *A. terreus* after caspofungin exposure [8,20,21]. In addition to visual turbidity, other assays have also measured this effect, including the XTT colorimetric assay [14,15,17,22], and nuclear magnetic resonance spectroscopy [23]. Interestingly, the range of echinocandin concentrations at which the paradoxical effect occurs in vitro appears to be consistent for *Candida* and *Aspergillus* species with these various assays.

**In-vivo phenotypic observations**

Increases in markers of invasive disease and fungal burden with increasing echinocandin doses have been reported in animal studies. In a neutropenic rabbit model of invasive pulmonary aspergillosis, Petraitiene et al. [24] reported no difference in mean pulmonary infarct score between controls and animals that received caspofungin doses of 3 and 6 mg/kg a day. A significant reduction in this marker of invasive disease was, however, observed at a dose of 1 mg/kg a day. In contrast, animals treated with caspofungin 6 mg/kg a day had the highest degree of hyphal cell wall damage, as assessed by histopathology. Using the same in-vivo model and strain of *A. fumigatus* the investigators reported an increase in serum galactomannan antigenemia in animals treated with micafungin at 2 mg/kg a day compared with a dose of 1 mg/kg a day [25]. Similar to the findings with caspofungin, increased doses of micafungin resulted in further hyphal damage. For micafungin, however, this increase in hyphal fragmentation was associated with significant reductions in pulmonary infarct scores.

A paradoxical increase in fungal burden as measured by quantitative polymerase chain reaction has been reported in a murine model of invasive pulmonary aspergillosis [8]. Despite reductions in pulmonary fungal burden at caspofungin doses of 1 mg/kg a day and 2 mg/kg administered every 48 h, increases in *A. fumigatus* conidial...
equivalents were noted at higher doses (1 mg/kg every 6 h, 4 mg/kg a day, and 8 mg/kg every 48 h), which corresponded to peak plasma levels between 4.6 and 22 μg/ml. It is important to note that in those studies of caspofungin and micafungin, the increases in the pulmonary infarct score, galactomannan, and conidial equivalents were not associated with increased mortality.

A similar effect has also been reported with anidulafungin in the treatment of invasive pulmonary aspergillosis. Petraitis et al. [26] observed similar mean total lung weights between immunocompromised rabbits administered control and anidulafungin doses of 10 and 20 mg/kg a day compared with reductions in lung weights with lower doses (1 and 5 mg/kg a day). As observed with caspofungin and micafungin, dose-dependent damage to hyphal structures did occur. Survival in animals administered anidulafungin 20 mg/kg a day did not, however, differ from that controls in contrast to improved survival with lower doses. Whether this lack of a survival benefit after this dose of anidulafungin was the result of a paradoxical effect or potential toxicity is unclear as increased pulmonary edema was reported in the lungs of animals that received doses of 10 and 20 mg/kg a day. Other investigators have reported necrotic lungs secondary to infarction in uninfected rabbits administered higher doses of anidulafungin [27].

The paradoxical effect has not been reported in each animal study of invasive aspergillosis involving echinocandin therapy [28]. Given the fact that this effect is not observed in every fungal isolate in vitro, it is not surprising that the paradoxical effect is not consistently observed in vivo. It is also important to note that the objective of those studies was not to assess this effect. In a study designed to assess the in-vivo consequence of the attenuation of caspofungin activity at higher concentrations, the paradoxical effect was not reproducible [29]. Although a significantly higher fungal burden was recovered in mice treated with higher doses of caspofungin and infected with a C. albicans isolate previously shown to demonstrate a paradoxical effect, similar results were not observed in an ensuing experiment. Caspofungin pharmacokinetic parameters were, however, not evaluated. Given the quadrifasiche nature of this effect in vitro [16], and the elevated and prolonged caspofungin concentrations observed in murine kidney tissue [30], it is unknown whether the exposures achieved in that study were similar to the in-vitro concentration range in which the paradoxical effect has been observed.

**Potential mechanisms**
The causes of the attenuation of echinocandin activity largely remain unknown. Potential mechanisms that have been suggested include an increase in the synthesis of the cell wall component chitin, upregulation of the protein kinase C (PKC) cell wall integrity pathway, and involvement of the calcineurin pathway. Two plausible explanations, including mutations in FKS1, a gene encoding a catalytic subunit of β-1,3-glucan synthase, and upregulation of β-1,3-glucan synthase activity to overcome the inhibitory effects of echinocandins, have been shown not to occur after exposure to an elevated concentration of caspofungin [31].

To evaluate whether the attenuation of echinocandin activity was associated with increases in cell wall constituents, Stevens et al. [32] quantified β-1,3-glucan, β-1,6-glucan, and chitin after caspofungin exposure at 12.5 μg/ml in a single C. albicans strain in which the paradoxical effect was previously shown to occur. Whereas both β-1,3-glucan and β-1,6-glucan content declined compared with untreated cells, chitin concentrations significantly increased (more than sixfold) after caspofungin exposure. This suggests that a compensatory increase in chitin is associated with the paradoxical attenuation of caspofungin activity in C. albicans. The extent to which chitin may have increased after exposure to lower caspofungin concentrations was not, however, determined. This is relevant because other investigators have described similar fold increases in chitin in C. albicans after exposure to cilofungin at concentrations similar to previously reported MIC₅₀ and MIC₉₀ for this agent (0.3 and 0.6 μg/ml) [10,33].

The roles of the PKC cell wall integrity and calcineurin pathways as potential mechanisms for this effect have also been evaluated. The core of the PKC cell wall integrity pathway is a mitogen-activated protein kinase cascade, which is responsive to perturbations of the cell wall in a number of fungi [34–38]. Studies in Saccharomyces cerevisiae and C. albicans have demonstrated a clear role for the cell wall integrity pathway in the coordination of the fungal response to cell wall damage. In S. cerevisiae rapid induction of SLT2 transcription, a gene encoding a mitogen-activated protein kinase of this pathway has been demonstrated after exposure to caspofungin [39,40]. In S. cerevisiae strains lacking Slt2p activity, a significant decrease in viable cells compared with the isogenic wild-type strain has been observed [40]. Increased expression of the C. albicans homologue of SLT2, MKC1, has also been reported at elevated caspofungin concentrations in which, a paradoxical attenuation of activity has been observed [17]. Furthermore, in a C. albicans Δmck1/Δmck1 homozygous mutant the paradoxical effect was no longer present. This same study also demonstrated that the paradoxical effect was no longer present when cyclosporine A was combined with caspofungin suggesting a potential role of the calcineurin pathway in the attenuation of antifungal activity at elevated echinocandin concentrations.
Recent data have demonstrated that an intact cell wall integrity pathway is required for increased chitin synthesis after cell wall damage. Munro et al. [41**] reported coordination of chitin synthase gene expression and synthesis of chitin by the PKC cell wall integrity, high-osmolarity glycerol mitogen-activated protein kinase, and calcineurin pathways within C. albicans. Activation of these pathways by cell wall damaging agents resulted in increased chitin synthase activity and elevated cell wall chitin content. It may thus be hypothesized that the increased chitin content observed upon exposure to elevated concentrations of caspofungin may be caused by the upregulation of different pathways in response to cell wall damage. Preliminary data in A. fumigatus demonstrating both an increased expression of MPKA, a putative homologue of SLT2, and elevated chitin content after exposure to caspofungin 8 μg/ml support this notion [42]. Others have reported an increased expression of genes for cell wall proteins in an A. fumigatus spontaneous mutant with restored sensitivity to caspofungin at elevated concentrations [20].

Clinical implications
The clinical implications of the paradoxical effect observed in vitro and in vivo are unknown. The concentrations at which this effect occurs may, however, be achieved with standard doses of the echinocandins approved for clinical use (Mycamine package insert, Astellas Pharma US Inc., 2006; Eraxis package insert, Pfizer Inc., 2006; Candidas package insert, Merck & Co., 2005). Currently, no data clearly demonstrate this effect in the treatment of invasive fungal infections. Whereas one case report has described an increase in serum galactomannan in a patient receiving caspofungin monotherapy for invasive aspergillosis despite no initial evidence of clinical deterioration [43], a small retrospective review of patients enrolled in a caspofungin salvage trial did not show evidence of a paradoxical increase in serum galactomannan antigenemia in patients who responded to therapy [44]. This lack of a paradoxical effect in patients receiving treatment for invasive aspergillosis may be caused by the use of echinocandins in combination to treat these infections. In-vivo data have demonstrated that the paradoxical increases in markers of disease burden observed with echinocandin monotherapy were no longer present with the addition of another antifungal agent [45]. A paradoxical attenuation of echinocandin activity has also not been observed in clinical studies of invasive candidiasis/candidemia [3–5]. Despite the high prevalence of this effect reported in vitro against certain Candida species [16,18*], the prevalence may be overestimated as this effect has been reported to be absent when isolates were tested in the presence of 50% human serum [46]. One potential clinical implication that has not been fully explored is a possible role of the paradoxical effect in the development of mutations in the glucan synthase gene FKS1 that have been associated with clinical failures [47–49]. Whereas in-vitro studies have reported fungicidal activity for these agents, this has not always been supported by in-vivo data. This raises the question as to whether the persistence of viable cells as a result of an attenuation of fungicidal activity may lead to mutations within fungi infecting patients who are exposed to prolonged or repeated echinocandin therapy. Further studies exploring this possibility are warranted.

Conclusion
A paradoxical attenuation of echinocandin activity at elevated concentrations has been observed in vitro. Whereas this effect has also been observed in vivo, further studies are warranted to determine the clinical implications, which remain unclear. Determining the mechanisms responsible for the paradoxical effect may increase our understanding of the fungal response to cell wall damage and allow for improved treatment strategies.

References and recommended reading
Papers of particular interest, published within the annual period of review, have been highlighted as:
• of special interest
•• of outstanding interest
Additional references related to this topic can also be found in the Current World Literature section in this issue (pp. 631–632).

Mould-active azoles: pharmacokinetics, drug interactions in neutropenic patients
Paul O. Gubbins

Purpose of review
Mould-active azoles are used to treat systemic mycoses in neutropenic patients because of their broad spectrum activity, their availability as intravenous or oral formulations, and their safety. These agents exhibit complex pharmacokinetics and interact with many drugs, however, which can make their use in neutropenic patients challenging. With the addition of two mould-active azoles to the marketplace in the past several years, this paper will provide an overview of the pharmacokinetics and drug–drug interaction profiles of these azoles and will review the issues surrounding the therapeutic drug monitoring of these agents.

Recent findings
New mould-active azoles have sparked interest in correlating their serum concentrations to efficacy and toxicity. Efforts to establish such correlations have, however, generally proved unsuccessful. All mould-active azoles interact significantly with calcineurin inhibitors.

Summary
When used in combination with mould-active azoles, calcineurin inhibitor doses should be reduced by at least 50% and their blood or serum concentrations should be closely monitored.

Keywords
drug interactions, itraconazole, posaconazole, voriconazole

Introduction
Itraconazole, voriconazole and posaconazole are mould-active azoles. They undergo extensive metabolism, interact with many drugs and their concentrations can be affected by mucositis and emesis associated with cytotoxic therapy. Clinicians should recognize mould-activeazole–drug interactions and understand the limitations of therapeutic drug monitoring (TDM) for these agents in neutropenic patients. This paper reviews the pharmacokinetics, drug–drug interaction profiles and the debate surrounding TDM of these agents in neutropenic patients.

Pharmacokinetics of mould-active azoles in neutropenic patients
Table 1 summarizes the pharmacokinetic properties of the oral mould-active azoles.

Itraconazole
Despite good compliance or high doses (400 or 600 mg), itraconazole capsules rarely achieve reliable, effective concentrations [1]. The oral solution quickly produces higher concentrations, but only with loading doses [1]. This formulation’s dilute concentration and poor taste offset its pharmacokinetic benefits and make it impractical for prolonged use. The intravenous solution eliminates the bioavailability concerns. Data suggest that the intravenous loading dose fails to produce concentrations exceeding 500 ng/ml in many patients, however, and may be insufficient to achieve ‘therapeutic concentrations’ rapidly [2]. Moreover, data also suggest that clinicians should not assume concentrations will be within the ‘therapeutic range’ for at least a week [2].

Voriconazole
In immunocompromised adults voriconazole is rapidly and well absorbed, exhibits non-linear elimination, and accumulates up to fivefold with multiple dosing for 2 weeks [3]. Unlike in adults, in immunocompromised children voriconazole exhibits linear pharmacokinetics, after multiple dosing [4]. Voriconazole should be dosed at 4 mg/kg every 12 h in children, to optimize drug exposure, and approximate the adult dosage [4]. Like all mould-active azoles voriconazole pharmacokinetics demonstrate significant intersubject variability (35–69%) [3,4]. Whether dose proportionality is maintained throughout childhood is unknown. Consequently, additional pediatric voriconazole pharmacokinetic data

Table 1: Pharmacokinetic properties of mould-active azoles in neutropenic patients

<table>
<thead>
<tr>
<th>Mould-activeazole</th>
<th>Oral formulation</th>
<th>Intravenous formulation</th>
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<tbody>
<tr>
<td>Itraconazole</td>
<td>Rarely achieves reliable, effective concentrations</td>
<td>Loading doses produce higher concentrations</td>
</tr>
<tr>
<td>Voriconazole</td>
<td>Rapidly and well absorbed</td>
<td>Linear pharmacokinetics in children</td>
</tr>
</tbody>
</table>

Abbreviations
BCRP breast cancer resistance protein
HSCT hematopoietic stem cell transplant
TDM therapeutic drug monitoring
UGT uridine diphosphate glucuronosyltransferase

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are needed before prescribed dosages can routinely exceed 4 mg/kg [4].

Posaconazole

In neutropenic hematopoietic stem cell transplant (HSCT) recipients increasing dosing frequency and doses up to 800 mg/day produced dose-related but less than dose proportional increases in maximum concentration and exposure [5]. At steady state, total exposure and mean apparent oral clearance increased with dose and dosing frequency [5]. In one study [5], most patients (85%) developed oral mucositis, but the majority of cases (70%) were mild to moderate (grades 1 to 2) in severity. Although mucositis reduced exposure, the effect was not significant and lessened with increasing total dose and dosing frequency.

In neutropenic patients with fever or refractory invasive fungal infection, posaconazole exposure increases proportionately up to 800 mg/day. From a pharmacokinetic standpoint there is no benefit to administering higher total doses singly or in divided doses [6]. Posaconazole 400 mg twice a day provides the best overall mean exposure and produces average serum posaconazole concentrations 57% higher than posaconazole 600 mg twice a day [6]. In neutropenic patients, like healthy volunteers, increasing total daily dose and dividing the dose increases exposure up to 800 mg/day. Administering 800 mg/day in at least two divided doses optimizes posaconazole pharmacokinetics. Differences in study design preclude direct comparisons, but between the studies there are notable disparities in pharmacokinetic values for similar total doses given at the same dosing frequency. These disparities may reflect the increased incidence of diarrhea, vomiting and mucositis seen among the neutropenic HSCT recipients, or perhaps there were differences between the studies in the patients’ nutritional intake during dosing.

Biotransformation of mould-active azoles

Table 1 summarizes the interactions between the mould-active azoles and phase I and II enzymes. All mould-azoles (and perhaps their metabolites) are cytochrome P450 substrates and inhibitors, but the degree to which they interact with cytochrome P450 proteins differs. To some extent, they all inhibit CYP3A4, which is the most common cytochrome P450 involved in drug metabolism.
Itraconazole

Itraconazole is converted by CYP3A4 to many metabolites including hydroxyitraconazole, keto-itraconazole, and N-desalkyl-itraconazole [7]. The principle metabolite, hydroxyitraconazole, is formed primarily in the intestine and is subsequently converted to keto-itraconazole, which undergoes further dealkylation to form N-desalkyl-itraconazole [7]. Itraconazole and perhaps these metabolites only inhibit CYP3A4 [7].

Voriconazole

Voriconazole is a CYP2C19, 2C9, and 3A4 substrate [7]. The principle N-oxide voriconazole metabolite is formed primarily by CYP2C19, CYP3A4 [7]. CYP2C19 and CYP2C9 exhibit genetic polymorphism, which if expressed result in deficient or absent enzyme activity. The CYP2C19 polymorphism is most prevalent among Asian, Polynesian and Micronesian populations, whereas the CYP2C9 polymorphism is most prevalent among Caucasians, and is less frequent among African-Americans, and absent in Asian populations. Voriconazole may potentially interact with many medicines because it inhibits CYP2C9, CYP2C19, and CYP3A4.

Posaconazole

Only 17% of a posaconazole dose is converted to more polar metabolites [8]. Posaconazole is minimally (2%) metabolized by cytochrome P450. The majority of posaconazole metabolites are glucuronide conjugates formed via uridine diphosphate glucuronosyltransferase (UGT) pathways [8,9]. Posaconazole inhibits CYP3A4 [10].

Mould-activeazole interactions with transporter proteins

Many transport proteins facilitate the uptake or efflux processes involved in drug disposition [11]. P-glycoprotein, an efflux protein that shares substrate specificity, and is extensively co-localized with CYP3A in the intestine, liver, kidney, and the cells of the blood–brain barrier [11]. Interactions between P-glycoprotein and the mould-active azoles vary. Itraconazole is a P-glycoprotein substrate and inhibitor, but voriconazole is neither a substrate nor an inhibitor [7]. Given its chemical similarities to itraconazole, posaconazole is probably a P-glycoprotein substrate [8].

Breast cancer resistance protein (BCRP), another transporter that functions like P-glycoprotein, is expressed in the placenta, small intestine and liver. Itraconazole is a potent BCRP inhibitor, but voriconazole is not. Whether posaconazole inhibits BCRP is unknown [12**].

Therapeutic drug monitoring of mould-active azoles

The significant interpatient variability observed with all mould-active azoles and genetic polymorphisms that can influence voriconazole pharmacokinetics have sparked interest in TDM for the mould-active azoles.

There is much interest in voriconazole TDM to help predict treatment outcomes, visual disturbances or transaminase abnormalities. Voriconazole produces visual disturbances or clinically significant transaminase abnormalities in approximately 30 and 12.7% of patients, respectively [13**]. The odds of experiencing visual disturbances are significantly related to serum concentrations [13**]. Estimates predict for every 1 µg/ml increase in serum voriconazole concentration up to 9 µg/ml, the odds of experiencing visual disturbances increase 4.7% [13**]. How much this probability increases at concentrations exceeding 9 µg/ml is unknown. The visual disturbances are transient, fully reversible, and are not associated with any long-term sequelae. Therefore the utility of TDM to prevent these effects is questionable.

Studies attempting to predict transaminase abnormalities have yielded conflicting results. Consequently, whether voriconazole is more hepatotoxic than other azoles, or requires routine TDM is debatable. The frequency of transaminase abnormalities may increase with voriconazole dose [3,14]. In a study of invasive aspergillosis, six of 22 patients (27%) with voriconazole concentrations exceeding 6 µg/ml developed transaminase abnormalities or liver failure [15].

Transaminase abnormalities occurred frequently in phase II and III voriconazole clinical trials, however, most cases were considered mild to moderate in severity and rarely (~3%) resulted in drug discontinuation [16]. A retrospective longitudinal logistic regression analysis provides compelling data suggesting that factors other than elevated serum drug concentrations contribute to transaminase abnormalities in patients receiving voriconazole. Serum voriconazole concentrations are significantly, but weakly, associated with the occurrence of transaminase abnormalities [13**]. Maximum rates of transaminase abnormalities are observed at the highest serum concentrations. For every 1 µg/ml increase in serum voriconazole concentrations, however, the probability of an abnormal alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, or bilirubin level increases only 7–17% [13**]. In the absence of a threshold concentration above which the risk of transaminase abnormalities increases compared with that of lower concentrations, voriconazole TDM is not useful in predicting subsequent transaminase abnormalities [13**].

Utilizing voriconazole TDM to optimize efficacy has proved equally challenging. Serum voriconazole concentrations ranging from 250 ng/ml to 2.05 µg/ml have been suggested as the minimum ‘effective’ threshold serum...
voriconazole concentration [15,17,18*]. These minimum thresholds are, however, based on data from small, poorly controlled, retrospectively designed studies of heterogeneous patient populations and invasive mycoses. Therefore a validated ‘therapeutic’ threshold concentration does not exist.

Rigorous, prospective data are needed to resolve the debate surrounding the role of TDM for the mould-active azoles. These agents pose several challenges for future studies designed at establishing threshold serum concentrations for safety or efficacy. All mould-active azoles exhibit considerable interpatient variability. Itraconazole and voriconazole (in adults) also exhibit non-linear pharmacokinetics making it difficult to predict their serum concentrations based upon dose. Furthermore, there are few data describing the penetration or activity of the mould-active azoles at the infection site (i.e. tissue). Whether serum mould-active azole concentrations are valid approximations of their concentrations or activity in tissue is unknown. Finally, susceptibility testing of invasive moulds is not standardized nor routinely performed.

Mould-activeazole drug interactions in neutropenic patients
Mould-active azoles are widely used in neutropenic patients, but they interact with many agents used for immunosuppression, and supportive care is unavoidable in this population. For a more detailed discussion of drug interactions between mould-active azoles and medicines in general, the reader is referred to more comprehensive reviews [19,20].

Itraconazole
In neutropenic patients the most clinically significant itraconazole drug interactions involve gastric pH modifiers, the calcineurin inhibitors, corticosteroids, and several chemotherapeutic and supportive care agents.

Gastric pH modifiers
H2-receptor antagonists, proton pump inhibitors and antacids reduce the absorption of itraconazole capsules up to 66%, but do not affect the absorption of the oral solution [7,21].

