The Efficacy of EBUS-Guided Transbronchial Needle Aspiration for Molecular Testing in Lung Adenocarcinoma

Julissa Jurado, MD, Anjali Saqi, MD, MBA, Roger Maxfield, MD, Alexis Newmark, BS, Matt Lavelle, MSE, Matthew Bacchetta, MD, Lyall Gorenstein, MD, Frank Dovidio, MD, Mark E. Ginsburg, MD, Joshua Sonett, MD, and William Bulman, MD

Department of General Thoracic Surgery, Department of Pathology and Cell Biology, and Division of Pulmonary and Critical Care Medicine, Columbia University Medical Center, New York, New York

Background. The purpose of the study was to assess the efficacy of obtaining adequate cytologic specimens by endobronchial ultrasound-guided transbronchial needle aspiration (EBUS-TBNA) for molecular testing of lung adenocarcinomas.

Methods. This was an institutional review board—approved study of all patients who had undergone EBUS-TBNA from April 2010 through March 2012 for the diagnosis, staging, or both of lung cancer. Patients with a diagnosis of adenocarcinoma were reflexively tested for molecular markers by polymerase chain reaction, sequencing, and fluorescence in situ hybridization (FISH). All procedures were performed with patients under conscious sedation in the bronchoscopy suite.

Results. Of 205 patients who underwent EBUS-TBNA, 56 patients (24 male, 32 female) had a diagnosis of adenocarcinoma warranting molecular analysis. Molecular analysis was available for epidermal growth factor receptor (EGFR), Kirsten rat sarcoma (Kras) mutation, and anaplastic lymphoma kinase (ALK) gene rearrangement. The institution’s clinical protocol involved initial testing for EGFR mutation with a reflex Kras test if the EGFR test result was negative. ALK FISH molecular testing was completed if both EGFR and Kras test results were negative. A total of 52 of 56 (93%) patients had sufficient cytologic material for complete or partial molecular testing, whereas 46 of 56 (82%) patients had sufficient material for all clinically indicated testing. EGFR, Kras, and ALK analysis yielded positive results in 5 (10%), 10 (25%), and 5 (12%) tested specimens, respectively. No complications were associated with EBUS-TBNA.

Conclusions. EBUS–TBNA performed with the patient under moderate sedation can be expected to yield sufficient tissue for sequential molecular analysis in the majority of patients. In an era of targeted therapy for lung adenocarcinomas, EBUS-TBNA is effective in clinical practice for complete diagnosis, staging, and treatment planning in these patients.


Over 226,000 new cases of lung cancer are expected in 2012 in the United States alone [1]. It remains the leading cause of cancer mortality, with the number of deaths in the U.S. expected to exceed 160,000, accounting for about 28% of all cancer deaths. The majority of patients have advanced disease at the time of their diagnosis. Accurate histologic subtyping and pathologic staging of patients at the time of diagnosis is essential for appropriate treatment selection. Approximately 85% of these malignancies will be non-small cell lung cancer (NSCLC), with adenocarcinomas accounting for over 50% [2]. Epidermal growth factor receptor (EGFR) mutations, Kirsten rat sarcoma (Kras) mutations, and translocations in the anaplastic lymphoma kinase (ALK) gene have been identified [3–5]. Discrete genetic alterations identified in adenocarcinomas have prognostic significance and present novel therapeutic targets [6].

Somatic mutations in the EGFR tyrosine kinase receptor can be targeted with small-molecule tyrosine kinase inhibitors (TKIs), such as erlotinib, and several recent studies have shown that TKI therapy in EGFR mutant adenocarcinoma results in better response rates and longer progression-free survival compared with conventional chemotherapy in patients with advanced disease [7, 8]. Approximately 30% of adenocarcinomas express Kras mutations, with mutual exclusivity with mutations in EGFR and ALK. The presence of Kras mutation has been associated with resistance to TKIs, with some studies showing that therapy with TKIs can be detrimental rather than beneficial in patients with this mutation [9, 10]. Several studies have demonstrated benefit in patients with lung adenocarcinoma bearing...
ALK gene rearrangements, with improved overall response rates and survival when crizotinib is given compared with traditional chemotherapy [5, 11]. In this context, we have entered an era of personalized therapy for patients with lung adenocarcinoma, with the results of molecular testing of a patient’s tumor potentially driving critical treatment decisions.