Immunosuppressive agents
Many case reports or small case series describe an interaction between oral itraconazole and oral calcineurin inhibitors. Because of the nature of these reports, the magnitude of the interaction between these agents varies substantially. Reported increases in blood cyclosporine concentrations range from 40 to 226% and have required up to 84% reductions in cyclosporine dosing [2*]. Likewise, reported increases in serum tacrolimus concentrations range two to sevenfold and have required up to 75% reductions in tacrolimus dosing [2*]. Similar to oral dosing, within 2–3 days of starting intravenous itraconazole with either intravenous cyclosporine A or tacrolimus, mean steady-state blood cyclosporine A or serum tacrolimus concentrations increase 80 and 83%, respectively. The increases in concentrations are variable for cyclosporine A (24–149%) and tacrolimus (49–117%). [2*]

The interaction probably results from the inhibition of CYP3A4-mediated calcineurin biotransformation. Case reports suggest that the interaction may persist for weeks or perhaps months after itraconazole therapy has been stopped.

Itraconazole inhibits the metabolism of oral and intravenous methylprednisolone and dexamethasone. Pharmacokinetically, the interactions produce up to fourfold increases in maximum concentration, half-life, or exposure, and pharmacodynamically the morning plasma cortisol concentration declines approximately 80% [7]. Itraconazole minimally affects the pharmacokinetics and pharmacodynamics of prednisolone [7].

An interaction between itraconazole and sirolimus manifesting within 6 days of initiating sirolimus (7 mg/day) has been described in an HSCT recipient. Sirolimus dosing was reduced and subsequently withheld for 8 days. Ultimately, sirolimus was re-initiated at a substantially reduced dosage (1 mg/day), and then empirically reduced 50% when the patient was switched from itraconazole to voriconazole [22*].

Miscellaneous chemotherapeutic agents
Safety and ethical concerns preclude conducting controlled trials to investigate potential drug interactions involving cancer chemotherapeutic agents. Information concerning the potential mould-active azole interactions must be gleaned from small case reports describing their concomitant use in patients receiving cancer chemotherapy.

Vinca alkaloids
Itraconazole reduces CYP3A4 metabolism and P-glycoprotein efflux of vincristine. This produces elevated vincristine concentrations that manifest as neurological toxicities (seizures, paresthesia, sensory deficits, muscle weakness, neuropathy), gastrointestinal disturbances (abdominal pain/distention, constipation, ileus) hyponatremia, and the syndrome of inappropriate antidiuretic hormone secretion [23]. This interaction develops approximately 2.5 weeks after starting itraconazole. Symptoms abate days to weeks after discontinuing itraconazole, but because of the severity of symptoms, during the interaction the antineoplastic regimen is usually interrupted [23]. Itraconazole also interacts with vinblastine, as illustrated in a recent report of a patient receiving vinblastine, doxorubicin, methotrexate and prednisone.
The patient developed profound neutropenia and neurological toxicity, including severe abdominal, lower back, and lower extremity neuropathic pain with paresthesias approximately 2 weeks after starting itraconazole [24**]. Itroconazole probably inhibited cytochrome P450 metabolism or P-glycoprotein efflux of doxorubicin, and vinblastine.

**Epidermal growth factor receptor tyrosine kinase inhibitor**

Gefitinib, a CYP3A4 substrate and a weak inhibitor of CYP2D6, is used in the treatment of advanced non-small-cell lung cancer. Depending on the gefitinib dose, concomitant itraconazole significantly increases its exposure 61–78%. Despite these increases, the interaction produced no significant increase in toxicity [25].

**Alkylating agents**

Cyclophosphamide, a prodrug used in myeloablative conditioning therapy before HSCT, undergoes a series of biotransformation reactions to yield the active forms that produce its cytotoxic effects. In addition to phase II enzymes, CYP2B6, CYP3A4, CYP2C9 and CYP2A6 are all involved in cyclophosphamide biotransformation [26]. Preliminary data suggest that itraconazole alters cyclophosphamide metabolism, and in doing so may predispose patients to specific toxicities related to the affected metabolites [27].

**Miscellaneous supportive care agents**

Phenytoin is given as seizure prophylaxis to patients receiving busulfan-containing preparative regimens. Phenytoin reduces serum itraconazole and hydroxyitraconazole concentrations 90%. In patients receiving this combination and a calcineurin inhibitor, however, the magnitude of this interaction may not prevent the interaction between itraconazole and calcineurin inhibitors [2**].

**Loperamide**

Loperamide, a peripherally acting μ-opioid receptor agonist used in the treatment of diarrhea in neutropenic patients, is a CYP3A4, CYP2C8 and P-glycoprotein substrate. Itraconazole increases the maximum concentration and exposure of loperamide approximately three to fivefold, respectively, and prolongs its half-life approximately 60% [28*]. The combination of itraconazole and gemfibrozil, a CYP2C8 inhibitor, synergistically increases the maximum concentration and exposure of loperamide approximately four to 12-fold, respectively, and prolongs its half-life approximately 210% [28*]. Although no increase in adverse effects occurred, clinicians using these agents in combination should be aware of any unexplained drowsiness or vomiting [28*].

**Voriconazole**

The most clinically significant voriconazole drug interactions in neutropenic patients involve the calcineurin inhibitors. Notable interactions also occur with prednisolone and supportive care agents.

**Immunosuppressive agents**

Voriconazole produced significant but variable (mean 70%; range 21–120%) increases in cyclosporine exposure in renal transplant recipients who completed a placebo-controlled crossover study. Trough cyclosporine concentrations were also elevated (mean 70%) in those who completed the study, whereas in patients who did not, the mean increase in trough cyclosporine concentrations was more substantial (mean 248%) [29]. A corresponding increase in treatment-related adverse effects was noted in patients receiving voriconazole, but many were deemed mild to moderate in severity. Case reports indicate that voriconazole also causes significant increases in tacrolimus minimum concentration that are much greater in magnitude than predicted by in-vitro studies [7,30].

Expert guidelines for preventing opportunistic infections among HSCT recipients recommend fluconazole (a mould-inactive azole), until engraftment to prevent candidiasis. If a breakthrough infection occurs, therapy must be switched to an agent active against fluconazole-resistant *Candida* spp. or invasive moulds (i.e. mould-active azoles, polyenes, echinocandins). HSCT recipients commonly receive calcineurin inhibitors, which interact with fluconazole and voriconazole. When switching from fluconazole to voriconazole in HSCT recipients the tacrolimus dose should be reduced perhaps as much as 80% [31**].

Voriconazole reportedly increases systemic sirolimus exposure 11-fold on average [32*]. Therefore, the coadministration of these agents is contraindicated. Retrospective case series suggest this significant interaction may be manageable [33,34**]. In the largest report (n = 11), the sirolimus dose was empirically reduced 90% in eight patients (73%) upon starting voriconazole [34**]. Patients receiving the reduced dose had many sirolimus concentrations measured (range 1–53; median 6.5), but only one concentration exceeded the targeted therapeutic trough range (3–12 ng/ml) [34**]. Furthermore, the median average steady-state trough concentrations were similar to pretreatment values [34**]. In contrast, patients who did not have the dose empirically reduced had fewer concentrations measured and plasma sirolimus concentrations routinely exceeded the targeted therapeutic range [34**]. Depending on the dose, voriconazole increases oral prednisolone exposure 13–30%. Like itraconazole these changes are not clinically significant [7].

**Miscellaneous supportive care agents**

Many neutropenic patients receive supportive care agents such as analgesics and gastric acid modifiers. Some
Many neutropenic populations require pain management, but there are few data regarding the effect of concomitant mould-active azoles on analgesics. Existing data are generally from non-neutropenic populations and provide little insight on the clinical consequences of any interaction.

The role of non-steroidal anti-inflammatory drugs (NSAIDs) in cancer pain is well established. Ibuprofen is a chiral compound and the S enantiomer is responsible for most of its pharmacological activity. This enantiomer is metabolized primarily by CYP2C9. Voriconazole significantly increases the plasma concentration and exposure of this enantiomer and prolongs its half-life approximately 45% [35]. Alfentanil is a short-acting, synthetic opioid analgesic that may be used as anesthesia during painful diagnostic and therapeutic procedures. The drug is primarily metabolized by CYP3A4. Voriconazole decreases the mean alfentanil plasma clearance 85%, and increases exposure sixfold. The interaction also significantly prolongs its half-life [36].

Methadone hydrochloride is a μ-opioid receptor agonist used in the management of cancer pain both in opioid-naive patients and in rotation from other opioids. Methadone is a chiral compound with a pharmacologically active R enantiomer. The oxidative metabolism of methadone involves multiple cytochrome P450 enzymes including CYP3A4, CYP2C19 and CYP2C9. Voriconazole significantly increases the exposure and maximum concentration of (R)-methadone 47.2 and 30.7%, respectively [37].

**Omeprazole**

Omeprazole, a proton pump inhibitor, is metabolized by CYP2C19 and CYP3A4 [7]. Voriconazole co-administration increases the steady-state maximum concentration and exposure of omeprazole approximately two and fourfold, respectively. The omeprazole half-life also increases approximately 1 h [7].

**Phenytoin**

Co-administration of oral voriconazole 400 mg twice a day for 10 days increased the steady-state maximum concentration and exposure of phenytoin approximately 70 and 80%, respectively [7]. The bidirectional nature of this interaction has been characterized. Phenytoin 300 mg a day for 2 weeks significantly reduces the steady-state plasma maximum concentration and systemic exposure of voriconazole approximately 53 and 72%, respectively, for up to 12 h postdose. The effect of phenytoin can be overcome by doubling the voriconazole dose from 200 mg twice a day to 400 mg twice a day; however, as described above, at that dosage voriconazole inhibits cytochrome P450-mediated phenytoin metabolism [7].

**Posaconazole**

There are few published data from properly controlled studies investigating posaconazole drug interactions in neutropenic patients.

**Immunosuppressive agents**

Posaconazole significantly interacts with the calcineurin inhibitors. In a small number of heart–lung transplant recipients, posaconazole increased cyclosporine exposure and necessitated cyclosporine dosage reductions of 14–29% [38**]. Like all mould-active azoles, posaconazole plasma concentrations exhibit considerable interpatient variability (71–141%) [38**]. The study was conducted with posaconazole tablets rather than the marketed suspension and used a lower dose (200 mg once a day) than is currently recommended. A simulation performed to predict what the interaction with cyclosporine would be at a clinically relevant dose (600 mg divided in three doses) revealed that cyclosporine concentrations would increase 50% [38**].

A significant interaction was observed between multiple dose posaconazole and single-dose tacrolimus in healthy subjects. Posaconazole increased the maximum concentration and exposure of tacrolimus 121 and 358%, respectively [38**]. The interaction also prolonged the tacrolimus half-life 23% (from 29.0 to 35.9 h), and decreased total body clearance from 79% (from 21.4 to 4.31 l/h) [38**].

**Miscellaneous supportive care agents**

Other posaconazole drug interactions in neutropenic patients remain to be elucidated, but two recent studies indicated that posaconazole has the potential to interact with CYP3A4 substrates even though it undergoes minimal CYP3A4 metabolism, and inhibits CYP3A4 less than other mould-active azoles. In one study, posaconazole increased the maximum concentration and exposure of rifabutin 31 and 72%, respectively [39**]. This interaction is bidirectional as the co-administration of rifabutin reduced the maximum concentration and exposure of posaconazole 43 and 49%, respectively [39**].

Posaconazole is primarily metabolized by UGT pathways (phase II enzymes). As CYP3A4 substrates may also interact with phase II enzymes, CYP3A4 inhibition may not be the sole cause of the interaction. This is illustrated by the interaction between posaconazole and phenytoin. At steady-state phenytoin significantly reduced the maximum concentration and exposure of
Mould-active azoles

Gubbins 585

posaconazole 44 and 52%, respectively \([40^*]\). There was also a corresponding 57% reduction in half-life and a 90% increase in steady-state clearance of orally administered posaconazole \([40^*]\). Although there was considerable interpatient variability in posaconazole concentrations, because the maximum concentration and exposure of phenytoin were not significantly altered by concomitant posaconazole administration, it is unlikely that the interaction was mediated solely by CYP3A4. Rather, the increase in posaconazole clearance may have resulted from phenytoin induction of UGT \([40^*]\).

Conclusion

Mould-active azoles are often used in neutropenic patients. Their pharmacokinetics are, however, complex and the role of TDM for these agents is debatable. Mould-active azoles can interact with many medicines. In neutropenic patients their drug interactions are somewhat unavoidable because they all significantly interact with the calcineurin inhibitors. If these agents are used in combination, depending on the calcineurin inhibitor, empirical dosage reductions of at least 50% may be required. Initially, empirical dosage adjustment may be necessary, but after the combination is started, measured calcineurin concentrations should guide dosage adjustments.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

\* of special interest

\** of outstanding interest

Additional references related to this topic can also be found in the Current Literature section in this issue (p. 632).


2. Leather H, Boyette RM, Tian L, Wingard JR. Pharmacokinetic evaluation of the drug interaction between intravenous itraconazole and intravenous tacrolimus or intravenous cyclosporin A in allogeneic hematopoietic stem cell transplant recipients. Biol Blood Marrow Transplant 2006; 12:325–334. This study provides the first controlled data regarding the interaction between intravenous itraconazole and the intravenous calcineurin inhibitors. It also provides compelling data that the intravenous itraconazole loading dose may be insufficient in neutropenic patients.


12. Gupta A, Usadkat JD, Mox G. Interactions ofazole antifungal agents with the human breast cancer resistance protein (BCRP). J Pharm Sci 2007; [E-pub ahead of print] doi: 10.1002/jps.20963. This paper provides a rigorous statistical analysis to address concerns regarding transaminase abnormalities and visual disturbances associated with voriconazole administration. In doing so it provides the first estimates regarding the probability of these adverse effects occurring with increasing serum concentrations. Before this paper such observations were based largely on opinion, or very small single institution experiences.


22. Said A, Garnick JJ, Dietz JN, et al. Sirolimus–itraconazole interaction in a hematopoietic stem cell transplant recipient. Pharmacotherapy 2006; 26:289–295. A notable case report because currently there are no published trials investigating the interactions between sirolimus and the mould-active azoles. The occurrence of this interaction is intuitive, but without published experience, the management of the interaction will be empirical or largely trial and error. Although it is likely that such studies may be published for recently marketed mould-active azoles, experience with itraconazole will probably have to be gleaned from case reports such as this.


An interesting paper regarding a compound that is metabolized by two different cytochrome P450 enzymes. It demonstrates clearly that pharmacokinetic consequences of the inhibition of both enzymes by specific inhibitors is greater than either one alone. It was interesting that a pharmacodynamic assessment was included in the study, but the pharmacokinetic changes did not produce any untoward pharmacodynamic effects.


This paper is the largest published experience with this interaction. It is of practical use in that, although as suggested by the manufacturer’s labeling the combination should be avoided, this is sometimes not possible in clinical practice. The paper offers a potential solution to how to manage this interaction.


In this interesting study, ibuprofen is well tolerated, but has notable toxicities (gastric, intestinal bleeding, etc.). The study shows that voriconazole can increase concentrations of the active enantiomer of ibuprofen and raises the possibility that the interaction could increase the incidence of adverse effects.


An interesting study demonstrating a significant interaction that could be overlooked.


An interesting study demonstrating a moderate interaction that could be overlooked.


This paper is notable in that it provides important data regarding the interaction between posaconazole and both cyclosporine and tacrolimus. The paper is also important because it illustrates that even drugs that are minimally metabolized by CYP3A4 can still be potent inhibitors of this important enzyme. Often this distinction is missed or confused, and clinicians can mistakenly think that as posaconazole is a poor CYP3A4 substrate it will be relatively devoid of drug interactions.


This paper is notable in that it provides important data regarding the interaction between posaconazole and rifabutin. The study demonstrates that although it is minimally metabolized, it inhibits rifabutin metabolism, which in turn accumulates and induces posaconazole metabolism. Even with the bidirectional nature of the interaction, the magnitude of the effects is surprising, and raises the question as to whether the interaction also involves phase II enzymes.


This paper is notable in that it provides important data regarding the interaction between posaconazole and phenytoin. The study demonstrates that cytochrome P450 inducers can also induce phase II enzymes such as UGT. This may partly explain interactions with the mould-inactiveazole fluconazole, which also interacts with the UGT pathway.
Purpose of review
Studies of antibiotic pharmacokinetics and pharmacokinetic–pharmacodynamics are useful in the design of optimal dosing strategies. This review examines recent advances in echinocandin pharmacokinetics and pharmacodynamics, and discusses how these studies could lead to newer dosing strategies for the treatment of invasive candidiasis.

Recent findings
Recent population pharmacokinetic analyses of caspofungin and micafungin suggest that the patient’s weight may affect the echinocandin serum drug concentrations achieved. This suggests that population pharmacokinetic studies in overweight and obese patients for all currently licensed echinocandins are needed. Pharmacokinetic–pharmacodynamic considerations suggest that high intermittent dosing of echinocandins may be desirable for the treatment of invasive candidiasis. For some agents such as micafungin, pharmacokinetic simulations have even identified human doses that could be used in these intermittent dosing schemes. These dosing strategies were subsequently found to be effective therapy in animal models. The potential limitations of high drug doses include a paradoxical decrease in microbial kill as well as the toxicity of high intermittent doses in patients. These will need to be addressed in clinical studies.

Summary
A better understanding of caspofungin, micafungin, and anidulafungin pharmacokinetic–pharmacodynamics will help in the design of new optimized dosing regimens for invasive candidiasis.

Keywords
candidiasis, echinocandins, intermittent dosing, pharmacokinetic–pharmacodynamics

Abbreviations
AUC area under the concentration–time curve
MIC minimum inhibitory concentration
PAE postantibiotic effect
PK–PD pharmacokinetic–pharmacodynamics

Introduction
The three echinocandins, caspofungin, micafungin, and anidulafungin, have been licensed and been shown to be highly effective in the treatment of invasive candidiasis [1–3]. The development of echinocandins has benefited from advances in the science of pharmacokinetic–pharmacodynamics (PK–PD). This review examines the pharmacokinetic and PK–PD literature over the past year (2006–2007) and discusses the potential of these new studies to provide solutions for new dosing strategies. The review will focus only on dosing strategies for the treatment of candidiasis.

Pharmacokinetic–pharmacodynamics principles applied to echinocandins
The relationship between dose (or exposure) and microbial kill is best described by the use of the inhibitory sigmoid maximum exposure (Emax) model (Fig. 1). The relationship between exposure and effect is a sigmoid shape, with the steep portion described by the Hill or slope factor (H). The maximal amount of kill by the drug is termed the Emax and denotes the efficacy of the drug. The dose associated with 50% of maximal kill is the EC50 and denotes the potency of the drug. The fourth parameter, Econ, denotes the fungal burden without treatment (i.e. controls). The inhibitory sigmoid Emax model will be utilized to discuss echinocandin dosing strategies.

The patterns of microbial kill are described by one of several PK–PD indices that include the area under the concentration–time curve (AUC) to the minimum inhibitory concentration (MIC), peak concentration to MIC (Cmax/MIC), the time that the drug concentration is above the MIC (T MIC), and the postantibiotic effect (PAE). If a drug’s microbial kill best correlates with the Cmax/MIC ratio, then administration of the same cumulative dose on a more intermittent basis, as opposed to more frequent dosing, would optimize efficacy. On the other hand, if the drug’s efficacy is best explained by the T MIC, then more frequent dosing would optimize efficacy, whereas
more intermittent dosing would reduce the efficacy. If efficacy is AUC/MIC linked, however, then the dosing schedule has little effect on microbial kill, and as long as the same cumulative dose is administered during a particular dosing interval intermittent dosing will have the same microbial effect as a more frequent dosing schedule. Drugs that have a Cmax/MIC or AUC/MIC linked effect often have a long PAE duration. In this paper, these PK–PD indices will be used to determine the best dosing schedule for echinocandins.

Impact of echinocandin population pharmacokinetics on individualization of doses

The existence of between-subject and within-subject variability of pharmacokinetic parameters means that a fixed dose of an echinocandin will result in a large distribution of drug exposures when that fixed dose is administered to a large population. Therefore, some patients will respond better than others based on the amount of exposure, and some patients will have a higher likelihood of experiencing a concentration-driven toxicity. The important sources of variability should be identified. Therefore, good population pharmacokinetic analyses are a prerequisite to proper echinocandin dose design and dose optimization. Nguyen et al. [4**] recently demonstrated a 24-fold difference in caspofungin trough levels in 40 surgical intensive care unit patients being treated for invasive fungal infection, consistent with a high between-subject variability. The two factors that explained the variability were a patient body weight of 75 kg or greater and serum albumin concentrations. We recently performed a population pharmacokinetic analysis of micafungin serum concentrations from 62 adult patients [5]. Patients with a weight of 66.3 kg or greater had 50% higher micafungin serum clearances than leaner patients. This means that a micafungin dose of 150 mg a day in the heavier patient would be needed to achieve the same AUC0–24 that is achieved by 100 mg a day administered to leaner patients. Studies that include large numbers of overweight, obese, and severely obese patients are needed for all three echinocandins, so that it can be determined how much to increase the dose for heavier patients, and if such dose individualization improves outcomes.

Impact of anidulafungin pharmacokinetic–pharmacodynamics on efficacy

Anidulafungin and fluconazole microbial responses were recently examined in neutropenic mice infected with three different strains of Candida glabrata [6**]. An example of a fungal response to anidulafungin is shown in Fig. 1. The microbial response to fluconazole was also examined. Inhibitory sigmoid Emax analysis revealed that anidulafungin’s Emax (efficacy) was superior to that of fluconazole, even in mice infected with the fluconazole-susceptible Candida strain. These PK–PD results in mice mean that anidulafungin is more efficacious than fluconazole even if infection was caused by fluconazole-susceptible Candida. These PK–PD conclusions lend experimental support to the potential superiority of anidulafungin to fluconazole, as recently demonstrated in a randomized controlled trial [2]. A second finding in the animal PK–PD study was that 96 h after a single anidulafungin dose of more than the ED50, the measured drug concentrations in tissues were still higher than the MIC50 to clinical isolates of Candida. These persistent concentrations were accompanied by a persistent fungal decline for the 96 h of study. This result is of particular importance in the design of anidulafungin dosing strategies for patients with candidemia. This is because anidulafungin has rather curious pharmacokinetics in that the half-life of approximately one day is the same in mice, humans, and indeed even in the test tube. This is because the important first step in anidulafungin’s elimination is by non-enzymatic degradation. In addition, anidulafungin’s volume of distribution in adult humans is 0.6 l/kg, 0.5 l/kg in children, and 0.5 l/kg in mice, approximating the total body water volume [6**,7,8*]. This means that the translation from anidulafungin exposures in mice to human exposures is relatively straightforward. These similarities, as well as the fact that the murine candidiasis model closely recapitulates events in humans with invasive candidiasis [9], means that it is also likely that fungicidal concentrations of anidulafungin will persist for many days in the tissues of patients with candidiasis, even after a single dose of anidulafungin. This provides a pharmacokinetic rationale for intermittent anidulafungin dosing in humans with invasive candidiasis.
inhibitory sigmoid E_{max} relationship derived in mice could be utilized in future to identify the optimal human dose. An approach could be utilized, based on the work of Bradley et al. [10] and Drusano and colleagues [11–13] with other anti-infective agents, to utilize the inhibitory sigmoid E_{max} relationship derived in mice and calculate the non-protein-bound exposures associated with 80% or greater of E_{max} of echinocandin. Monte Carlo simulations of several thousand patients are then performed to determine how likely it is that a particular human dose will achieve the exposures associated with 80% or more of the maximal effect, given the between-subject variability in pharmacokinetic parameters, as well as the distribution of anidulafungin MICs in Candida clinical isolates. Recently published studies can be used to calculate the non-protein-bound fractions of anidulafungin [14**,15**].

Andes and Marchillo [16] recently examined the effect of anidulafungin dose scheduling in neutropenic mice with disseminated candidiasis. They demonstrated that mice treated with the most intermittent regimen (once every 96 h) had less kidney fungal burden than those treated with more frequent dosing, consistent with a C_{max}/MIC linked effect. Unfortunately, as of yet, that study has only been published in abstract form, so details and a full analysis are not available. Although the result differs from the PK–PD index linked to effect that we have identified (AUC/MIC) for caspofungin and micafungin [17,18**], it is consistent with results from others who have identified C_{max}/MIC as being linked to caspofungin and aminocandin effect [19,20]. The differences in the PK–PD index identified are probably the result of differences in study design and schedules for killing infected mice. The practical point, however, is that both C_{max}/MIC-driven activity and AUC/MIC-driven activity offer strong PK–PD support for designing intermittent anidulafungin dose schedules. If anidulafungin effect were C_{max}/MIC linked then intermittent dosing with high doses would be more efficacious than the current standard of daily dosing.

**Micafungin pharmacokinetic–pharmacodynamics and the design of intermittent dosing regimens**

In the past, micafungin has been demonstrated to have an in-vivo PAE of several days [21]. This is despite the fact that micafungin has a relatively modest half-life in mice (6.3 h). We infected persistently neutropenic mice with C. glabrata, and then treated them with single micafungin intraperitoneal doses between 0 and 100 mg/kg, and examined the kidney fungal burden on days 1, 4 and 7 after the single dose [18**]. Doses (ED_{50}) were associated with persistent fungal decline for the entire one-week dosing interval, with no re-growth. Optimal exposures were associated with a remarkable E_{max} of 6.7 CFU/g at the end of the week. We administered weekly doses as one dose given as a single dose at the start of therapy, the weekly dose divided into two and administered at the start of the week and midweek, or the entire weekly dose divided into seven equal portions and administered daily. Once a week dosing was as effective as daily dosing for doses equal to or greater than the ED_{50}. The effect of micafungin was best explained by the AUC/MIC ratio. This means that in the design of dosing schedules, intermittent therapy would not compromise the effect, as long as a high enough dose was administered. We were interested in mimicking the human micafungin serum concentration–time profiles in mice and examining the efficacy, as has been done in the past with antibacterial agents [22]. We performed a population pharmacokinetic analysis on micafungin serum concentrations from patients treated with 12.5–200 mg micafungin a day [23], and used the results of the analysis in Monte Carlo simulations to predict how doses of 700 mg or more would be handled in a population of approximately 10 000 patients. The study demonstrated that a weekly micafungin dose greater than 700 mg and 1400 mg or less would be the minimum dose associated with persistent fungal killing.

This approach could be used to study the effect of different echinocandin human doses in any animal model, despite the higher drug clearances often encountered in small animals. Figure 2 illustrates this for micafungin in the rabbit model. With such a model, the effectiveness of different ‘human’ intermittent doses can be studied in animals infected with different Candida isolates chosen from a broad representation of clinical strains.

An important reality of clinical care is inadvertent delays in the initiation of therapy, usually as a result of the late recognition of a Candida infection [24]. This leads to poor outcomes [24]. Dosing strategies are needed to address this important clinical problem. Hope et al. [25**] recently examined these questions in a PK–PD study of neutropenic mice treated with either amphotericin B, fluconazole, or micafungin. Inhibitory sigmoid E_{max} modeling for amphotericin B demonstrated a higher E_{max} in neutropenic mice compared with non-neutropenic mice, but no changes in ED_{50} (potency). There was, however, an exponential decline in the E_{max} with progressive delay in amphotericin B therapy, so that a treatment delay of 5 h resulted in an E_{max} of approximately 4\log_{10}CFU/g; a treatment delay of 24 h resulted in an E_{max} of 1.23\log_{10}CFU/g with a decrease in potency, and a delay of 36 h or more resulted in no dose response (i.e. E_{max}≈0). Similarly, there was no dose response with a 24 h delay in the treatment with fluconazole. Micafungin still achieved an E_{max} of 2.3\log_{10}CFU/g with a 24 h delay (versus 4.78\log_{10}CFU/g with a 5 h delay) with no changes in
A potential pharmacodynamic limitation to the concept of high intermittent dosing schedules is the paradoxical effect in which there is a relative resistance to killing at some echinocandin concentrations above the MIC, but not at or near the MIC. This may be caused by a compensatory increase in cell wall chitin as the β-1,3 and β-1,6-glucan levels decrease in response to high caspofungin concentrations [27]. The paradoxical effect has been demonstrated to occur most commonly with caspofungin (seen in 60% C. albicans, 90% C. parapsilosis, 40% C. tropicalis, and 10% C. krusei), and less often with anidulafungin (seen with 40% C. albicans and 20% C. tropicalis) and micafungin (seen with 70% C. tropicalis and 60% C. krusei) [28*]. If these in-vitro paradoxical effects also occur in vivo, they could be a limitation to high intermittent echinocandin dosing, because there might be a paradoxical decrease in microbial kill with high intermittent doses. A recent in-vivo pharmacodynamic study demonstrated that C. albicans isolates showing paradoxical response to caspofungin in vitro do not necessarily exhibit this in vivo, and even when they did the effect was not reproducible [29*]. Further studies are still needed, but the paradoxical effect will probably play a relatively minor role in limiting intermittent high-dose therapy.

Potential limitations to intermittent echinocandin dosing

Although the PK–PD studies we have discussed point towards the potential for intermittent echinocandin therapy, the important potential limiting factor is likely to be the safety of high intermittent doses. The PK–PD studies reviewed in this article were in animal studies, therefore it must not be assumed that patients would tolerate high intermittent doses to the same extent as rodents and rabbits. Clinical studies will be needed to determine the safety of these dosing strategies. It is likely that intermittent high-dose regimens for some of the echinocandins will be limited by dose-dependent toxicity, potentially differentiating one echinocandin from another. However, even if patients were only able to tolerate intermittent doses two or three times the current daily doses, that would still be an important advancement compared with daily therapy, potentially allowing single one to two intermittent doses before stepping down to an oral alternative after microbiological clearance of the Candida.

Conclusion

Population pharmacokinetic studies suggest that optimal doses for echinocandins for patients who are overweight need to be studied in the future. PK–PD studies suggest that all three currently licensed echinocandins have the potential for intermittent therapy. Potential doses to be studied in intermittent therapy regimens have been determined for some echinocandins by the use of PK–PD
modeling methods. Clinical studies are needed to establish the safety of the proposed dosing schemes.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

• of special interest
** of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (pp. 632–633).


A significant effect of patient’s weight and serum albumin concentrations on caspofungin trough concentrations in patients in the surgical intensive care unit was identified. This study suggests that the effect of weight on drug concentration and persist on such pharmacokinetic parameters as clearance, should be examined in obese and overweight patients.


This murine study established that anidulafungin concentrations persist for many days at sites of infection such as kidney tissues where they continue to exert an antifungal effect. The study also established the exposures associated with optimal antifungal effect, which can be used in subsequent studies for dose selection. Finally, the study established the superior efficacy of anidulafungin compared with fluconazole in vivo in mice with invasive candidiasis and invasive aspergillosis.


Good pharmacokinetic studies in children and neonates are often lacking. This study identified the pharmacokinetic parameters of anidulafungin in neutropenic children. These results will be useful in the design of new anidulafungin dosing regimens for children.


Serum protein binding needs to be taken into consideration when translating drug exposures from animal models to patients. This study examined a large number on Candida clinical isolates, and demonstrated that serum protein binding decreases the effectiveness of anidulafungin and micafungin. This was demonstrated to be caused by a decrease in the sensitivity of fungal glucan synthase to echinocandins in the presence of serum.


The authors examined the effect of human and mouse serum on anidulafungin and caspofungin, and demonstrated that human and mouse serum had equivalent effects in reducing the effectiveness of anidulafungin.


This murine study established that micafungin effect is AUC/MIC linked. Using mathematical modeling, a human dose was designed for once a week dosing, and the effectiveness was tested in mice in which human concentration time profiles of micafungin 700–1400 mg once a week were mimicked.


This elegant study examined the dose–response relationships of amphotericin B, fluconazole, and micafungin, in the context of different durations of delay of therapy in mice with disseminated candidiasis. The efficacy of all drugs decreased with delays in therapy, although the micafungin continued to have a dose response with a 24 h delay of therapy. There was no decrease in the potency of micafungin with a delay in therapy, suggesting that doses do not need to be increased even if micafungin therapy is inadvertently initiated late.


This in-vitro study examined many clinical Candida isolates and demonstrated that the paradoxical effect was most often seen with caspofungin, and less so with micafungin and anidulafungin. No paradoxical effect was seen with C. glabrata.


This well-designed study was an attempt to correlate in vitro the paradoxical effect of caspofungin with the in-vivo decrease in killing of C. albicans with high caspofungin doses. Isolates with an in-vitro paradoxical effect did not necessarily exhibit paradoxical effect in vivo, and even for the isolate with in-vivo paradoxical effects, the effect was not consistently reproducible.
Antifungal therapy for neonatal candidiasis
Theoklis Zaoutis\textsuperscript{a,b} and Thomas J. Walsh\textsuperscript{c}

Purpose of review
This review focuses on recent data regarding antifungal prophylaxis and antifungal treatment of neonatal candidiasis.

Recent findings
Candida species are the leading cause of invasive fungal infection in the neonatal intensive care unit, and are the third most common blood culture isolates recovered from cases of late-onset sepsis in the neonatal intensive care unit. Neonatal candidemia is associated with significant morbidity and mortality. Risk factors for neonatal candidiasis have been elucidated, and prophylaxis of high-risk infants has been found to be effective, particularly in neonatal intensive care units with a high incidence of candidemia. Amphotericin B has been the mainstay of antifungal therapy for candidemia in the neonatal intensive care unit but newer agents such as echinocandins and azoles are currently being evaluated.

Summary
Neonatal candidiasis is a serious infection especially in extremely low-birthweight infants and prophylaxis has been shown to be effective in preventing candidiasis in high-risk infants. Newer pharmacokinetic, safety and efficacy data for both older and newer antifungal agents are emerging in neonates, and these data may help us achieve more effective management of neonatal candidiasis.

Keywords
antifungal therapy, candidiasis, fungal infections, neonatal, pediatric

Introduction
Candida species are the leading cause of invasive fungal infections in the neonatal intensive care unit (NICU), and are the third most common blood culture isolates recovered from cases of late-onset sepsis in the NICU. Invasive candidiasis is frequently associated with dissemination to various organs with resultant end-organ damage [1]. The incidence of neonatal candidiasis (candidemia or disseminated candidiasis) in extremely low-birthweight (ELBW) infants (<1000 g) is 7–20%, and it decreases with increasing birthweight to less than 1% in neonates with a birthweight greater than 1500 g [2].

Neonatal candidiasis is associated with significant morbidity and mortality. A recent study suggested that the mortality attributable to candidiasis in ELBW infants is 12% and such infants with candidiasis were two times more likely to die than matched control infants [3]. In addition to extremely low birthweight, other risk factors for neonatal candidiasis are listed in Table 1 [4].

This review will focus on antifungal prophylaxis and treatment of neonatal candidiasis.

Treatment of neonatal candidiasis
Treatment of Candida infections in neonates is based largely on extrapolation of data from adults. There is a paucity of randomized trials or controlled observational studies evaluating the treatment options for neonatal candidiasis. Antifungal therapy with one or more of the agents shown in Table 2 [4] is the mainstay of therapy. Newer agents such as the echinocandins and broad-spectrum azoles are currently being evaluated. In addition to antifungal therapy, the removal of a central venous catheter is an important adjunctive intervention. Failure to remove or delayed removal of central venous catheters in neonates with candidiasis has been associated with persistent candidemia and worse outcomes.

Polyenes
Amphotericin B is the only member of the polyene class of medications used to treat deeply invasive fungal infections. Amphotericin B has classically been considered the ‘gold standard’ for the treatment of invasive fungal infections in adults and children, including neonates. The molecule binds to ergosterol (a sterol component unique to fungal cell membranes), alters cell permeability, and induces pore formation, leading to the leakage of cytoplasmic contents and fungal death. There are limited data on the pharmacokinetics of amphotericin B in neonates.
The disposition of amphotericin B is more variable in neonates, and the serum half-life tends to be longer than in older children or adults. Neither serum concentrations nor pharmacokinetic properties appear to predict efficacy or safety. Dosages are similar to those used in older children and adults. Amphotericin B achieves concentrations in the central nervous system (CNS) sufficient to eradicate *Candida albicans* from tissue. Cerebrospinal fluid (CSF) levels are variable and not necessarily predictive of outcome. Neonates seem to tolerate amphotericin B better than adults with less nephrotoxicity and fewer fevers, chills, and rigors associated with infusion of the drug. Hypokalemia may, however, be severe and extended usage may lead to renal impairment.

Data on the use of the lipid preparations of amphotericin B in neonates are limited. A study of amphotericin B lipid complex found distribution similar to that of other groups and clearance that varied according to weight. The study concluded that dosages of 2.5–5.0 mg/kg per day were appropriate for the treatment of invasive candidiasis in neonates [5]. The pharmacokinetic properties of liposomal amphotericin B have not been studied in neonates. The successful use of these preparations in the treatment of neonatal candidiasis is limited to case series. Results from such case series suggest that lipid formulations of amphotericin B are effective antifungal agents for the treatment of neonates with invasive candidiasis.

### 5-Fluorocytosine

5-Fluorocytosine is a fluorinated analogue of cytosine. It is converted to 5-fluorouracil by fungal cells and then further converted to metabolites that inhibit fungal RNA and DNA synthesis. 5-Fluorocytosine is thought to enhance the antifungal activity of amphotericin B, especially in the CNS, and it has been used in neonates primarily in combination with amphotericin B for the treatment of candidemia. The lack of an intravenous formulation of 5-fluorocytosine limits its usefulness in the NICU population because many ELBW infants with candidiasis are not fed orally. The major side effects of 5-fluorocytosine are dose-dependent myelosuppression and gastrointestinal toxicity; the latter may lead to necrotizing enterocolitis. Serum levels of 5-fluorocytosine should be monitored in order to minimize toxicity. In addition, 5-fluorocytosine is not recommended as monotherapy because resistance develops rapidly. Moreover, as there are no prospective controlled trials of 5-fluorocytosine in combination with amphotericin B, it should be used with caution in this population because of the potential risks.

### Azoles

The azoles are a class of antifungal agents that inhibit the production of ergosterol, the major sterol component of the fungal cell membrane. As the sterol target of azoles is a cytochrome P-450 enzyme, azoles interact with other medications and can cause hepatotoxicity. Of the azoles, fluconazole is the only agent with significant data on use in neonatal populations. The use of voriconazole in neonates is limited.

Fluconazole is available as an oral or intravenous form; the bioavailability of the oral form of fluconazole is 90%. Metabolism accounts for only a minor proportion of fluconazole clearance, and unchanged drug is predominately cleared by the kidneys. Fluconazole rapidly passes into all body fluids including the CSF. The recommended dosage of fluconazole for children (>2 years of age) is 6–12 mg/kg per day. Because fluconazole exhibits a faster clearance rate, larger volume of distribution, and favorable safety profile, a dose of 12 mg/kg per day may be more appropriate in full-term neonates 2 weeks or more postpartum. In preterm neonates less than 1500 g, however, in whom renal immaturity is an important factor, a dosing regimen of 6–12 mg/kg per day once every 72 h has been advocated during the first week of life, based on the initially decreased clearance of fluconazole. This dosage regimen has not been validated. There are several published studies including 10 or more patients that suggest that fluconazole is effective in the treatment of neonatal candidiasis including meningitis [6–10]. Fluconazole has

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**Table 1 Risk factors for neonatal candidiasis**

<table>
<thead>
<tr>
<th>Risk Factor</th>
<th>Description</th>
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<tbody>
<tr>
<td>Extremely low birthweight</td>
<td>Birthweight below 1500 g</td>
</tr>
<tr>
<td>Use of third-generation cephalosporins</td>
<td>Use of antibiotics during the first week of life</td>
</tr>
<tr>
<td>Central venous catheters</td>
<td>Use of central lines</td>
</tr>
<tr>
<td>Previous use of intralipids</td>
<td>Use of fat emulsions</td>
</tr>
<tr>
<td>Parenteral nutrition</td>
<td>Use of parenteral nutrition</td>
</tr>
<tr>
<td>Shock/disseminated intravascular coagulation</td>
<td>Presence of shock or DIC</td>
</tr>
<tr>
<td>Histamine blockers</td>
<td>Use of histamine blockers</td>
</tr>
<tr>
<td>Corticosteroids</td>
<td>Use of corticosteroids</td>
</tr>
<tr>
<td>Intubation or length of stay greater than 7 days in NICU before candidiasis</td>
<td>Length of stay in NICU</td>
</tr>
<tr>
<td>5-Min Apgar score less than 5</td>
<td>Low Apgar score</td>
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<tr>
<td>Corticosteroids</td>
<td>Use of corticosteroids</td>
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<tr>
<td>Parenteral nutrition</td>
<td>Use of parenteral nutrition</td>
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<td>Use of central lines</td>
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<tr>
<td>Use of third-generation cephalosporins</td>
<td>Use of antibiotics during the first week of life</td>
</tr>
<tr>
<td>Extremely low birthweight</td>
<td>Birthweight below 1500 g</td>
</tr>
<tr>
<td>Gastrointestinal tract colonization with <em>Candida</em></td>
<td>Enteric colonization</td>
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</table>

NICU, Neonatal intensive care unit. Reprinted from Zaoutis et al. [4], with permission.

**Table 2 Antifungal agents for neonatal candidiasis**

<table>
<thead>
<tr>
<th>Agents currently in use</th>
<th>Description</th>
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<tbody>
<tr>
<td>Amphotericin B</td>
<td>Antifungal agent</td>
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<tr>
<td>Lipid formulations of amphotericin B</td>
<td>Lipid formulation</td>
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<tr>
<td>Amphotericin B lipid complex</td>
<td>Lipid formulation</td>
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<tr>
<td>Amphotericin B colloidal dispersion</td>
<td>Lipid formulation</td>
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<tr>
<td>Liposomal amphotericin B</td>
<td>Lipid formulation</td>
</tr>
<tr>
<td>Fluconazole</td>
<td>Oral or intravenous form</td>
</tr>
<tr>
<td>Fluocytosine</td>
<td>Oral or intravenous form</td>
</tr>
<tr>
<td>Echinocandins</td>
<td>Antifungal agent</td>
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<tr>
<td>Caspofungin</td>
<td>Antifungal agent</td>
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<tr>
<td>Micafungin</td>
<td>Antifungal agent</td>
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<tr>
<td>Anidulafungin</td>
<td>Antifungal agent</td>
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<tr>
<td>Azoles</td>
<td>Antifungal agent</td>
</tr>
<tr>
<td>Voriconazole</td>
<td>Antifungal agent</td>
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Reprinted from Zaoutis et al. [4], with permission.
been compared with amphotericin B in a prospective randomized trial in premature neonates with systemic candidiasis. Neonates were treated with fluconazole, 10 mg/kg per day initially, followed by 5 mg/kg per day, or amphotericin B, 1 mg/kg per day. The groups had equivalent survival and clearance of infection [11]. Concurrent with the increased use of fluconazole prophylaxis in the 1990s, non-albicans Candida spp. with intrinsic azole resistance, such as C. glabrata and C. krusei have become more common in adult populations. Fortunately, these species are still uncommon in neonates and children.

Voriconazole, a synthetic derivative of fluconazole, is more potent and has a broader spectrum of antifungal activity than fluconazole. Voriconazole may be active against fluconazole-resistant Candida spp., but caution should be used if azole resistance is suspected because the mechanism of resistance to all triazoles is similar. The plasma pharmacokinetics have not been defined in neonates. Children older than 2 years require higher doses of voriconazole than adults to obtain similar serum concentrations because the pharmacokinetics of voriconazole in children are linear, whereas in adults they are non-linear over the range of 3–4 mg/kg. In a case report by Muldrew and colleagues [12], a preterm infant with refractory disseminated candidiasis was treated with 6 mg/kg every 8 h of intravenous voriconazole. Serum concentrations using this dosing regimen were comparable to those observed in adults receiving 4 mg/kg twice a day.

Echinocandins
The echinocandins are a new class of antifungal agents that act by selectively and non-competitively inhibiting 1,3-β-D-glucan synthase, an enzyme responsible for fungal cell wall synthesis. As this enzyme is not found in mammalian cells, this unique mechanism of action has resulted in a favorable toxicity profile in humans compared with current antifungal agents, and has prompted the use of echinocandins in combination with other antifungal drugs [13]. Echinocandins are fungicidal against Candida spp. and are not metabolized through the cytochrome P-450 enzyme system thus reducing the potential for drug interactions [13,14]. Although echinocandins achieve low or undetectable levels in CSF, they may achieve sufficiently high concentrations to be effective in Candida meningoencephalitis [15]. Therefore their role in treating patients with CNS infections in comparison with amphotericin B remains to be determined. Amphotericin B should remain the first line agent for the treatment of Candida meningoencephalitis in neonates. Echinocandins are only administered parenterally. Data regarding the pharmacokinetics, safety, and efficacy of these agents in neonates are limited. Currently three echinocandins are approved by the US Food and Drug Administration for the treatment of systemic candidiasis in children: caspofungin, micafungin, and anidulafungin.

Caspofungin has linear pharmacokinetics and is excreted primarily by the liver. Plasma concentrations of caspofungin are observed in patients with moderate hepatic insufficiency, and a dose reduction is recommended in such patients [16]. Preliminary pharmacokinetic data in neonates and infants less than 3 months of age suggest that the plasma half-life of caspofungin in neonates is longer than that seen in older children and adults. The observed data suggest that a dose of 25 mg/m² or 2 mg/kg in neonatal patients may result in plasma concentrations that are roughly similar to the range of concentrations in adults who are treated effectively and safely with caspofungin [17]. Further confirmation of these data are needed before a firm dosage recommendation can be made for neonates. The clinical efficacy and safety of caspofungin has been evaluated in two uncontrolled, observational studies. Odio and colleagues [15] described the successful use of caspofungin in nine premature infants and one term infant with invasive candidiasis refractory to standard antifungal therapy. Caspofungin was administered intravenously at a dose of 1 mg/kg for 2 days followed by 2 mg/kg per day. All positive blood cultures cleared between 3 and 7 days. No clinical or laboratory adverse events occurred during the course of caspofungin therapy. Natarajan and colleagues [18] performed a retrospective chart review of 12 infants (gestational age 24 weeks to term) with persistent candidemia (6–30 days) despite conventional antifungal therapy. After the addition of caspofungin at 1 mg/kg intravenously once a day, sterilization of blood cultures was achieved in 11 of 12 infants in a median of 3 days. Adverse events included thrombophlebitis (one patient), hypokalemia (two patients) and elevation of liver enzymes (four patients).

Micafungin exhibits linear pharmacokinetics, is metabolized by the liver, and fecal excretion is the major route of elimination [19]. A phase I pharmacokinetic study in premature neonates weighing more than 100 g found that a single dose of 3.0 mg/kg was well tolerated [20]. Premature infants displayed a shorter half-life and a more rapid rate of clearance compared with adults and older children. Area under the curve comparisons suggest that doses of 5–7 mg/kg per day in neonates weighing more than 1000 g will approximate the area under the curve drug exposure of adults receiving daily doses of 100 and 150 mg as used to treat invasive candidiasis and esophageal candidiasis. Further studies are needed to define the appropriate dose of micafungin in neonates. Clinical data on the safety and efficacy of micafungin in the treatment of candidiasis in neonates are not available at this time.

Anidulafungin is unique among the echinocandins because it slowly degrades in human plasma rather than
being metabolized [21]. There are currently no data available on the use of anidulafungin in neonates or children less than 2 years of age. In pediatric patients between 2 and 17 years of age, doses of 0.75 or 1.5 mg/kg per day have anidulafungin concentration profiles similar to those of adult patients receiving 50 or 100 mg/kg a day [22*].

Antifungal prophylaxis in the neonatal intensive care unit

The associated morbidity and mortality of neonatal invasive candidiasis coupled with its relatively insensitive and untimely clinical and laboratory diagnostics make this disease an attractive candidate for prophylaxis. Accordingly, the effect of prophylactic treatment both on colonization and infection with Candida spp. has been the subject of many studies (Table 3) [23–32,33*,34,35]. Of note is the fact that the influence of an antifungal agent on colonization, although a logical intermediary in the pathogenesis of invasive disease, is not directly relevant to what are, ostensibly, the true goals of these investigations: the prevention of the morbidity and mortality associated with invasive candidiasis. Therefore, when interpreting these data, it is helpful to focus on the influence of disease incidence on the efficacy of fungal prophylaxis. Likewise, the following discussion of the current state of prophylaxis reflects such a bias.

Initial studies that focused on the oral agents nystatin [23] and miconazole [24] demonstrated a reduction in fungal colonization, but had no appreciable effect upon invasive disease outside of the urinary tract. More recently, the concept of treating gastrointestinal colonization with an oral, non-absorbable antifungal agent was revisited. In that study [25], the use of nystatin for prophylaxis against and treatment of known Candida-colonized neonates was associated with lower rates of invasive candidiasis. The inclusion of neonates of variable birth weight and variable start/endpoints of prophylaxis make it difficult to generalize these findings to the population that is generally presumed to be at highest risk of invasive candidiasis, i.e. the very low-birthweight (VLBW), premature infant. A novel approach to influence candidal colonization employed the oral delivery of lactobacillus to VLBW infants [36]. The decrease in colonization rates seen in neonates given lactobacillus, possibly secondary to a competitive effect, is intriguing, although a reduction in invasive disease should be demonstrated before considering such an approach.

Since these pioneering studies, multiple retrospective analyses of outcomes before and after the adaptation of fungal prophylaxis have been performed [26–28]. Although these data are consistent with a beneficial effect of prophylaxis, the comparison to historical controls is subject to considerable bias. For example, as these studies range in time from 4 to 6 years, changes in general infection control practices could influence outcomes. One intriguing study employed a strategy of targeted prophylaxis to patients indentified by previous studies to be at higher risk of invasive candidiasis [29]. In that case, only VLBW neonates exposed to broad-spectrum antibiotics for 3 days or more were given fluconazole. In that retrospective analysis using historical controls, rates of invasive fungal infection were reduced from 6.3 to 1.1%. By shortening the patients’ drug exposure time, the investigators hoped to limit the emergence of resistance, drug toxicity, and cost, variables that were not measured in that particular study.

In terms of prospective or randomized trials, two single-center, prospective, randomized trials addressed these topics. In 2001, Kicklighter et al. [30] randomly assigned 103 neonates with birthweights under 1500 g (VLBW) to 28 days of fluconazole or placebo, demonstrating a significant reduction in rectal colonization (15 versus 46%). Although there was no difference in the development of invasive disease, the rate was extremely low in both groups. Later that year, by randomizing 100 neonates weighing less than 1000 g (ELBW) who had endotracheal tubes and intravenous access to 6 weeks of fluconazole or

Table 3 Summary of studies evaluating antifungal prophylaxis in the neonatal intensive care unit

<table>
<thead>
<tr>
<th>Study</th>
<th>Design</th>
<th>Population</th>
<th>Drug regimen</th>
<th>Outcomes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sims et al. [23] 1988</td>
<td>Randomized, controlled</td>
<td>67 &lt; 1250 g</td>
<td>Nystatin while intubated</td>
<td>Reduced IFI; 32 vs 6%</td>
</tr>
<tr>
<td>Wainer et al. [24] 1992</td>
<td>Randomized, controlled</td>
<td>600 &lt; 1750 g</td>
<td>Miconazole while in NICU</td>
<td>No IFI difference; 2 vs 2.6%</td>
</tr>
<tr>
<td>Kicklighter et al. [30] 2001</td>
<td>Randomized, controlled</td>
<td>103 &lt; 1500 g</td>
<td>Fluconazole 4 weeks</td>
<td>Reduced FC; 46 vs 15.1%</td>
</tr>
<tr>
<td>Kaufman et al. [31] 2001</td>
<td>Randomized, controlled</td>
<td>100 &lt; 1000 g</td>
<td>Fluconazole 6 weeks</td>
<td>Reduced IFI; 20 vs 0%</td>
</tr>
<tr>
<td>Kaufman et al. [32] 2005</td>
<td>Randomized, controlled</td>
<td>81 &lt; 1000 g</td>
<td>Fluconazole 6 weeks</td>
<td>Similar low IFI rate as with 2001 study (low dose)</td>
</tr>
<tr>
<td>Bertini et al. [27] 2005</td>
<td>Retrospective, historical controls</td>
<td>255 &lt; 1500 g</td>
<td>Fluconazole 4 weeks</td>
<td>Reduced IFI 7.8 vs 0%</td>
</tr>
<tr>
<td>Healy et al. [28] 2005</td>
<td>Retrospective, historical controls</td>
<td>446 &lt; 1000 g</td>
<td>Fluconazole 4–6 weeks</td>
<td>Reduced IFI 7 vs 2%</td>
</tr>
<tr>
<td>Manzoni et al. [26] 2006</td>
<td>Retrospective, historical controls</td>
<td>465 &lt; 1500 g</td>
<td>Fluconazole 4 weeks</td>
<td>Reduced IFI 16.7 vs 4.4%</td>
</tr>
<tr>
<td>Uko et al. [29] 2006</td>
<td>Retrospective, historical controls</td>
<td>384 &lt; 1500 g</td>
<td>Fluconazole if on BSA</td>
<td>Reduced IFI 6.3 vs 1.1%</td>
</tr>
<tr>
<td>Aghai et al. [34] 2006</td>
<td>Retrospective, historical controls</td>
<td>277 &lt; 1000 g</td>
<td>Fluconazole 6 wks</td>
<td>Reduced IFI 6.6 vs 0%</td>
</tr>
<tr>
<td>Ozturk et al. [25] 2006</td>
<td>Randomized, controlled</td>
<td>3991 all NICU</td>
<td>Nystatin variable</td>
<td>Reduced IFI 14.1 vs 1.8%</td>
</tr>
<tr>
<td>Manzoni et al. [33*] 2007</td>
<td>Multicenter randomized</td>
<td>322 &lt; 1500 g</td>
<td>Fluconazole 4 weeks</td>
<td>Reduced IFI 13.2 vs 2.7%</td>
</tr>
</tbody>
</table>

BSA, Broad-spectrum antibiotics; FC, fungal colonization; IFI, invasive fungal infection; NICU, neonatal intensive care unit. Reproduced from Gerber and Zaoutis [38].
placebo, Kauffman et al. [31] were the first to demonstrate a decreased incidence of invasive candidiasis (20 versus 0%), the primary endpoint of the study. The study by Kauffman et al. [31] was notable for a high (20%) background rate of invasive fungal disease (approximately double the rate of similar historical cohorts), 90% of which were *Candida* species. A similarly designed follow-up study by the same group [32] compared twice weekly prophylactic fluconazole with the original, more frequent dosing schedule. Although there was no placebo group, the low rates (5 versus 3%) of invasive candidiasis in the two treatment groups suggested that the less frequent dosing regimen was equally effective (compared with historical controls) and provided an alternative regimen that might reduce the development of resistance.

The first multicenter, prospective, randomized trial of fluconazole prophylaxis was published in 2007. The three-arm study of 3 mg/kg per day, 6 mg/kg per day, and placebo demonstrated a significant reduction in invasive candidiasis in the recipients of fluconazole [33**,33]]. The placebo arm in that study had a frequency of 13% of invasive candidiasis. Interestingly, prophylaxis of neonates previously colonized had no appreciable effect on the development of invasive disease, a result consistent with previous studies that were able to assess this. Also consistent with past investigations, there was no effect on overall mortality between groups. The potential benefit of instituting preventive or prophylactic strategies in a large group of patients with significantly lower event rates must be weighed against the potential risks (e.g., antifungal resistance and toxicity). Investigators have suggested that preventive measures should be targeted to populations with a baseline rate of candidiasis of more than 10% [37].

To date, only one study, a retrospective report published in 2006 [34], has offered evidence of harm from prophylactic fluconazole, citing a significant increase in cholestasis in ELBW neonates following a protocol similar to that used in the studies discussed above. In that study, 42.9% of the fluconazole-treated infants developed conjugated hyperbilirubinemia compared with 8.8% of historical controls. Both the retrospective design of the study and the considerable potential for cholestasis in parenterally fed infants are notable. Previous studies, if data were provided, suggest that only a small, transient, clinically insignificant elevation of hepatic enzyme levels occurred with fluconazole prophylaxis. In addition, the recent multicenter, prospective trial demonstrated no significant change in hepatic function between fluconazole and placebo groups, casting doubt on this association [33**]. The emergence of fluconazole resistance was not detected during this observation, but the study was not powered to detect this outcome.

**Conclusion**

The echinocandins have largely moved into a first-line role for invasive candidiasis and candidiasis based on four randomized clinical trials in adults that have been published or presented in abstract form at international meetings. Although there is a wealth of data regarding the use of these agents in critically ill adult patients, the use of this class of drugs in preterm neonates should be approached with caution because of the relative lack of clinical data. Neonatal candidiasis is a serious infection, especially in ELBW infants, and prophylaxis has been shown to be effective in preventing candidiasis in high-risk infants. Although antifungal agents believed to be effective against candidiasis in neonates are available, rigorously obtained data are lacking. Future efforts should focus on obtaining pharmacokinetic, safety and efficacy data for both older and newer antifungal agents in this unique population.

**References and recommended reading**

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest

Additional references related to this topic can also be found in the *Current World Literature* section in this issue (p. 633).


Antifungal therapy for neonatal candidiasis Zaoutis and Walsh

Zaoutis and Walsh 597
Strategies to reverse drug resistance in malaria
Timothy J. Egan and Catherine H. Kaschula

Purpose of review
Despite the current success of artemisinin combination therapy, the threat of drug-resistant falciparum malaria remains severe. Reversal of resistance to old drugs remains one strategy to deal with this problem. This review highlights recent significant findings.

Recent findings
This review provides a brief description of current antimalarials, their known or putative targets and mechanisms of resistance (where applicable). The main focus is recent reports on chloroquine resistance-reversing agents, including primaquine, so-called ‘reversed chloroquines’, novel resistance reversers such as xanthenes and two new mefloquine resistance-reversing compounds. A number of patents also report interesting new chloroquine resistance reversers, most notably HIV protease inhibitors. The review is confined to Plasmodium falciparum.

Summary
Only chlorpheniramine has so far shown some clinical utility as a chloroquine resistance reverser. Recent observations, however, that both primaquine and HIV protease inhibitors are chloroquine resistance reversers may eventually prove to be of clinical significance. ‘Reversed chloroquines’ are a scientifically innovative new class of antimalarial that both kill malaria parasites and have the potential to reverse resistance to their own antimalarial pharmacophore.

Keywords
antimalarial, chloroquine, malaria, Plasmodium falciparum, resistance, resistance reverser

Introduction
Clinical treatment of falciparum malaria currently relies on a limited number of drugs acting against an even more limited number of targets. In the case of sulfadoxine, dapsone, pyrimethamine, cycloguanil, chlorocycloguanil [1] and atovaquone [2], the targets are known to be specific proteins, while for doxycycline the target is protein synthesis, probably involving ribosomal binding [3]. The target of chloroquine, amodiaquine, quinine, quinidine, mefloquine, halofantrine and lumefantrine is thought to be haem [specifically in the form of Fe(III)-protoporphyrin IX] [4]. In the case of the artemisinins, activity has been variously attributed to inhibition of haemozoin formation, Fe(II)haem-generation of carbon-centred drug radicals or inhibition of PfATPase6 with the peroxide group possibly being activated by nonhaem Fe²⁺ [5]. Current evidence seems to favour PfATPase6 as the likely target.

Antimalarial drug resistance
Resistance to antifolate drugs arises from point mutations in their target enzymes, dihydrofolate reductase (DHFR) and dihydropteroate synthase (DHPS) [1]. In DHFR the process of acquiring resistance begins with a single point mutation at amino acid position 108 [1]. Such mutants show elevated IC₅₀ values for pyrimethamine, cycloguanil and chloroguanil, but remain sensitive to the sulfadoxine-pyrimethamine combination (Fansidar). Acquisition of a further mutation at position 51 or 59 results in even lower activity of the individual drugs and confers resistance to sulfadoxine-pyrimethamine. Attainment of a triple mutation (at positions 108, 51 and 59 in most African strains) or even quadruple mutation results in strongly resistant parasites. In the case of DHPS, mutations at positions 436, 437, 540, 581 and 613 have all been shown to confer resistance to sulfonamides, including sulfadoxine and dapsone [1]. Resistance to atovaquone also arises from point mutations in the target protein, cytochrome bc₁ [2]. In this case, in some strains a single point mutation confers resistance, accounting for the very rapid appearance of atovaquone resistance when used as a monotherapy.

Resistance to quinolines appears to be more complex, but seems to be related to the appearance of mutations in two food vacuole membrane proteins, which may lower the concentration of the drug at the target. The gene pfCRT encodes a 424-amino acid protein called Plasmodium falciparum chloroquine resistance transporter (PfCRT)
Directed to several recent reviews on this topic [6,7]. Chloroquine resistance is associated with a spectrum of mutations in this protein, which differ depending on the geographical origin of the strain [6]. All, however, contain a critical K76 mutation. The other mutations are believed to be necessary to maintain the normal function of the protein. The mechanism by which chloroquine resistance is conferred is not definitively established. As detailed in a recent review [6], one hypothesis proposes that there is a change in vacuolar pH. An increase in pH would result in reduced accumulation of drug through the weak base pH trapping effect, but evidence supporting this is lacking [6]. Claims that chloroquine resistance is associated with an unexpected decreased pH have been controversial, with the experimental observations being disputed [6]. Alternative hypotheses are that chloroquine is actively transported out of the food vacuole, or that PfCRT acts as a channel, allowing the drug to exit the food vacuole down its concentration gradient [6]. Space constraints preclude a detailed discussion of these hypotheses, and readers are directed to several recent reviews on this topic [6,7]. Reduced susceptibility to quinine is also associated with PfCRT mutations [8,9]. No such cross-resistance is observed with mefloquine which, in fact, appears to often exhibit an inverse relationship to chloroquine resistance [10].

A second food vacuole transmembrane protein, P. falciparum P-glycoprotein homolog 1 (Pgh1), encoded by the pfmdr1 gene, has also been linked to resistance to quinolines [11]. This protein is a member of the family of ATP-binding cassette (ABC) transport proteins that have been linked to multidrug resistance in mammalian cancer cells [11]. Earlier studies linked chloroquine resistance to mutations at codon 86 in this transporter; however, later investigations involving a genetic cross between a resistant strain with a mutation at position 86 and a sensitive strain lacking this mutation maintained resistance in the absence of the mutation at this position [12]. Nonetheless, there is evidence that increased copy number of the pfmdr1 gene is associated with mefloquine resistance [13]. Several recent reviews are available that discuss the roles of PfCRT and Pgh1 in further detail [6,11,14].

Clinical resistance to artemisinins, lumefantrine and doxycycline has not been reported to date.

**Approaches to reverse resistance**

Resistance reversal refers to the application of a compound that restores the activity of a drug, without itself having any activity against the organism. This is only possible where resistance does not arise from an irreversible change in the target itself, but rather in a protein that modulates the concentration of the drug at the target site. The strategy of reversing resistance to malaria can be viewed in a broader context, however. For the purposes of this review, we focus on resistance-reversers, but brief mention is also made of strategies that may be used to restore the usefulness of old drugs. From this point of view, four approaches can be envisaged: drug rotation, combination therapy, redesign of existing drugs to overcome resistance, and resistance reversing agents.

There is evidence that parasites with the critical PfCRT K76 mutation are at a fitness disadvantage. For example, in Malawi, the first African country to replace chloroquine with sulfadoxine-pyrimethamine as first-line therapy, a recent study has shown that malaria is again susceptible to chloroquine treatment [15]. This suggests that after a sufficient period of nonuse, susceptibility to chloroquine might be restored. At first sight, this could indicate that drug rotation would be a useful approach to reversing resistance in a given geographical area. Similar observations have been made previously in Gabon, Vietnam, China and Thailand [16]. Available evidence, however, indicates that recovery of chloroquine-sensitivity arises from expansion of wild-type PfCRT in the population, rather than back-mutation [17]. It therefore seems doubtful that drug rotation would be a viable strategy as re-expansion of resistant forms of the pfcrf gene is likely to spread rapidly upon resumption of drug pressure [18]. Furthermore, such reversal has not been seen in any South American strains [14].

Use of combination therapy is currently mandated by the World Health Organization [19]. This is the only viable method for restoring or maintaining the usefulness of antifolates and atovaquone, where resistance arises from mutations in the target proteins themselves. Examples of successful combinations are sulfadoxine-pyrimethamine (which has been a useful and cheap first-line treatment in Africa for almost two decades, although now waning owing to the onset of resistance), chlorproguanil-dapsone (Lhapda) [20] and atovaquone-proguanil (Malarone) [2]. Similar attempts at combining existing drugs with chloroquine (e.g. chloroquine-sulfadoxine-pyrimethamine) have proved less successful [21]. Combinations of other quinolines and related compounds have proved successful, however. These include mefloquine-artesunate (in Southeast Asia) [22] and lumefantrine-artemether (in Africa), which continue to cure disease despite mutations pointing towards future resistance [23]. Amodiaquine-sulfadoxine-pyrimethamine has also shown encouraging clinical activity [21]. Recently, azithromycin, a semisynthetic derivative of erythromycin, has also been considered for combination...
therapy with quinolines [24]. The drug is safe for use in children and pregnant women and has been studied in a clinical trial against Plasmodium vivax. A recent in-vitro study of azithromycin and erythromycin in chloroquine-resistant P. falciparum has shown synergistic activity of the chloroquine-azithromycin or erythromycin and quinine-azithromycin or erythromycin combinations, suggesting probable clinical utility of these combinations. The authors, however, suggest that the widespread occurrence of chloroquine resistance may deleteriously impact on the efficacy of the chloroquine-azithromycin or erythromycin combinations [24].

Combination therapy is based on the assumption that while a mutation conferring resistance to a single agent might occur with a certain low but finite frequency, the statistical probability of simultaneous mutations conferring resistance to two agents active against different targets is negligibly small. This principle is sound under certain circumstances, but suffers pitfalls when the elimination half-life ($t_{1/2}$) of the drug combination is very long or when the pharmacokinetics of the two drugs are not well matched [25**]. In the latter case, the one drug will be completely eliminated while the other remains at significant concentrations. In these situations, organisms can develop resistance to one or both of the drugs. Recent reports by Hastings and Watkins have described the process of resistance development as first passing through a stage of drug tolerance [25**,26]. Both drug-tolerant and sensitive organisms are eliminated by curative doses, but tolerant organisms have the evolutionary advantage of being able to re-infest patients earlier posttreatment when sub-therapeutic concentrations of drug linger. This phenomenon can only occur when the drug or drugs have long half-lives. Further mutations then lead from tolerance to resistance. This process is suggested to have been involved in the development of resistance to sulfadoxine-pyrimethamine. There is also recent evidence that a similar process may be unfolding with respect to artemisinin combination therapy. Sisowath et al. [23] have investigated lumefantrine-artemether in Zanzibar and have found a significant increase in pfmdr1 86N following drug treatment, which may be a sign of developing lumefantrine tolerance. Even more worrisome is evidence from Cambodia suggesting possible cross-resistance between mefloquine and artesunate [22]. Development of resistance to artemisinin drug combinations would be an unparalleled disaster given their current importance in malaria treatment.

The third approach, redesigning existing drugs to overcome resistance, really involves drug discovery using existing drugs as leads. The approach has been successful in the field of antifolates as well as quinolines. In the former case, structural modelling of DHFR revealed that mutations in this enzyme sterically destabilize binding of pyrimethamine and cycloguanil to the enzyme [27]. Yuthavong and co-workers have shown that suitable analogues of pyrimethamine in which the para-chloro group on the drug is moved to the meta position are active against resistant strains of parasite [28], restoring the inhibition constants of these analogues to drug-sensitive levels. Increasing the flexibility of antifolate drug analogues also restores activity against mutant enzymes as this allows the compound to again fit into the drug-binding site [29]. It has been pointed out that the parasite does not have unlimited scope for mutation of the enzyme, as many mutations will tend to reduce activity or result in an unstable protein [29]. A similar, although more empirical, approach has been successful in obtaining chloroquine analogues with restored activity against malaria parasites. These include compounds with shortened side chains [30,31] or with substantially altered side chains, for example, containing an organometallic ferrocene group [32]. None of these compounds is yet in clinical use, but both reengineered antifolates and chloroquine analogues are at various stages of development.

The fourth strategy for tackling resistance is to use a resistance reversing agent. This is potentially viable in the case of quinoline and related drugs. Following the discovery of the chloroquine resistance reversing properties of verapamil in 1987 [33], more than 40 compounds have been identified as chloroquine resistance reversers, and four as mefloquine resistance reversers. Three recent reviews provide detailed descriptions of this approach and include comprehensive lists of known resistance reversers [11,34,35*]. Here we only concentrate on recent developments from 2005 onwards. It should be noted, however, that with the exception of chlorpheniramine, resistance reversing agents have yet to be used clinically, because of the unacceptably high concentrations required, mainly owing to serum $\alpha_1$-acid glycoprotein binding [36]. A recent study has confirmed that the antihistamine chlorpheniramine significantly restores the efficacy of chloroquine in a setting of almost universal chloroquine resistance [37]. Thus, the concept of developing a resistance reverser appears to be a sound one, and reports of new resistance reversers over the last 2 years are of considerable interest.

In this regard, six research papers and five patents are of importance. The most significant publications are those of Bray et al. [38] and Burgess et al. [39**]. The first shows that the antimalarial primaquine (Fig. 1), which is used clinically in combination with chloroquine in the treatment of P. vivax malaria, reverses chloroquine resistance in P. falciparum in vitro. The significance here is that an existing registered cheap drug combination may be suitable for treatment of drug-resistant P. falciparum. It must be emphasized, however, that this has not been demonstrated in vivo, let alone clinically. Obstacles to
implementation of chloroquine-primaquine combination therapy include pharmacokinetic issues, as primaquine is rapidly metabolized to carboxyprimaquine, which does not reverse resistance. In addition, serious adverse drug effects are observed with primaquine in people with impaired glucose-6-phosphate dehydrogenase activity, which would probably limit wholesale use of this combination.

Burgess et al. report on a highly innovative hybrid molecule that combines the pharmacophore of an active 4-amino-7-chloroquinoline antimalarial with a resistance reversing group based on imipramine [39**] (Fig. 2). This compound has been shown to be highly active against both chloroquine-sensitive and resistant strains of P. falciparum in vitro and was shown to be effective against P. chabaudi in mice, with no obvious signs of toxicity. The authors have dubbed this class of compound a 'reversed chloroquine'. An extensive patent on this class of compound has also been filed [40**]. The basis of the 'reversed chloroquine' idea is that the fundamental pharmacophore for haem binding (4-amino-quinoline), inhibition of haemozoin formation (4-amino-7-chloroquinoline) and accumulation through pH trapping (a basic tertiary amino group in the side chain) is preserved, as is the pharmacophore for a resistance reverser, namely two suitably positioned aromatic rings with an amino group separated by a short chain. It must be emphasized, however, that alterations to the side chain of chloroquine in many cases result in compounds that are active against resistant parasites, an effect recently shown to be directly associated with the mutations in PfCRT that confer chloroquine resistance [8]. As a result, the activity of the 'reversed chloroquine' reported by Burgess et al. may
simply reflect the altered side chain. These compounds have yet to be shown to actually reverse resistance, which ought to be demonstrated by showing a synergistic effect with chloroquine in resistant, but not sensitive, strains of parasite.

A number of novel resistance reversing agents have also been reported (Fig. 1). These include a series of xanthene derivatives that show weak, but significant, in-vitro antimalarial activity, but resistance reversing activity superior to that of verapamil, the archetypal
chloroquine resistance reverser [41]. Here there are increased cellular concentrations of chloroquine in resistance reverser-treated compared with untreated parasites. An attempt has also been made to reverse chloroquine resistance with the flavonoid derivatives dehydroisolybin and 8-(1;1)-DMA-kaempferide based on evidence that flavonoids reverse MDR-1-induced multidrug resistance in cancer cells [42]. This was unsuccessful, however, possibly reflecting the fact that chloroquine resistance is not primarily determined by Pgh-1, but rather by the unrelated PfCRT. It would have been interesting to know whether mefloquine resistance is affected by flavonoids. In this regard, fluoxetine (Fig. 1), which is also a P-glycoprotein inhibitor, was shown to be active in reversing both mefloquine and chloroquine resistance [43]. Additionally, an in-vivo investigation in rhesus monkeys has shown that ketoconazole (Fig. 1) reverses mefloquine resistance [44]. These data are of considerable interest, as to date only four other compounds have been shown to reverse mefloquine resistance.

Four patents have been registered claiming new classes of resistance reversers. These include a wide range of protease inhibitors used in the treatment of HIV-AIDS (Fig. 3) [45], inactive antihistamine isomers (Fig. 1) [46], vocamine, its metabolites and derivatives (Fig. 1) [47], and a series of (diphenylmethyl)piperazines (Fig. 1) [48]. The first of these patents is of particular significance as the widespread occurrence of HIV in parts of Africa means that many malaria patients are likely to be receiving protease inhibitors.

Finally, as a cautionary note, it is worth pointing out that there is recent evidence that the sensitivity of P. falciparum to the weak intrinsic antimalarial activity of the resistance reverser verapamil, is itself subject to mutations in pfmdr1 [49]. This leads one to wonder whether resistance to combinations of chloroquine with resistance reversers would eventually also arise if ever these were to be used clinically.

Conclusion

The current success of artemisinin combination therapy has seen a waning of interest in chloroquine resistance reversers; however, the persistent threat of resistance leaves no room for complacency. Several interesting reports have appeared recently, ranging from potential clinical application in the not too distant future represented by primaquine combination therapy, to new discoveries that may be useful in the longer term. Perhaps the most interesting of these are the new hybrid molecules containing both antimalarial and resistance-reversing pharmacophores. Such a compound could combine both types of activity without the drawbacks of differing pharmacokinetic profiles that necessarily accompany combinations of more than one molecule.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

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- of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (pp. 642–643).


An interesting review presenting competing arguments for the mechanism of chloroquine resistance by PICRT.
Antimicrobial agents: viral/parasitic


An important publication exploring the role of amino acid identity at position 76 on chloroquine, quinine and quinidine sensitivity and the role of additional mutations in quinine resistance associated with restored chloroquine sensitivity. The work indicates that mutations occur on the hydrophobic faces of three amphipathic transmembrane helices.


A paper describing recovery of chloroquine sensitivity in Malawi.


An important publication explaining the origin of drug resistance in the malaria parasite arising through a stage of tolerance to sub-therapeutic doses following treatment for malaria. The paper describes the process in a readily understandable, nonmathematical way.


A comprehensive review of resistance reversing agents for quinoline antimalarial drugs and their structure-activity relationships.


A paper reporting on a highly innovative class of compound combining both the antimalarial pharmacophore of chloroquine and that of a chloroquine-resistance reversal. The compound is shown to be active against both chloroquine sensitive and resistant parasites.


An important patent that provides data on a family of ‘reversed chloroquines’ that combine antimalarial and resistance reversing motifs in one compound.


One of the few publications in the literature describing mefloquine resistance reversal.


A patent reporting on the chloroquine resistance reversing effect of vocamine.


A patent reporting chloroquine resistance reversing effects of a series of diphenylmethylpiperazines.

Artemisinins and synthetic trioxolanes in the treatment of helminth infections
Jennifer Keiser and Jürg Utzinger

Purpose of review
Helminthiases, including schistosomiasis and food-borne trematodiases, affect millions of people. Just a few drugs are used for the treatment and control of these diseases. We review recent in-vitro and in-vivo observations with the artemisinins and synthetic trioxolanes against major trematode infections, update clinical findings, and discuss the potential impact that artemisinin-based combination therapy might have on trematode infections in settings where malaria and helminthiases are co-endemic.

Recent findings
The artemisinins and synthetic trioxolanes possess a broad spectrum of activity against trematodes. High worm-burden reductions were obtained with these drugs in rodents with acute or chronic infections of Schistosoma japonicum, S. mansoni, Clonorchis sinensis, Fasciola hepatica and Opisthorchis viverrini. Clinical trials carried out in Africa, utilizing artemether or artesunate singly or as artemisinin-based combination therapies, following recommended malaria treatment schedules, found an effect against schistosomiasis.

Summary
Artemisinin-based combination therapies are increasingly deployed against malaria, and hence there is a need to assess the potential auxiliary effects against schistosomiasis in settings where both diseases are endemic. Also, the effect of artemisinin-based combination therapies on food-borne trematodiases should be assessed. In-vitro and in-vivo findings with the synthetic trioxolanes provide data to launch preclinical investigations.

Keywords
artemether, artemisinin-based combination therapy, artesunate, helminth infections, schistosomiasis, synthetic trioxolanes

Abbreviations
ACT  artemisinin-based combination therapy
RCT  randomized controlled trial
WHO  World Health Organization

Introduction
Helminths or parasitic worms are invertebrates that are characterized by elongated, flat or round bodies. Helminths include cestodes (tapeworms), nematodes (roundworms) and trematodes (flukes) [1]. Here, we focus on trematode infections, particularly those that are of medical and veterinary significance. From a clinical and public-health perspective, the most important trematodes are the blood flukes of the genus Schistosoma. There are five species parasitizing humans; these are Schistosoma haematobium, S. mansoni, S. japonicum, S. intercalatum and S. mekongi. The first three show the widest geographical distribution and cause the highest burden [2,3,4]. More than 200 million individuals are infected; an estimated 779 million are at risk of schistosomiasis, and the disease has spread to previously non-endemic areas due to water-resource developments [5,6]. Estimates of the global burden of schistosomiasis range from 1.7 to 4.5 million disability-adjusted life years (DALYs) [2,7,8]. New epidemiological data and a recent meta-analysis, however, suggest that even the higher estimate is a considerable underestimation of the true burden of schistosomiasis [9,10,12,13].

The second class of trematodes comprises the food-borne trematodes, also known as tissue flukes. The liver flukes (Clonorchis sinensis, Fasciola gigantica, F. hepatica and Opisthorchis viverrini) and the lung flukes (Paragonimus spp.) are the most significant species clinically and from a public-health point of view, followed by the intestinal flukes (Echinostoma spp., Fasciolopsis buski and heterophyids). In the mid-1990s, the World Health Organization (WHO) estimated that 40 million individuals were infected, and 750 million were at risk of food-borne trematodiases [13]. It is conceivable that both estimates have risen as a result of population growth and the rapid expansion of aquaculture in Southeast Asia [14]. Although food-borne trematode infections are associated with serious morbidities (e.g. different hepatobiliary diseases and bile duct cancer) [15], the global burden remains to be assessed [7,16].
A number of trematode species are of great veterinary importance, and some are significant zoonoses (e.g. *S. japonicum*). This issue can be illustrated by the following statistics. An estimated 165 million cattle in Africa and Asia are infected with schistosomes (i.e. *S. bovis* and *S. mattheei*) [17]. In China, more than 100,000 bovines are infected with *S. japonicum*, which poses an additional challenge for the control of human schistosomiasis [18–20]. The annual economic loss due to *F. hepatica* infections in livestock is US$2 billion [21].

The control of helminthiases, including schistosomiasis and food-borne trematodiases, relies on regular administration of anthelmintic drugs. In the mid-1980s, the WHO endorsed chemotherapy-based morbidity control as the new strategy for schistosomiasis control [22]. Praziquantel is the current drug of choice; over the past 20 years, more than 100 million doses have been administered in just two countries (China and Egypt), and new efforts are underway for large-scale administration in several African countries [3,23]. The control of food-borne trematodiases is facilitated by either praziquantel or triclabendazole [24,25]. Although triclabendazole is currently registered in only four countries, Novartis Pharma announced a donation for the treatment of Fasciola-infected patients in endemic countries (website: http://www.who.int). Preventive chemotherapy is the new buzzword at the WHO for the control of helminth infections and other so-called neglected tropical diseases [26]. The danger of relying on just two drugs for the control of schistosomiasis and food-borne trematodiases has been emphasized, as well as the need to develop new, safe and efficacious antitrematocidal drugs [16,27].

Here, we briefly review the discovery and development of artemisinin and its semisynthetic derivatives and synthetic peroxides as antimalarials. We highlight key laboratory findings of the trematocidal properties of two semisynthetic artemisinins (artemether and artesunate) and an important class of synthetic peroxides, the 1,2,4-trioxolanes (secondary ozonides, abbreviation OZ). Results of the clinical use of artemether and artesunate in the chemoprophylaxis and therapy of trematode infections are summarized. Finally, we speculate that the new antimalaria treatment policies, that is, artemisinin-based combination therapies (ACTs), will result in auxiliary benefits against trematode infections in settings where malaria and helminthiases are co-endemic.

**Artemisinin, semisynthetic artemisinins and synthetic peroxides**

Artemisinin (*qinghao*) is a sesquiterpene trioxane found in the medicinal plant *Artemisia annua* (*qinghao*). *A. annua* has been described in the Chinese materia medica during the Tang, Song, Yuan and Ming dynasties for the treatment of bone steaming and heat vexation [28]. Moreover, extracts of the plant have been used for treating haemorrhoids, lice, boils and fever [29]. The antimalarial activity of artemisinin was ‘rediscovered’ in 1971 [30,31]. Treatment of *Plasmodium*-infected mice with an ether extract of *A. annua* resulted in cure rates of 95–100%. Subsequently, 21 patients with malaria were treated in Beijing with an extract of *qinghao* and most of the patients were cured [28]. By the late 1970s, more than 2000 patients had been enrolled in clinical studies [32,33]. Earlier attempts to demonstrate the antimalarial properties of the plant were unsuccessful, which was probably explained by the insolubility of artemisinin in most solvents [28].

In the early 1970s, the first artemisinin analogues were synthesized with an emphasis on improving the poor solubility of the parent compound. This first generation of semisynthetic artemisinins – dihydroartemisinin, artemether and artesunate – have become the most widely used antimalarials today [34,35]. Figure 1 shows the chemical structure of artemether and artesunate. These semisynthetic artemisinins reduce the parasite burden more rapidly than any other known antimalarial drug, and they have good therapeutic indices. Semisynthetic artemisinins have a number of drawbacks, however. First, their production requires artemisinin as the starting material; it takes 10–13 months to cultivate *A. annua*, and extract and produce the final product [36]. Second, the semisynthetic artemisinins have short half-lives and low bioavailabilities [27**,37–39], as summarized in Table 1.

![Figure 1 Chemical structure of the semisynthetic artemisinins: artesunate and artemether](image-url)
Today, artemether and artesunate are mainly employed as ACTs in order to avoid or delay the induction of parasite drug resistance, and to prevent recrudescence [35]. The rationale for combining two or more drugs with independent mechanisms of actions was to prevent the emergence of resistant mutants, a strategy first developed in the treatment of tuberculosis. While the artemisinin component causes a rapid and substantial reduction in parasite biomass, the remaining parasites are killed by high concentrations of the long-acting companion drug [40]. Since 2001, worldwide, a total of 56 malaria-endemic countries have adopted ACT as the first-line (or second-line) treatment policy [36]. The demand for ACTs has witnessed an exponential growth in the new millennium. This is illustrated by the estimated 120 million adult treatment courses of artemether–lumefantrine (Coartem) administered in 2005. Coartem is the ACT most widely used, constituting approximately 70% of all clinically administered ACTs [41]. These 120 million doses of Coartem required more than 100 tons of artemisinin to produce approximately 57 tons of artemether [41]. It has been predicted for 2008 that 200 million adult doses of Coartem will be needed [42].

In the meantime, a number of synthetic artemisinin analogs have been developed, motivated by the quest for a simplified chemical structure, economic and scalable synthesis, and improved biopharmaceutical properties [27,38]. Comprehensive reviews pertaining to the synthesis and evaluation of second-generation artemisinin derivatives are available [43,44]. The secondary ozonides are an important class of synthetic peroxides [43]. After several years of investigations, pursuing a rigorous lead compound progression approach, OZ277 had been selected as a new antimalarial drug-development candidate [38]. Importantly, selected secondary ozonides (e.g. OZ78 and OZ288; Fig. 2) have also been subjected to in-vitro and in-vivo activity trials against several trematode species, and key results are discussed in the next section. In Table 1, the chemical and biopharmaceutical properties of OZ78 are juxtaposed with those of the artemisinins.

It is important to note that the exact mechanism of action of the artemisinins against malaria is still being elucidated. Several possible mechanisms of action have been proposed, for example, interference with the parasite’s sarcoplasmic/endoplasmic reticulum Ca\(^{2+}\)-ATPase PfATP6 (SERCA) [45], disruption of parasite mitochondrial function, modulation of host immune function, and an inhibition of angiogenesis [46]. In addition, it is well known that the peroxide bond undergoes reductive cleavage by haem released by the parasite’s haemoglobin digestion, which results in carbon-centred free radicals or carbocations that are capable of alkylating parasite biomolecules [47]. New research suggests that haem-mediated cleavage of the peroxide bond plays an important role in the mechanism of action of the secondary ozonides against malaria [48,49].

**Trematocidal activity of semisynthetic artemisinins and secondary ozonides**

The artemisinins have been tested against blood flukes and tissue flukes *in vitro* and *in vivo*, as well as in clinical studies, which we summarize below.

**In-vitro and in-vivo investigations**

With the exception of one study that reported a lack of in-vitro activity of 0.5 μg/ml of artemether against the...
nematode *Gnathostoma spinigerum* [50], we found no indications that the semisynthetic artemisinins and secondary ozonides had been investigated for in-vitro and in-vivo activity against cestodes and nematodes. The paucity of these kinds of studies is somewhat surprising as most helminths are blood-feeding parasites that digest haemoglobin which, as mentioned before, is a possible activator/receptor for the semisynthetic artemisinins and the secondary ozonides. Hence, the remainder of this section focuses on the trematocidal properties of peroxidic compounds, where detailed investigations have been carried out.

In-vitro studies with *S. mansoni* and *F. hepatica* confirmed the role of haemin in the activation of the semisynthetic artemisinins and secondary ozonides. While no effect on the motor activity and the tegument was observed in schistosomes incubated in a medium containing arte- mether or OZ78 for as long as 72 h, supplementing the culture medium with haemin resulted in reduced worm motor activity, tegumental alterations or even death of the worms [51,52]. Damage to the tegument of *F. hepatica* worms was more pronounced after haemin was added to the artemether-containing or artesunate-containing culture medium [53].

Here we briefly summarize key results of in-vivo activities of artemether, artesunate, OZ78 and, if available, OZ288 against selected trematodes. For further details, the reader is referred to recent articles/reviews published elsewhere [16,27*,52*,54*,55]. With regard to schistosomes, three issues are worth highlighting. First, similar activities were observed between the semisynthetic artemisinins (artemether and artesunate) and secondary ozonides (OZ78 and OZ288), although the latter are characterized by improved chemical and biopharmaceutical properties. For example, a single 400 mg/kg oral dose of artemether administered to mice infected with 35-day-old *S. mansoni* resulted in a worm-burden reduction of 48.9% [56]. The corresponding worm-burden reductions with single 400 mg/kg oral doses of OZ78 or OZ288 to mice infected with 49-day-old adult *S. mansoni* were 0% and 52.2%, respectively [52*] (Table 2). Second, schistosomula (the young development stages of the parasite) are more susceptible to semisynthetic artemisinins and secondary ozonides than the adult worms [52*,54*,56]. Third, there is a remarkable difference in the activity of artemether and secondary ozonides against adult schistosomes depending on the rodent model; significantly higher worm-burden reductions were found in the hamster than in the mouse model [52*,54*]. The reason why particularly high adult schistosome worm-burden reductions are found in hamsters remains to be elucidated.

Quite different results were obtained when semisynthetic artemisinins and secondary ozonides were administered

<table>
<thead>
<tr>
<th>Parasite</th>
<th>Host animal</th>
<th>Drug</th>
<th>Dose (mg/kg)</th>
<th>Day of treatment postinfection</th>
<th>Worm-burden reduction (%)</th>
<th>Dose (mg/kg)</th>
<th>Day of treatment postinfection</th>
<th>Worm-burden reduction (%)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Schistosoma mansoni</em></td>
<td>Mouse</td>
<td>Artemether</td>
<td>800</td>
<td>49</td>
<td>15.8</td>
<td>200</td>
<td>21</td>
<td>81.0</td>
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<td>82.0</td>
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<td></td>
<td>OZ288</td>
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<td>200</td>
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<td>95.4</td>
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<td>83.0</td>
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<td>84.0</td>
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<td>90.0</td>
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<tr>
<td><em>Fasciola hepatica</em></td>
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<td>Artemether</td>
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<td>56</td>
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<tr>
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<td>300</td>
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<td>78.5</td>
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n.d., not determined.
to rodents infected with tissue flukes (C. sinensis, F. hepatica and O. viverrini) rather than schistosomes. First, markedly higher worm-burden reductions were observed against adult C. sinensis and adult F. hepatica when compared with juvenile flukes [57,58]. For example, while a chronic C. sinensis infection in rats could be cleared with single 150 mg/kg oral doses of artemether or artesunate [58], only low worm-burden reductions (15.2–20.5%) were observed with these artemisinins given at the same doses to rats infected with 7-day-old C. sinensis (unpublished observation) (Table 2). These findings are in contrast to the stage-specific susceptibility of schistosomes against artemether in the mouse model, as described before [54*]. Second, different species of food-borne trematodes have distinctively different susceptibilities to the semi-synthetic artemisinins and secondary ozonides. For example, only moderate worm-burden reductions (39.7–65.5%) were observed when single 400–600 mg/kg oral doses of artemether were administered to hamsters infected with O. viverrini, whereas complete cures were achieved in the C. sinensis–rat and F. hepatica–rat models (Table 2) [57,59]. Third, differing activities were observed between the semisynthetic artemisinins and OZ78. For example, while single 200–400 mg/kg oral doses of arte-sunate and artemether cured chronic F. hepatica infections in the rat model [57], a two-fold to four-fold lower dose of OZ78 (i.e. 100 mg/kg) resulted in a worm-burden reduction of 100% in rats harbouring adult F. hepatica [60]. On the other hand, C. sinensis was found to be more susceptible to artemether and artesunate, when compared with OZ78.

Another important observation is that rodents infected with schistosomes or food-borne trematodes showed a better tolerance to secondary ozonides than to artemether and artesunate. In several experiments, artemether and artesunate at doses of 200 mg/kg and above showed toxic effects. For example, in an experiment with five F. hepatica-infected rats, three died following a 400 mg/kg oral dose of artesunate, while OZ78 was well tolerated at a single 400 mg/kg oral dose [60].

The promising trematocidal activities of the secondary ozonides, coupled with the lower toxicity, enhanced metabolic stability and better pharmacokinetic properties than the semisynthetic artemisinins, indicate their potential, and provide a rationale for further progressing these compounds into preclinical investigations.

**Clinical trials**

To our knowledge, no randomized controlled trials (RCTs) have been carried out to investigate whether the semisynthetic artemisinins or ozonides have an effect on cestode or nematode infections in humans. In two RCTs that assessed the effect of repeated oral artemether (six doses at 6 mg/kg each, administered once every 3 or 4 weeks) to prevent patent infections with S. mansoni [65] or S. haematobium [66], however, the presence of nematodes (Ascaris lumbricoides, hookworms and Trichuris trichiura) was investigated at the beginning and the end of the trials. Artemether showed no effect on any of these nematodes.

With regard to food-borne trematodiasis and peroxidic drugs, only one clinical trial has been carried out thus far. Twenty-one Vietnamese patients with clonorchiasis were given artemisinin at 500 mg twice daily for 5 days. Although the mean egg count pretreatment [1103 eggs per gram (EPG) of stool] was more than halved 5 weeks posttreatment (542 EPG), this reduction showed no statistical significance. Moreover, 90% of the patients still excreted C. sinensis eggs at end of study evaluation [67]. In mid-2007, a proof-of-concept trial was launched in Egypt to assess whether oral arte- mether (6 × 80 mg) has any effect on F. hepatica and/or F. gigantica infections.

The RCTs carried out in China and Africa with arte- mether and artesunate for the prevention of patent S. japonicum, S. mansoni and S. haematobium infection have been reviewed [18,54*]. Regarding S. japonicum, random summary protective efficacies of 96% were found for the 1-week artemate, and 85% and 86% for the 2-week treatment intervals with artesunate and artemether, respectively. The protective efficacy against S. mansoni (artemether given at 3-week intervals) was 50% and that against S. haematobium (artemether given at 4-week intervals) was 25% [2,54*].

In addition to the above-mentioned RCTs assessing the preventive effect of the semisynthetic artemisinins, a number of studies in different African settings focused on the therapeutic effect of artemether and artesunate against S. haematobium and S. mansoni [54*]. Usually, the artemisinins were administered according to recommended malaria treatment schedules at the time of investigation; the latest two studies used different ACTs [68*,69]. Although comparison among the different studies is not possible (e.g. different parasite species, different age groups, varying treatment schedules, different diagnostic approaches, etc.), it is interesting to note that some trials reported significant cure and egg-reduction rates. Summarizing the available data, the cure rates ranged between 23% and 100% and the egg-reduction rates between 55% and 100%.

**The role of artemisinins in the treatment of schistosomiasis**

Since the semisynthetic artemisinins not only possess antimalarial properties, but are also active against trematode infections, it has been emphasized that the effect of the artemisinins, particularly ACTs used against malaria,
should be assessed against schistosomiasis and food-borne trematodiasis in areas co-endemic for malaria and helminthiases [2,16,27**,54**]. Here, we further illustrate this issue using recent statistics and health-policy deliberations, coupled with cartographic tools. Figure 3 shows the country-specific prevalence of schistosomiasis on the African continent as of mid-2003 [5*]. More than 80% of the global burden of schistosomiasis is concentrated in Africa, similar to malaria [2,70]. Juxtaposing this schistosomiasis-prevalence map with malaria risk across Africa [71], it is clear that large parts are co-endemic for malaria and schistosomiasis. In the meantime, 38 African countries and the islands of Sao Tome and Principe and Zanzibar have adopted ACT treatment policies (Fig. 3). Twenty-five of these territories have furthermore implemented these policies and are deploying the ACTs in the public-health sector [36]. In this connection, it should be noted that the challenges from adopting a new malaria-treatment policy until the policy takes effect are formidable. In Kenya, for example, it took more than 2 years from the first announcement of a drug-policy change towards ACT treatment until early implementation was completed. Reasons for the long duration with regard to ACT implementation included funding delay and missing clarity regarding sustainable funds, lack of sufficient clinical data regarding the efficacy of ACTs in Kenya, tendering and procurement procedures, regulatory issues, national and international interests of pharmaceutical drug companies and a complex drug-ordering process [72*]. Notwithstanding these challenges, an ever-increasing fraction of clinical malaria episodes in Africa will be treated with ACTs, and hence the potential auxiliary benefits on schistosomiasis should be assessed.

**Conclusion**

We have summarized the promising trematocidal properties of the artemisinins and ozonides. With regard to the ozonides, further drug discovery and development work should be initiated. It has been argued that the use of the artemisinins should be restricted for the control of malaria, as the use of the artemisinins against helminth infections might generate drug-resistant malaria parasites. The implementation of ACTs in settings where malaria and schistosomiasis are co-endemic (large parts of Africa), however, offers the opportunity to rigorously assess the likely auxiliary benefit of this strategy on schistosome infections at the individual patient level in trial set-ups, as well as on the population level by monitoring schistosomiasis transmission dynamics. Although very few settings are co-endemic for malaria and food-borne trematodiasis (parts of Southeast Asia), it will be interesting to monitor the effect of ACTs used against malaria on clonorchiasis, fascioliasis, opisthorchiasis and other food-borne trematode infections.

**Acknowledgements**

Many of the ideas and data summarized here have been developed through discussions and exchanges with Professor Marcel Tanner (Swiss Tropical Institute; Basel, Switzerland), Professor Xiao Shu-Hua (National Institute of Parasitic Diseases, Chinese Center for Disease Control and Prevention; Shanghai, China) and Professor Jonathan L. Vennerstrom (College of Pharmacy, University of Nebraska Medical Center; Nebraska, USA). We thank Dr Barbara Matthys for drawing Fig. 3. We are grateful to the Swiss National Science Foundation for financial support via projects PPOOA-114941 (J. Keiser) and PPOOB-102883 (J. Utzinger).


This paper provides a good update of recent research pertaining to the epidemiology and control of schistosomiasis, including the important issue of the possible development of praziquantel resistance.


Excellent paper summarizing the current state of affairs regarding vaccine development for major trematode species.


This paper provides a good update of recent research pertaining to the epidemiology and control of schistosomiasis, including the important issue of the possible development of praziquantel resistance.

Concluding that further underscores why the 'true' burden of schistosomiasis has been underestimated.


This paper provides a good update of recent research pertaining to the epidemiology and control of schistosomiasis, including the important issue of the possible development of praziquantel resistance.
Antimicrobial agents: viral/parasitic


Excellent update on the possible mechanisms of action of artemisinins against malaria.


Interesting study analysing the iron-mediated reactivity of OZs and their in-vitro antimalarial activity.


The first study documenting the in-vitro and in-vivo activities of representative OZs against major human schistosome species.


Comprehensive review summarizing the trematocidal activity of the artemisinins in laboratory investigations and their safety and efficacy in clinical trials.


First study showing that artesinin-based combination therapies (ACTs), when used in preschool children with a clinical malaria and concurrently infected with Schistosoma haematobium have a significant effect on schistosomiasis.


Interesting paper that highlights the difficulties implementing the ACT treatment policy in Kenya.
Intermittent preventive therapy for malaria: progress and future directions
Martin P. Grobusch, Andrea Egan, Roly D. Gosling and Robert D. Newman

Purpose of review
This review summarizes recent evidence regarding the efficacy of intermittent preventive treatment with focus on infancy (IPTi) and the rationale behind such a control strategy.

Recent findings
Pooled safety and efficacy analyses of all six trials of IPTi with sulfadoxine-pyrimethamine conducted between 1999 and 2007 have demonstrated a 30% protective efficacy against clinical malaria, a 24% protective efficacy against all-cause hospital admissions, a 37% protective efficacy against malaria-related hospital admissions, and a 15% protective efficacy against anemia, all in the first year of life. Rebound in malaria following discontinuation of the intervention has not been noted in pooled analyses of the IPTi trials.

Summary
Given the efficacy, excellent safety and tolerability of the intervention and the fact that it is inexpensive and easily deliverable if linked to the Expanded Programme on Immunization, IPTi-sulfadoxine-pyrimethamine appears to add a valuable tool to the malaria-control armamentarium in endemic areas of Africa. Routine monitoring of sulfadoxine-pyrimethamine efficacy will be required to guide future IPTi programme implementation. Variations of IPTi that target older children may be required for areas of Africa with highly seasonal malaria transmission.

Keywords
Africa, expanded programme on immunization, infants, intermittent preventive treatment, malaria

Introduction
Plasmodium falciparum malaria continues to cause an enormous public health burden in Africa, killing an estimated 1 million children below 5 years of age annually [1]. The indirect death toll attributable to malaria is also high. Malaria is a major cause of anemia in pregnant women, infants and older children; anemia and malaria-associated sequelae may account for another 400 000 to 1.7 million deaths annually in Africa [2]. Until relatively recently, malaria control in Africa relied almost exclusively on attempting to deliver prompt and efficacious treatment to persons with symptomatic malaria. Since the African Summit on Roll Back Malaria held in Abuja in 2000 [3], there has been an increased emphasis on malaria prevention through the use of insecticide-treated mosquito nets (ITNs) [4] and, more recently, indoor residual spraying [5]. In addition, the strategy of intermittent preventative treatment (IPT) – the administration of treatment doses of antimalarials to a target population at regularly scheduled intervals – became an integral part of antenatal care practice in malarious areas to prevent the adverse consequences of malaria during pregnancy. Since then, the concept of IPT has been broadened to include infants (IPTi) [6] and children (IPTc) [7]. A number of trials [8,9,10–13] (Table 1) have now demonstrated the potential benefit of IPTi. Some of these were conducted under the auspices of the IPTi Consortium [14], which was formed to generate an evidence base on the safety and efficacy of IPTi upon which the World Health Organization (WHO) could base a decision on whether to recommend IPTi as an additional malaria control strategy [6]. Here, we look into the potential role of this new intervention in Africa.

Why not chemoprophylaxis?
IPT is an attractive form of malaria control in pregnant women, infants, and children because giving limited
<table>
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</thead>
<tbody>
<tr>
<td>Trial, country</td>
<td>Ifakara, Tanzania</td>
<td>Navrongo, Ghana</td>
<td>Manhica, Mozambique</td>
<td>Kumasi, Ghana</td>
<td>Lambaréné, Gabon</td>
<td>Tamale, Ghana</td>
</tr>
<tr>
<td>Transmission</td>
<td>Perennial</td>
<td>Highly seasonal</td>
<td>Perennial with seasonal peaks</td>
<td>Perennial</td>
<td>Perennial with seasonal peaks</td>
<td>Perennial with seasonal peaks</td>
</tr>
<tr>
<td>Incidence rate/year of clinical malaria in placebo group (all episodes)</td>
<td>0.43</td>
<td>1.0</td>
<td>0.55</td>
<td>1.29</td>
<td>0.22</td>
<td>0.88</td>
</tr>
<tr>
<td>Use of bed nets, % placebo/sulfadoxine-pyrimethamine treated (untreated)</td>
<td>67/68</td>
<td>7/19</td>
<td>0/0 (14/15)</td>
<td>20/20 estimate (39/38)</td>
<td>5/5 (80/80)</td>
<td>&lt;1%</td>
</tr>
<tr>
<td>Iron supplementation</td>
<td>Daily unsupervised from 2 to 6 months of age (2 mg/kg/day)</td>
<td>Twice weekly unsupervised for 1 month after each IPTi dose, (2.5 ml, 15 mg elemental iron)</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>Ages at dosing, months</td>
<td>2, 3, 9 (at time of DPT2, DPT3 and measles)</td>
<td>3, 4, 9, 12 (at time of DPT2, DPT3 &amp; measles +extra at 12 months)</td>
<td>3, 4, 9 (at time of DPT2, DPT3 and measles +extra at 15 months)</td>
<td>3, 9, 15 (at time of DPT3 and measles +extra at 15 months)</td>
<td>3, 9, 15 (at time of DPT3 and measles +extra at 15 months)</td>
<td></td>
</tr>
<tr>
<td>Method and duration of follow up</td>
<td>PCD to 24 months of age (CSS at 12 and 18 months of age)</td>
<td>PCD to 24 months of age (CSS at 2, 9, 12 and 18 months of age)</td>
<td>PCD to 24 months of age (CSS at 12 and 24 months of age)</td>
<td>ACD monthly to 21 months of age and PCD</td>
<td>ACD monthly to 30 months of age and PCD</td>
<td>ACD every 3 months to 24 months of age and PCD</td>
</tr>
<tr>
<td>Study design</td>
<td>Individual randomization</td>
<td>Cluster randomization</td>
<td>Individual randomization</td>
<td>Individual randomization</td>
<td>Individual randomization</td>
<td>Individual randomization</td>
</tr>
<tr>
<td>EPI serology analysis</td>
<td>100,000 IU at time of measles vaccination</td>
<td>100,000 IU at 6 months of age, then every 6 months (not until what age)</td>
<td>100,000 IU at 6 months of age, then every 6 months of age, once every 6 months up to 5 years of age</td>
<td>Every 6 months, and for treatment of measles (6–11 months = 100,000 IU; 12–59 months = 200,000 IU)</td>
<td>None</td>
<td>None</td>
</tr>
</tbody>
</table>

IPTi, intermittent preventive treatment delivered to infants; ACD, active case detection; PCD, passive case detection; EIR, entomological inoculation rate; CSS, cross-sectional surveys; DPT, diphtheria pertussis tetanus; EPI, Expanded Programme on Immunization.
treatment doses of antimalarial drugs may circumvent the problems associated with chemoprophylaxis. Chemoprophylaxis is effective at preventing clinical malaria [15] and mortality [16] among young children in endemic African settings. Among infants receiving weekly chemoprophylaxis with pyrimethamine plus dapsone for 1 year, however, the rates of severe anemia and malaria experienced after discontinuing chemoprophylaxis during the second year of life were higher than in those infants who never received chemoprophylaxis [15]; by 4 years of age these differences had essentially disappeared, however [17**]. A similar rebound in malaria morbidity was observed among Gambian children during the first year following discontinuation of weekly chemoprophylaxis with pyrimethamine plus dapsone given during the first 5 years of life [18]; no rebound in mortality was seen among these children. This so-called rebound effect is thought to be the result of a loss, or delay, in the acquisition of naturally acquired immunity due to a lack of exposure to parasites [15]. Chemoprophylaxis also poses logistical delivery problems, is costly, and might accelerate the spread of drug resistance through the use of sub-therapeutic – rather than treatment – doses of antimalarial drugs.

**Evidence from pregnant women that intermittent preventive treatment is an efficacious and effective strategy**

Multiple trials have now demonstrated that IPT delivered to pregnant women (IPTp) is successful at preventing adverse consequences of malaria during pregnancy, including placental parasitemia [19–21,22*], maternal anemia [20,21,23], and low birth weight [21]. Most trials have been conducted using two doses of sulfadoxine-pyrimethamine delivered at antenatal care visits during the second and third trimesters of pregnancy. Among HIV-positive pregnant women, there is now evidence from pooled studies that monthly dosing of IPTp-sulfadoxine-pyrimethamine results in less placental parasitemia and higher mean birth weights when compared with a two-dose regimen of IPTp-sulfadoxine-pyrimethamine [24**].

Programme effectiveness evaluations have demonstrated similar benefits of IPTp-sulfadoxine-pyrimethamine, likely because of the fact that a high percentage of women attend antenatal clinics in Africa, and sulfadoxine-pyrimethamine can be easily administered as directly observed therapy [25]. With IPTp-sulfadoxine-pyrimethamine was born the idea that treatment doses of an antimalarial drug, delivered at regular intervals to asymptomatic individuals already attending facility-based care, might prove to be a cost-effective platform for a new approach to malaria control.

**Recent evidence regarding efficacy and effectiveness of intermittent preventive treatment delivered to infants**

Results are now available from five additional trials of IPTi-sulfadoxine-pyrimethamine delivered alongside EPI vaccines across a range of malaria-transmission settings in Africa [9,10*–13*]. The IPTi Consortium has conducted pooled safety and efficacy analyses of all six trials of IPTi-sulfadoxine-pyrimethamine at 12 months of age (Brekenridge et al.; Aponte et al., in preparation). These analyses have demonstrated a 30% protective efficacy against malaria, a 23% protective efficacy against all-cause hospital admissions, a 38% protective efficacy against malaria-related hospital admissions, and a 15% protective efficacy against anemia – all in the first year of life.

**Overall evidence regarding safety and serologic interactions of IPTi-sulfadoxine-pyrimethamine with serologic response to Expanded Programme on Immunization antigens**

Sulfadoxine-pyrimethamine has been extensively used in Africa over many years, and its safety in all age-groups and in later stages of pregnancy is well established [24*,26–28,29*]. Approximately 4000 infants received ~12000 doses of IPTi-sulfadoxine-pyrimethamine in the six trials. The total number of deaths in the IPTi-sulfadoxine-pyrimethamine groups and in the control groups was similar, although the trials were not powered to measure the effect of IPTi on mortality (Brekenridge et al., in preparation). There was one death in the six trials that was considered possibly attributable to sulfadoxine-pyrimethamine [11*]. Overall, there was a statistically significant 19% reduction in the risk of serious adverse events (SAE) among those receiving IPTi-sulfadoxine-pyrimethamine compared with placebo across all trials (pooled relative risk 0.82, 95% confidence interval 0.74–0.9). Only one trial [11*] noted skin reactions considered due to IPTi; two in the sulfadoxine-pyrimethamine group (both Stevens Johnson Syndrome), and one in the placebo group; all three infants recovered fully.

A committee convened by the WHO to investigate possible interactions between IPTi and the serological response to EPI vaccines found no difference in the geometric mean titer between the sulfadoxine-pyrimethamine and placebo groups for the following antigens: measles, diphtheria, pertussis, tetanus, polio, hepatitis B and yellow fever (limited results only for yellow fever). The committee concluded that IPTi-sulfadoxine-pyrimethamine delivered at the time of routine vaccination does not have an adverse impact on the serological responses to EPI vaccines when delivered alongside those vaccines.
How does intermittent preventive treatment delivered to infants work?
While the mechanism by which IPTi works is not known, several trials have reported that the period of greatest protective efficacy against malaria is in the 30 days following an IPTi dose, suggesting that the primary mechanism of action is prophylaxis [9,10]. This conclusion has also been supported by modeling exercises [30]. By decreasing the risk of a first malaria episode, IPTi may reduce the risk for subsequent episodes [31]. Additionally, sulfadoxine-pyrimethamine, when administered to uninfected infants in endemic areas who have maternal antibody against malaria and also still have fetal hemoglobin, may effectively prevent new blood-stage infections. Finally, as malaria episodes are not entirely prevented by IPTi, there may be an increase in subclinical infections among those receiving IPTi that may enhance the development of protective antibody responses [31].

Why has there not been a rebound observed with intermittent preventive treatment delivered to infants?
A possible rebound effect following termination of an IPTi intervention did not occur in the initial Tanzanian trial, which demonstrated a sustained protective efficacy of 36% against clinical malaria during the follow-up period from 10 to 24 months of age [31]. No rebound effect was reported from the Mozambiquan trial [10] during follow-up until the age of 24 months. Chandramohan et al. [9] reported a 19% increase in high-density (≥5000 parasites per microliter) malaria from 16 to 24 months of age, corresponding to 4–12 months after the last dose of IPTi, but did not identify any other evidence of rebound in terms of overall burden of clinical malaria, hospitalizations, or anemia. In the Kumasi and Tamale trials [11], rebound in anemia was seen in the second year of life but these effects were only seen in select subgroup analyses, and do not represent an overall pattern of rebound.

In the pooled analysis of the six trials, there was no significant rebound in episodes of clinical malaria, anemia, or all-cause hospital admissions or with parasites in the 5-month period after the IPTi schedule was finished. Based on the evidence to date, it seems that IPTi is not associated with a rebound effect as was seen in earlier chemoprophylaxis trials. It appears that while IPTi protects against clinical malaria and anemia, it does not interfere with the acquisition of natural immunity to P. falciparum. Further analysis of the results from extended follow-up of the IPTi trials may help to elucidate the complex interplay between various potential determinants of protection such as transmission intensity and the use of ITNs.

Is intermittent preventive treatment delivered to infants the appropriate strategy in all epidemiological settings in Africa?
While IPTi clearly appears to be a promising strategy for much of Africa where malaria transmission is stable and perennial, there are legitimate concerns about its role in areas with seasonal transmission. Chandramohan et al. [32] have predicted the impact that IPTi might have in West Africa where transmission of malaria is often seasonal. These authors estimate that of the 18.6 million episodes of malaria expected annually in 10 West African countries, only 10% would be averted through an EPI-linked IPTi delivery mechanism. This estimate, lower than one would expect based on the efficacy results from published trials, is attributed to the low coverage with EPI vaccines, the highly seasonal nature of transmission in much of the sub-region, and the fact that the disease burden in such epidemiological settings is not necessarily concentrated in infancy. The authors suggest that alternative delivery strategies, such as IPTc (also labeled seasonal IPT by some), may be required.

In an area of intense, highly seasonal (5 months) malaria transmission in West Africa, however, IPTi delivered through EPI resulted in a 25% reduction in clinical malaria, a 40% reduction in hospital admissions for malaria, and a 35% reduction in hospital admissions for anemia [9], demonstrating that IPTi delivered through the EPI can be efficacious in such areas with up to a 5-month malaria transmission season.

To date, one published trial, conducted in Senegal [7], in an area where the malaria season lasts for 3 months, examined the efficacy of seasonal IPT with sulfadoxine-pyrimethamine (single dose) + artemisin (single dose) given at monthly intervals for 3 months. This intervention resulted in an 86% protective efficacy against clinical malaria in children 2–59 months of age.

There are two primary concerns regarding IPTc or seasonal IPT as a potential malaria-control strategy. The first is whether such an approach – which does not build on an existing healthcare platform such as antenatal care clinics or EPI – is feasible and can be scaled up at reasonable cost outside of a trial setting to achieve the desired coverage levels. The second concern is whether the administration of multiple closely spaced doses of antimalarials during a short transmission season will completely impede the generation of natural immunity. If, in such settings, the strategy is coupled with aggressive transmission reduction through the use of ITNs, with the ultimate goal of actually interrupting transmission, then these concerns might be allayed.
Will intermittent preventive treatment with sulfadoxine-pyrimethamine continue to work as sulfadoxine-pyrimethamine fails for treatment?

There are two concerns relating to IPT and drug resistance. First, how does the efficacy of an antimalarial when measured using the standard in-vivo measure of efficacy for treatment relate to the efficacy of IPT, that is, prevention? Second, what role does the administration of antimalarials to asymptomatic individuals have in the progression of parasite drug resistance?

Sulfadoxine-pyrimethamine, when used as first-line treatment for malaria, exerts a strong selection pressure on parasite populations, increasing the frequency of mutations associated with parasite resistance to sulfadoxine-pyrimethamine, namely those mutations found in DHFR and DHPS in the parasite folate pathway [33]. These mutations are associated with a reduction in the efficacy of sulfadoxine-pyrimethamine for the treatment of uncomplicated malaria in children less than 5 years old [34,35], and this rise in resistance has been well documented in in-vivo studies throughout the African continent including the IPTi study areas [36–40]. Many countries have therefore changed their recommended first-line antimalarial drug regimen for treating uncomplicated malaria to artemisinin combination therapy (ACT) [41]. When used for malaria prevention, however, sulfadoxine-pyrimethamine appears to continue to function despite high rates of resistant haplotypes and reduced efficacy in treatment of symptomatic malaria. In studies of IPTp, sulfadoxine-pyrimethamine was associated with a reduced risk of placental malaria and maternal anemia, and increased birth weight in areas where day-14 clinical and parasitologic failure rates were as high as 19–26% [24**].

In studies of IPTi, the lack of association between sulfadoxine-pyrimethamine preventive and treatment efficacy is illustrated in Fig. 1. At levels of at least 31% clinical and parasitologic failure with sulfadoxine-pyrimethamine, there does not appear to be a correlation between the protective efficacy of sulfadoxine-pyrimethamine used as IPTi and treatment efficacy. The mechanism of action of sulfadoxine-pyrimethamine may be different in the context of prevention as opposed to treatment. In the case of asymptomatic infections, sulfadoxine-pyrimethamine may be able to suppress lower levels of parasitemia despite the presence of resistant haplotypes. Studies examining this question are currently being undertaken in Tanzania and Gabon (ClinicalTrials.gov identifiers NCT00361114 and NCT00453856).

The second concern to policy makers is how the implementation of IPT-sulfadoxine-pyrimethamine will affect parasite resistance to sulfadoxine-pyrimethamine. There is no doubt that widespread use of sulfadoxine-pyrimethamine for treatment leads to selection of resistant parasites. Now that sulfadoxine-pyrimethamine monotherapy is no longer generally used as a first-line treatment antimalarial, the drug pressure on the parasite is substantially reduced, as is the primary concern of waning sulfadoxine-pyrimethamine efficacy for treatment of clinical malaria. The IPTi Consortium is currently examining the relationship between widespread use of sulfadoxine-pyrimethamine for IPTi and molecular markers of drug resistance in a community-randomized effectiveness trial of IPTi-sulfadoxine-pyrimethamine delivered to 12 000 infants a year in Tanzania; effects on drug resistance will be measured in all age groups of the population in the trial area.

Figure 1 IPTi protective efficacy up to 3 months after last dose compared with estimated Plasmodium falciparum resistance to sulfadoxine-pyrimethamine across six sites in Africa

![Figure 1](image-url)
What are likely successor drugs beyond sulfadoxine-pyrimethamine for intermittent preventive treatment?

It is possible that rates of *P. falciparum* resistance to sulfadoxine-pyrimethamine will continue to rise to levels that will render IPTi with sulfadoxine-pyrimethamine less efficacious, although that scenario is by no means assured. Prudence dictates, however, that the safety and efficacy of alternative drugs to sulfadoxine-pyrimethamine be explored for use as IPT. Three trials, two in Africa and one in Oceania, exploring alternatives to sulfadoxine-pyrimethamine for IPTi are currently underway. One, in Kenya, is testing sulfadoxine-pyrimethamine (single dose)+artesunate (given over 3 days), amodiaquine (given over 3 days)+artesunate (given over 3 days), and chlorproguanil-dapsone (given over 3 days). Another, in Tanzania, is examining sulfadoxine-pyrimethamine (single dose), mefloquine (single dose) and chlorproguanil-dapsone (given over 3 days). The third trial in Papua New Guinea is testing sulfadoxine-pyrimethamine (single dose)+artesunate (given over 3 days), and sulfadoxine-pyrimethamine (single dose)+amodiaquine (given over 3 days).

Finding a replacement for sulfadoxine-pyrimethamine will not be easy as it has properties that make it ideal for IPT. It has a long half-life of at least 5 days; it can be administered as a single dose, has few acute unpleasant side effects (such as vomiting) that might limit acceptability, and it is very inexpensive. In addition, the lack of a correlation between moderate levels of sulfadoxine-pyrimethamine treatment failure and IPTi efficacy, coupled with the lack of a rebound effect seen in the IPTi trials, may indicate that a drug like sulfadoxine-pyrimethamine that is ‘leaky’ or failing to completely clear parasitemia, is ideal for a preventive strategy such as IPT. Such a drug used preventively may actually function more like a vaccine, allowing extended exposure to parasites while preventing clinical illness and the associated counter-productive immunologic cascade.

Recent evidence from the pooled analyses suggests that the effect of IPTi is more related to intermittent chemoprophylaxis, rather than clearance of parasitemia. Therefore, the addition of short-acting drugs like artesunate to drugs such as sulfadoxine-pyrimethamine or amodiaquine, or the use of short-acting drugs alone, such as chlorproguanil-dapsone, are unlikely to yield promising results. Mefloquine, with its long half-life and ability to be administered as a single dose, is promising, although vomiting and other adverse effects may limit its acceptability when used for prevention. Dihydroartemisinin-piperazine may also prove to be a viable candidate for IPT. There may be risks, however, associated with using a drug that has great promise as a first line in the treatment of symptomatic malaria simultaneously for mass prevention. In addition, it remains unclear if using a drug as efficacious and long-acting as dihydroartemisinin-piperazine may result in sufficiently complete prevention of malaria to result in clinical rebound in the second year of life, due to the lack of exposure to parasites and resultant effect on limiting the development of natural immunity.

What other questions need to be answered?

Acceptance by caregivers and communities is critical for the successful uptake of any new intervention, which must also be cost-effective. A series of acceptability trials is currently being carried out at several IPTi Consortium sites. The first of these studies to be published showed that while there were some concerns related to the IPTi trial itself, the intervention was generally well accepted, did not affect perceptions of EPI, and was not misperceived as a malaria vaccine. A large-scale community effectiveness trial is underway in Tanzania, the results of which will elucidate how well the protective effect of IPTi holds when the intervention is scaled up programmatically. Results of this trial are expected in late 2007 or early 2008. A group of health economists are examining the cost effectiveness of IPTi. Preliminary data suggest that the cost per DALY averted is less than US$4 using IPTi-sulfadoxine-pyrimethamine, making this public-health intervention a very good investment by any measure (Hutton et al., submitted for publication). UNICEF teams are currently conducting large-scale pilot implementation of IPTi-sulfadoxine-pyrimethamine involving over 300,000 infants in six African countries (Benin, Ghana, Madagascar, Malawi, Mali and Senegal). These pilot experiences will provide valuable lessons for programmers on how best to scale up IPTi if the strategy is ultimately adopted by National Malaria Control Programmes.

Conclusion

A solid evidence base demonstrated in a pooled analysis of 6 trials that IPTi-sulfadoxine-pyrimethamine provides a 30% protective efficacy against malaria, a 24% protective efficacy against all-cause hospital admissions, a 37% protective efficacy against malaria-related hospital admissions, and a 15% protective efficacy against anemia – all in the first year of life. Given the excellent safety and tolerability of sulfadoxine-pyrimethamine, and that the intervention is inexpensive and easily deliverable if linked to the EPI, IPTi appears to add a valuable tool to the malaria-control armamentarium. Variations of IPTi that target older children may be required for areas of Africa with highly seasonal malaria transmission. There is a need to be alert to the potential detrimental effect an increase in *P. falciparum* resistance to sulfadoxine-pyrimethamine might have on the efficacy of IPTi with sulfadoxine-pyrimethamine. This threat demands monitoring of the efficacy of IPTi with sulfadoxine-pyrimethamine and the prompt evaluation of other suitable antimalarial agents.
Acknowledgements
Roly Gosling is currently paid from a grant from the Bill and Melinda Gates Foundation (Grant number: 205/79) for a study called ‘Drug options for intermittent preventive treatment for malaria in infants in an area with high resistance to sulfadoxine/pyrimethamine: An evaluation of short and long-acting antimalarial drugs’.

Andrea Egan works for the Hospital Clinic Foundation (Barcelona) as the coordinator of the IPTi Consortium. She receives her salary from the Hospital Clinic Foundation, which receives the funds for her position from the Bill and Melinda Gates Foundation.

References and recommended reading
Papers of particular interest, published within the annual period of review, have been highlighted as:

* of special interest
** of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (pp. 641–642).

7 This review offers an insight into and an overview on the policy-making process required for turning a researched intervention into policy and action.
9 Seasonal IPT with 3 doses of sulfadoxine-pyrimethamine-ir-artesunate given in monthly intervals resulted in a 86% PE against clinical malaria in Senegalese children 2–59 months of age.
13 One of four recent IPTI-sulfadoxine-pyrimethamine trials demonstrating PE efficacy against malaria in Southern East Africa.
15 One of four recently published IPTI-sulfadoxine-pyrimethamine trials, demonstrating PE efficacy against malaria and malaria-associated anemia in a hyperendemic area of West Africa.
17 One of four recent IPTI-sulfadoxine-pyrimethamine trials, demonstrating PE efficacy against malaria in a hyperendemic area in Central Africa.
19 One of four recently published IPTI-sulfadoxine-pyrimethamine trials, demonstrating PE efficacy against malaria and malaria-associated anemia in another hyperendemic area of Ghana.
24 This paper is of utmost importance as it links the development of natural immunity following repeated malaria episodes to clinical presentation, and as it offers important insight into what constitutes rebound and of what importance it is in the long term.
30 In HIV-positive pregnant women, monthly IPTP-sulfadoxine-pyrimethamine proved to be more efficacious than a 2-dose regimen in preventing placental malaria.
33 Detailed overview looking into how sulfadoxine-pyrimethamine efficacy may afflict and alter efficacy of IPTP.
39 This paper gives an updated overview on sulfadoxine/pyrimethamine safety and toxicity and discusses implications for IPTP.
43 This paper stresses that in areas of highly seasonal transmission, delivery mechanisms other than EPI may be needed to facilitate for optimal effectiveness of IPT to young children.

Intermittent preventive therapy for malaria Grobusch et al. 619

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Antimicrobial agents: viral/parasitic


The implications of antiviral drugs with activity against hepatitis B virus and HIV
Marcelle Bottecchia, Javier Garcia-Samaniego and Vincent Soriano

Purpose of review
Around 10% of individuals infected with HIV suffer from chronic hepatitis B virus infection. This represents at least 4 million people worldwide. HIV infection modifies the course of hepatitis B virus associated liver disease with faster progression to cirrhosis. The number of anti-hepatitis B virus drugs has increased within the last few years, and some of them also exert activity against HIV-1. The aim of this article is to update the current knowledge on antiviral therapy for chronic hepatitis B in HIV-infected patients.

Recent findings
In the absence of successful anti-hepatitis B virus therapy, morbidity and mortality associated with liver disease are increased in hepatitis B virus/HIV coinfected individuals. Data derived from studies using new more potent anti-hepatitis B virus drugs are very promising, and strategies to use these antivirals sequentially and/or in combination are being developed. Hopefully, this success will help bring a halt to liver-related complications and death in the hepatitis B virus/HIV coinfected population.

Summary
Appropriate diagnosis and monitoring of chronic hepatitis B, including the use of noninvasive tools for assessing liver fibrosis, measurement of serum hepatitis B virus-DNA, and drug resistance testing, along with wise use of antivirals may convert hepatitis B virus/HIV coinfection into a manageable disease.

Keywords
antiviral drugs, coinfection, hepatitis B virus, HIV-1, liver

Introduction
Among the estimated 40 million persons infected with HIV worldwide, approximately 4 million (10%) are chronically infected with hepatitis B virus (HBV) [1]. The prevalence of HBV/HIV coinfection demonstrates geographical variations, largely due to differences in the predominant routes of transmission. Studies focused on the natural history of chronic hepatitis B (CHB) in the HIV setting have demonstrated a greater risk of liver disease progression and death in coinfected individuals [2]. In the USA and Europe, more than half of HIV-infected homosexual men have evidence of past HBV infection, and 5–10% suffer from chronic HBV infection [3,4], which is defined as the persistence of the surface antigen (HBsAg) in the serum for over 6 months. In endemic regions of Africa and Asia, the majority of HBV infections occur perinatally (vertical transmission) or before the age of 5 years through close contact within households (horizontal transmission), medical procedures and traditional scarifications [5]. The relatively low rate of vertical transmission in Africa compared with Asia may be due to a lower prevalence of serum HBeAg in African women with CHB, which is a major determinant of perinatal HBV transmission [6]. Rates of HBV/HIV coinfection seem to be slightly lower among intravenous drug users and much lower among people infected through heterosexual contact [7].

HBV strains can be classified into eight genotypes, designated A to H, based on a minimum sequence divergence of 8% of the entire genome. HBV genotypes have a distinct geographical distribution, with genotype A predominant in northern Europe, North and South America, and some African regions. This genotype may be subdivided into three subgenotypes which differ by at least 4% in their nucleotide sequence and also show a distinct geographical distribution [8]. Genotypes B and C are commonly found in East Asia, and the latter has been associated with an increased risk of hepatocellular carcinoma [9]. Genotype D is more frequent in the Mediterranean basin, genotype E in Africa, genotype F in Central and South America, genotype G in France and USA, and genotype H in North and Central America. The geographic distribution of HBV genotypes should be viewed as a dynamic phenomenon due to increased population migrations. A different susceptibility to antiviral agents has been reported for distinct HBV genotypes [9,10]; for example, genotype A tends to respond better to
Elevated
Significant

The relevance of occult HBV infection in HIV-positive patients has been a matter of debate and discordant results may be explained by differences in methods used and definition criteria [11]. Ideally, occult HBV infection should be defined as the presence in serum of detectable HBV-DNA in the absence of HBsAg [12]. Using strict criteria, occult HBV infection is rare and does not account for significant liver damage in most instances.

Hepatitis delta virus (HDV) is a subviral satellite of HBV that depends on the HBsAg for the encapsidation of its own genome. As HDV shares the same routes of infection as HIV, HBV and HCV, coinfection with all these viruses is not uncommon, especially in high-risk populations such as intravenous drug users [12]. Of note, HIV coinfection may drive a more severe course of chronic delta hepatitis [13,14]. Therefore, delta superinfection should always be investigated in all HBsAg carriers.

When to treat chronic hepatitis B in HIV-infected patients?
The decision to treat chronic hepatitis B (CHB) in HIV-coinfected individuals must be based on a careful consideration of the need for antiretroviral therapy for HIV infection, the severity of liver disease, the likelihood of response to anti-HBV agents and potential adverse events [12,15]. HBV/HIV coinfected individuals with active HBV replication and elevated aminotransferases should be considered for anti-HBV therapy, even if the criteria for starting antiretroviral therapy for HIV infection have not yet been satisfied. In the context of HIV infection, CHB progresses more rapidly to cirrhosis and the response to HBV therapy worsens as immunodeficiency progresses [12]. HBV treatment objectives are the same for individuals with and without HIV coinfection: alanine aminotransferase (ALT) normalization, improvement in liver histology, and sustained suppression of serum HBV-DNA [12,15,16,17,18,19].

Recent studies have highlighted the benefits of inhibiting HBV replication and have established a direct association between serum HBV-DNA levels and the risk for developing liver cirrhosis and hepatocellular carcinoma, regardless of HBeAg status and/or liver enzyme elevations [22,23]. The most recent guidelines recommend starting anti-HBV treatment in individuals positive for the hepatitis B e antigen (HBeAg) when serum HBV-DNA is above 2 × 10^4 IU/ml. In contrast, in patients with negative serum HBeAg, the threshold above which therapy should be recommended is 2 × 10^5 IU/ml [17,18,19]. In view of the suppressive rather than curative nature of HBV therapeutics in most cases, medication has to be provided for long periods and even indefinitely to maintain its benefit through persistent HBV suppression. Treatment is most beneficial and efficacious when patients are in the immunoreactive phase [17**]. Patient characteristics that contribute to treatment success have been identified, and include low serum HBV-DNA levels, HBeAg positivity and elevated liver enzymes [17**,18,19].

Given the accelerated course of CHB in HIV-infected individuals [2], treatment should be considered more openly than in HIV-negative counterparts [12,15]. Figure 1 records an algorithm for anti-HBV treatment in HIV-positive patients, which is based on three parameters. In order of importance, these are serum HBV-DNA, ALT and liver fibrosis staging. When viremia is above 2000 IU/ml and/or ALT are elevated, significant liver damage must be expected, and therefore treatment should be advised. On the other hand, advanced liver fibrosis or cirrhosis can sporadically be recognized in patients with low serum HBV-DNA and/or normal ALT; and accordingly these patients may also benefit from antiviral treatment.

As previously mentioned, all patients with CHB should be tested for serum HDV antibodies, regardless of HIV status. Almost all seropositive individuals show active HDV replication, which is associated with more severe liver damage, and even worse in the HIV setting [13,14]. HIV-positive patients with delta hepatitis should therefore always be considered as candidates for treatment, although therapeutic options are very limited at this time and information on the potential efficacy of drugs other than interferon is scarce.

Antivirals for the treatment of chronic hepatitis B in HIV-infected patients
Seven drugs have been approved by the US Food and Drug Administration (FDA) for the treatment of CHB.

Figure 1 Therapeutic algorithm for chronic hepatitis B in HIV

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Interferon α-2b
Interferon α-2b was the first drug approved for treating CHB; however, standard interferon has been replaced by pegylated interferon in most instances. Interferon (or pegylated interferon) is particularly effective for HBeAg⁺ CHB individuals with high ALT levels and low serum HBV-DNA titers \[17^{**},18,19\]. Frequent side effects of the drug (flu-like symptoms, psychiatric effects, bone marrow suppression and thyroid dysfunction) have limited its use and it is contraindicated in decompensated cirrhotic patients, as it may exacerbate decompensation events. Liver enzyme flares during interferon treatment are more common in HIV-infected persons than in HIV-negative counterparts for unclear reasons \[24\]; moreover, the efficacy of interferon is lower in HBV/HIV coinfec-
tion, most likely as result of immune abnormalities \[25,26\]. Pegylated interferon-α-2a
Pegylated forms of interferon-α have a longer half-life and higher potency than standard interferon. In individuals with HBV monoinfection, pegylated interferon is more effective than standard interferon. In HBeAg⁺ patients, nearly one-third may lose serum HBeAg and normalize ALT upon 12 months of therapy \[27\]. Trials comparing pegylated interferon and lamivudine have shown that rates of HBeAg seroconversion, serum HBV-DNA suppression and ALT normalization are significantly higher using pegylated interferon than lamivudine, but interestingly there is no further benefit using both drugs in combination \[28,29\].

In HBV/HIV coinfec-
tion, pegylated interferon is asso-
ciated with lower rates of therapeutic success and increased toxicity \[25,26\]. Therefore, the drug may only be advised in nondecompensated cirrhotic patients with no need for antiretroviral therapy and those with good chances of interferon response, such as those with elevated liver enzymes and low serum HBV-DNA \[12,15\]. Lamivudine
Lamivudine is an oral cytosine nucleoside analog with both anti-HIV and anti-HBV activities, although the doses needed to suppress HBV (100 mg/day) are much lower than those required for suppressing HIV (300 mg/ day). The effectiveness of lamivudine in the treatment of CHB is very well documented, providing significant reductions in serum HBV-DNA and ALT levels, improvement in liver histology, and enhanced rate of serum HBeAg loss. A major problem with the long-term use of lamivudine, however, is selection of viral resistance (Fig. 2), which is inherently associated with rebounds in serum HBV-DNA and liver enzyme flare-ups \[30\]. For treating HBV/HIV coinfection, the recommended dose of lamivudine is 300 mg/day and the drug should always be given with at least two other anti-HIV agents, otherwise HIV resistance mutations would rapidly emerge.

Given its oral administration, excellent tolerability and posology (one pill once daily), lamivudine has been widely used as anti-HBV agent, including in patients coinfected with HIV, many of whom have received long-term lamivudine therapy and unfortunately harbor lamivudine-resistant HBV \[31^{*},32\]. Overall, HBV resistance mutations can be recognized in 94% of viremic patients with HIV infection who have received lamivudine treatment for over 4 years.

An intriguing phenomenon which has recently attracted much attention is that lamivudine resistance mutations may result in changes in HBV antigenicity. The reason for this observation is that the polymerase and envelope genes of HBV overlap and drug resistance mutations in the polymerase may simultaneously alter the HBsAg, causing a diminished HBs antigen-antibody binding. This may result in failure using diagnostic tests or vaccine escape \[33^{**},34\]. These mutations are more frequently found in individuals infected with HBV genotype A, which is the most prevalent in European and North American HBV/HIV coinfected patients \[34\]. Adefovir
Adefovir was the first nucleotide analog approved for the treatment of HBV infection. The drug may also inhibit HIV at doses greater than approved for treating HBV, but is then associated with an increased risk of nephrotoxicity. At doses of 10 mg/day, adefovir suppresses HBV replication and, interestingly, is associated with a low rate of resistance \[35,36,37^*\], as recorded in Fig. 2.

In HBV/HIV coinfected individuals, the performance of adefovir was examined in 35 patients with ongoing antiretroviral therapy, including lamivudine. After 144 weeks of adding adefovir, a decrease in serum HBV-DNA levels.
was observed in 45% of subjects, which is lower than the 56% observed in HBV monoinfection [38]. Selection of mutation K65R in HIV using adeovir monotherapy in HBV/HIV coinfected patients not taking antiretroviral therapy has been a matter of concern, but at least one study has excluded this possibility after examining minor virus populations using endpoint dilutions [39].

Some 5–10% of CHB patients do not respond to adeovir [40–42]. Several reasons may explain this failure (see Table 1), including pharmacokinetic/pharmacodynamic limitations of the low adeovir dosing, presence of genetic polymorphisms (I233V and L217R), and cross-resistance with lamivudine upon selection of changes at codon 181 (A→STV) [33**,43].

**Entecavir**

Entecavir is a guanosine analog that inhibits HBV replication at three different steps (priming, reverse transcriptase and positive strand synthesis) [44]. Entecavir shows more potency in suppressing serum HBV-DNA than lamivudine and adeovir, and is effective against wild-type and lamivudine and adeovir-resistant HBV [45–47]. Entecavir resistance generally results from the accumulation of multiple changes in the HBV polymerase, including lamivudine resistance mutations [48]. For this reason, entecavir doses of 0.5 mg/day are recommended for lamivudine-naive patients, but 1 mg/day is advised for patients with lamivudine-resistant HBV.

The data on entecavir use in HBV/HIV coinfected individuals are conflicting. While the drug was not thought to be active against HIV [49], a recent report has highlighted that it can produce 1 log reduction in plasma HIV-RNA and occasionally select for mutation M184V in HIV [50**,51]. Another report has not confirmed this observation in an individual with HIV/HBV/HDV coinfecion, in whom entecavir did not cause any significant change in plasma HIV-RNA nor select for the M184V mutation in HIV after more than 24 weeks of treatment [52]. Further research is therefore needed to elucidate the potential clinical relevance of any anti-HIV activity of entecavir at the doses currently approved for treating HBV. In the meantime, a warning from the FDA has alerted against the use of entecavir in coinfected patients in the absence of antiretroviral therapy. This recommendation has however been made in the absence of information about potential interactions of entecavir with other antiretrovirals, especially abacavir, given that both drugs are guanosine analogs and may be subject to inhibitory competition phenomena (see Table 2) [53].

**Telbivudine**

Telbivudine is a thymidine L-analog with no activity against HIV. Telbivudine has significantly greater antiviral efficacy than either lamivudine or adeovir in patients with CHB, and selects for resistance mutations at intermediate rates (see Fig. 2) [54]. In studies used for the registration of the drug, up to 60% of HBeAg+ CHB individuals achieved undetectable serum HBV-DNA after 12 months of telbivudine treatment compared with 40% treated with lamivudine. In the second year of treatment this rate lowered to 54% due selection of telbivudine resistance [55]. Characteristically, telbivudine selects for mutation M204I, which causes cross-resistance to lamivudine, which may select for either M204V or M204I; therefore, telbivudine should not be used following lamivudine failure, and vice versa. Interestingly, there is no evidence so far of cross-resistance between telbivudine and adeovir. Finally, no studies have been conducted yet to test the activity and safety of telbivudine in HBV/HIV coinfection.

**Emtricitabine**

Like lamivudine, emtricitabine is a cytosine analog with antiviral activity against both HBV and HIV. It has a longer half-life than lamivudine and similarly induces a rapid and sharp reduction in serum HBV-DNA levels at doses of 300 mg/day. Suppression of HBV replication is maintained over 48 weeks of treatment in more than half of patients [56,57]. No data are available on emtricitabine used alone in HBV/HIV coinfection, although large experience already exists derived from using the drug in combination with tenofovir in a single pill formulation (Truvada; Gilead Sciences, Foster City, CA, USA). In fact, Truvada is the preferred choice for treating CHB in HBV/HIV coinfected patients with need for antiretroviral therapy, as shown in Fig. 3 [12]. This combination provides potent anti-HBV activity along with a solid backbone for a triple combination antiretroviral regimen. Like lamivudine, emtricitabine should not be used as

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**Table 1 Reasons to fail adeovir**

<table>
<thead>
<tr>
<th>Low antiviral activity ± Poor drug exposure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polymorphisms causing natural resistance (I233V)</td>
</tr>
<tr>
<td>HBV genotype A2 (L217R)</td>
</tr>
<tr>
<td>Lamivudine cross-resistance (A181T/V)</td>
</tr>
</tbody>
</table>

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**Table 2 Classification of nucleos(t)ide analogs used as antiviral agents**

<table>
<thead>
<tr>
<th>Pyrimidine analogs</th>
<th>Purine analogs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cytidine</td>
<td>Thymidine</td>
</tr>
<tr>
<td>AZT</td>
<td>d4T</td>
</tr>
<tr>
<td>ddi</td>
<td>Tenovir</td>
</tr>
<tr>
<td>Entecavir</td>
<td></td>
</tr>
<tr>
<td>Telbivudine</td>
<td></td>
</tr>
<tr>
<td>Adeovir</td>
<td></td>
</tr>
<tr>
<td>Emtricitabine</td>
<td></td>
</tr>
<tr>
<td>Clevudine</td>
<td></td>
</tr>
<tr>
<td>Trifluridine</td>
<td></td>
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<tr>
<td>Vidarabine</td>
<td></td>
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<tr>
<td>Ribavirin</td>
<td></td>
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<tr>
<td>Gancyclovir</td>
<td></td>
</tr>
<tr>
<td>Famicyclovir</td>
<td></td>
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<tr>
<td>Acyclovir</td>
<td></td>
</tr>
<tr>
<td>Valacyclovir</td>
<td></td>
</tr>
</tbody>
</table>

CMV, cytomegalovirus; HBV, hepatitis B virus; HCV, hepatitis C virus; HIV, human immunodeficiency virus
monotherapy in HBV/HIV coinfected persons due to high risk for selecting the M184V resistance mutation in HIV. As emtricitabine and lamivudine show almost total cross-resistance, emtricitabine should not be prescribed after lamivudine failure.

**Tenofovir**

Tenofovir is an adenosine nucleotide analog, already approved for the treatment of HIV infection. It also shows potent activity against HBV in patients with and without lamivudine resistance [58–63]. HBV resistance to tenofovir has been occasionally described in HBV/HIV coinfected patients with lamivudine resistance mutations. In this subset of patients, selection of one additional change, A194T, resulted in more than 10-fold loss of susceptibility to tenofovir [64]. Large clinical trials are currently ongoing to prove the safety and efficacy of the drug in HBV-monoinfected patients. The ACTG A5127 trial was recently interrupted prematurely after showing that tenofovir was noninferior to adefovir, with clear evidence that in fact tenofovir could be superior to adefovir [65]. Figure 4 summarises the potency and genetic barrier for resistance of current approved anti-HBV agents [26].

**Preferred treatment choice for chronic hepatitis B in HIV-infected patients**

When HBV infection requires treatment but HIV infection does not, generally based on elevated CD4 counts (>350 cells/mm³), treatment options for HBV should include agents with no clinical activity against HIV, such as pegylated interferon, adefovir or telbivudine (Fig. 3). At the moment, entecavir should not be used until its controversial activity against HIV is elucidated. A 12-month course of pegylated interferon may be advisable for patients with elevated ALT, low serum HBV-DNA and minimal liver fibrosis, particularly when infected by HBV genotype A. Up to one-third of these patients may show sustained suppression of serum HBV-DNA upon stopping therapy, a benefit which cannot be achieved with any other drug. The limitation of pegylated interferon is its poor tolerability and its lower efficacy in the HIV setting. Moreover, the drug is contraindicated in decompensated cirrhosis, although it can be used with caution in individuals with compensated cirrhosis [7].

For the rest of HBV/HIV coinfected patients who do not need antiretroviral therapy, long-term nucleos(t)ide therapy is the only available option. At the moment, either adefovir or telbivudine may be good alternatives; however, given the risk of selecting drug resistance, a ‘very early add-on’ strategy (Fig. 5), as named by Fabien Zoulim, should be considered for patients who do not reach undetectable serum HBV-DNA at week 24 of therapy. Adding a drug rather than replacing it should be advised, as there is evidence for a protective activity against selection of resistance and less for a synergistic or additive antiviral activity with drug combination. Drugs with dual antiviral effect, such as lamivudine,
emtricitabine or tenofovir should never be used as single agents, given the high risk for selecting resistance in HIV.

An alternative option in this subset of HBV/HIV coinfected patients needing anti-HBV therapy but with a relatively well-preserved immune status is to advance the time to initiate antiretroviral therapy, and then include the combination of tenofovir plus emtricitabine (or lamivudine) as nucleos(t)ide backbone as part of a triple regimen [66]. This option may be particularly reasonable for patients with high plasma HIV-RNA and/or with active risk behaviors, for whom the risk of progression and/or transmission to others, respectively, is enhanced.

When both HIV and HBV fit criteria to be treated, the main caveat is whether prior exposure to lamivudine has occurred. In drug-naïve patients, following what has been advised in HIV-negative individuals, lamivudine (or emtricitabine) should no longer be prescribed as the only anti-HBV agent, given the risk for selecting resistance mutations that may compromise future therapeutic options, such as entecavir, telbivudine and occasionally adefovir [67]. At the moment, the combination of tenofovir plus emtricitabine (or lamivudine) is by far the preferred choice for this subset of patients.

For individuals with prior exposure to lamivudine and uncontrolled HBV replication, lamivudine resistance is almost always present and therefore the only available options are rescue interventions based on tenofovir or entecavir. The latter should be used at doses of 1 mg/day in this subset of patients, and viral load should be monitored periodically in order to assure that undetectable viremia is achieved relatively shortly, otherwise drug pressure will drive selection of resistance. With respect to tenofovir, several studies have clearly established its activity in the face of lamivudine-resistant HBV (Table 3) [58–63]. Of note, although serum HBV-DNA may decline on average 4 logs in this population, it may take a long time (several months or even more than a year) to achieve undetectable serum HBV-DNA. During this period of ongoing virus replication under tenofovir exposure, selection of resistance mutations that may compromise tenofovir activity (e.g. A194V) may rarely be seen [64].

A final consideration regards HBV/HIV coinfected patients with delta hepatitis. As mentioned earlier, all HBsAg+ individuals should be tested for HDV antibodies. In southern and central Europe, and especially in intravenous drug users, one-third of HBsAg+ patients will be HDV-seropositive. Recent studies have shown that almost all these patients are viremic for delta virus and, accordingly, are particularly at risk for more severe liver disease [13,14]. Treatment with the new potent anti-HBV nucleos(t)ide analogs may be beneficial in patients with delta hepatitis, although improvements in liver enzymes, serum delta viremia and liver histology may only begin to be recognized after several months or years of successful treatment [68].

**Expert opinion**

Treatment of CHB in HIV-infected individuals is challenged by the more accelerated course of liver disease in this population and the complexity and limited efficacy of antiviral treatment options. Most treatment decisions should be guided primarily by monitoring HBV viremia, although liver enzymes and hepatic fibrosis staging may help to make more appropriate treatment decisions. Selection of drug resistance is challenging and rescue interventions often should be made, adding rather than replacing drugs. In drug-naïve HBV/HIV coinfected patients, the treatment plan should be individualized based on the need to treat HIV infection. If so, the combination of tenofovir plus emtricitabine (or lamivudine) must be considered the preferred option. In contrast, when only anti-HBV therapy is advisable, early initiation of antiretroviral therapy or prescription of agents with selective anti-HBV activity may be considered. Among the latter, a course of pegylated interferon may be advisable for HBeAg+ patients with low HBV-DNA and elevated ALT levels. In the rest, either adefovir and/or telbivudine may be a good option. The role of entecavir in this subset of patients is currently on hold due to its potential anti-HIV activity. The role of combination therapy in HBV and the relevance of selecting drug-resistant HBV strains with HBsAg antigenic changes merit further research. Finally, delta hepatitis should always be excluded in all HBsAg+ patients.

**Conclusion**

Chronic HBV infection is relatively common in HIV-infected individuals and is associated with an increased risk of liver-related morbidity and mortality. The management of chronic hepatitis B in these individuals is challenging, as some drugs share activity against both HIV and HBV, and correspondingly may select for drug resistance in each virus. Treatment decisions should be based on serum HBV-DNA, ALT and liver fibrosis.

### Table 3 Tenofovir against hepatitis B virus in HBV/HIV coinfected patients

<table>
<thead>
<tr>
<th>Study</th>
<th>No. of patients</th>
<th>Week 12*</th>
<th>Week 24*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Núñez et al. [58]</td>
<td>12</td>
<td>–</td>
<td>–3.78</td>
</tr>
<tr>
<td>Nelson et al. [59]</td>
<td>20</td>
<td>–2.47</td>
<td>–4.00</td>
</tr>
<tr>
<td>Dare et al. [60]</td>
<td>10</td>
<td>–</td>
<td>–4.9</td>
</tr>
<tr>
<td>Von Bormel et al. [61]</td>
<td>35</td>
<td>–3.9</td>
<td></td>
</tr>
<tr>
<td>Restig et al. [62]</td>
<td>6</td>
<td>–3.1</td>
<td>–4.3</td>
</tr>
<tr>
<td>Benhamou et al. [63]</td>
<td>65</td>
<td>–4.56 (HBeAg+)</td>
<td>–2.53 (HBeAg-)</td>
</tr>
</tbody>
</table>

* Serum HBV-DNA decline (log IU/ml).
staging, while drugs of choice should be selected based on the need for antiretroviral therapy and prior lamivudine resistance, among other factors.

Acknowledgements
We would like to thank Julie Sheldon, Jose Martinez-Alarcon and Eugenia Vispo for their help and support with writing this review article.

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References and recommended reading
Papers of particular interest, published within the annual period of review, have been highlighted as:
* of special interest
** of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (pp. 643–645).


This bibliography is compiled by clinicians from the journals listed at the end of this publication. It is based on literature entered into our database between 1 August 2006 and 31 July 2007. Articles are generally added to the database about two and a half months after publication. In addition, the bibliography contains every paper annotated by reviewers; these references were obtained from a variety of bibliographic databases and published between the beginning of the review period and the time of going to press. The bibliography has been grouped into topics that relate to the reviews in this issue.

- Papers considered by the reviewers to be of special interest
- Papers considered by the reviewers to be of outstanding interest

The number in square brackets following a selected paper, e.g. [7], refers to its number in the annotated references of the corresponding review.

Bibliography

Current World Literature

Vol 20 No 6 December 2007

Contents

Antimicrobial agents: bacterial/fungal

629 Management of Gram-positive bacteraemia

630 Approaches to serious methicillin-resistant Staphylococcus aureus infections with decreased susceptibility to vancomycin: clinical significance and options for management

631 Therapy for resistant C. difficile-associated diarrhoea

631 Strategies for improved antibiotic use in lower respiratory tract infection

631 Clinical relevance of echinocandin paradoxical antifungal effect

632 Mould interactions with immunosuppressive agents, anti-cancers and comparative pharmacokinetics

632 Impact of pharmacodynamics and pharmacokinetics on echinocandin dosing strategies

633 Antifungal therapy for neonatal candidiasis

633 Treatment strategies for miscellaneous bacterial infections

634 Detecting trends in antimicrobial susceptibility or resistance patterns

636 Prophylactic use of antimicrobials

636 Antimicrobial resistance mechanisms and molecular characteristics

638 Adverse reactions

638 Antibiotic policies and management

639 New antimicrobials

640 Antibiotic administration and assimilation

640 Miscellaneous

Antimicrobial agents: viral/parasitic

640 Development of protease inhibitors as anti-parasitic agents

640 Do artemisinin derivatives have a role in the treatment and control of helminth infections and malaria?

641 Intermittent preventive therapy for malaria: progress and future directions

642 Strategies to reverse drug resistance in malaria

643 The implications of antiviral drugs with activity against hepatitis B virus and HIV

645 The potential of chemokines and chemokine receptors 5 inhibitors for HIV

647 The biology of viral-host cell fusion

647 Hepatitis B vaccination

648 Microbicides

648 Parasites and the immune system

649 Schistosomiasis

650 Chronic viral hepatitis

651 Parasitic infections: general

652 Trypanosomiasis

652 Malaria: general

653 Viral hepatitis: general

654 Miscellaneous

Antimicrobial agents: bacterial/fungal

Management of Gram-positive bacteraemia

Review: (pp. 561–567)


Antimicrobial agents: bacterial/fungal

Approaches to serious methicillin-resistant Staphylococcus aureus infections with decreased susceptibility to vancomycin: clinical significance and options for management

Review: (pp. 568–573)


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Antimicrobial agents: bacterial/fungal Clinical relevance of echinocandin paradoxical antifungal effect

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632 Antimicrobial agents: bacterial/fungal

Impact of pharmacodynamics and pharmacokinetics


Antifungal agents: bacterial/fungal 

Treatment strategies for miscellaneous bacterial infections

633


Antimicrobial agents: bacterial/fungal

Detecting trends in antimicrobial susceptibility or resistance patterns


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Antimicrobial agents: bacterial/fungal

Detecting trends in antimicrobial susceptibility

635

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Antimicrobial resistance mechanisms and molecular characterisation


Antimicrobial agents: bacterial/fungal


Antimicrobial agents: viral/parasitic

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Antibiotic administration and assimilation


Antimicrobial agents: viral/parasitic

Development of protease inhibitors as anti-parasitic agents


Do artemisinin derivatives have a role in the treatment and control of helminth infections and malaria?

Related review: Artemisinins and synthetic trioxolanes in the treatment of helminth infections (pp. 605–612)

Antimicrobial agents: viral/parasitic

Intemperate preventive therapy for malaria

Intermittent preventive therapy for malaria: progress and future directions

Review: (pp. 613–620)


642 Antimicrobial agents: viral/parasitic

Strategies to reverse drug resistance in malaria


Antimicrobial agents: viral/parasitic
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The potential of chemokines and chemokine receptors 5 inhibitors for HIV


The biology of viral-host cell fusion


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Antimicrobial agents: viral/parasitic

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TRYPANOSOMIASIS


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Viral hepatitis: general


Antibody responses to hepatitis B virus and HBeAg in children and adolescents: a 20-year follow-up study of a cohort infected in the 1980s.


Erratum

A typographical error was published in the review ‘Preerythrocytic malaria vaccine development’ by Mikolajczak et al. [1] which appeared on pp. 461–466 of Current Opinion in Infectious Diseases, Volume 20, issue 5.

The following text under the Introduction ‘Drug resistant parasites, insecticide resistant anopheline vectors, and elaborate immune evasion mechanisms of the parasites’ pathogenic blood stages foil successful malaria control and [1,2]. Doubtlessly programs an effective malaria vaccine would be the major breakthrough to reduce the global burden of malaria should read as follows ‘Drug resistant parasites, insecticide resistant anopheline vectors, and elaborate immune evasion mechanisms of the parasites’ pathogenic blood stages foil successful malaria control programs [1,2]. Doubtlessly, an effective malaria vaccine would be the major breakthrough to reduce the global burden of malaria.

Reference