Cervical mediastinoscopy has traditionally been viewed as the gold standard for pathologically staging the mediastinum in patients with lung cancer [12]. Increasingly, endobronchial ultrasound with transbronchial needle aspiration (EBUS-TBNA) is being favored over mediastinoscopy for the diagnosis and staging of thoracic malignancy [13]. EBUS-TBNA can accurately stage the mediastinum in patients with NSCLC [14], is able to access hilar nodes that mediastinoscopy does not routinely biopsy, and can be performed with the patient under moderate sedation in an outpatient setting. Multiple studies have reported excellent sensitivity and specificity of EBUS-TBNA, comparable with those reported for mediastinoscopy [15, 16], although the majority of these studies were done in patients who underwent EBUS-TBNA under general anesthesia. The performance characteristics of this procedure done when patients are under moderate sedation are less well described, as is its efficacy in obtaining sufficient tissue for molecular analysis in patients with adenocarcinoma.

The rationale of this study was to assess the efficacy of EBUS-TBNA, performed with patients under moderate sedation, for obtaining sufficient tumor for molecular analysis in patients with lung adenocarcinoma. The primary endpoint was the percentage of specimens with sufficient cytologic material to undergo analysis for one or more molecular markers using a clinically relevant sequential testing algorithm.

**Material and Methods**

This was an institutional review board—approved review of data for all patients who underwent EBUS-TBNA at our institution from April 2010 through March 2012. The subset of patients with a final diagnosis of NSCLC, adenocarcinoma, or adenosquamous carcinoma was identified for this study. The subset consisted of patients who underwent EBUS-TBNA for initial diagnosis and staging, patients with a known diagnosis requiring mediastinal staging, and patients with a known diagnosis who required additional tumor sampling for molecular testing. In some cases, targeted sampling of a specific site was used; in the remainder, sequential ultrasound inspection and TBNA sampling of mediastinal nodes from highest to lowest nodal station, N3 to N1, was performed.

All procedures were performed with the patients under moderate sedation with Versed and fentanyl. Patients receiving antiplatelet therapy with aspirin or clopidogrel had these agents withheld for 5 and 7 days, respectively, before the procedure, and anticoagulant agents were discontinued before the procedure; patients who were not permitted to withhold these agents did not undergo EBUS-TBNA. Patients with a specific contraindication to a bronchoscopic procedure (refractory hypoxemia, active cardiac arrhythmia, or recent myocardial infarction) were excluded from EBUS-TBNA. All procedures used an on-site cytopathologist or cytopathologist for rapid on-site cytologic evaluation (ROSE) of each individual aspirated sample. Aspirated material was triaged in a manner designed to optimize the amount of tissue available for ancillary studies, including immunohistochemistry and molecular analysis. The procedures for cytologic specimen handling and processing have been described in detail by Bulman and colleagues [17].

After careful assessment of the patient and administration of adequate sedation, a BF-UC180F Olympus bronchoscope (Olympus America Inc, Center Valley, PA) was introduced into the oropharyngeal airway and advanced to the tracheobronchial tree. Aspirations were performed with a 21-gauge or 22-gauge needle at the discretion of the bronchoscopist, and ROSE was used for each aspirate. Each aspirate was discharged onto a glass slide, either by blowing air with a syringe through the needle or by replacing the needle stylet, and then saline was passed through the EBUS needle into Cytolyt solution to discharge any remaining cells. From the material dispelled onto the glass slide, a few tan-white color tissue particles were selected from the blood/mucus and smeared on two slides. The remaining specimen on the original slide was allowed to clot, and the clot was placed in formalin. One slide smear was air-dried for Diff-Quik staining for ROSE, and the second was placed immediately in alcohol for Papanicolaou staining. Cell blocks were made from the material in formalin, Cytolyt solution, or both [17]. Immunohistochemistry was performed for diagnosis by use of the cell block specimen at the discretion of the cytopathologist. Specifically, immunostains were performed to differentiate squamous cell carcinoma from adenocarcinoma, especially in cases of poorly differentiated carcinoma, and to determine the origin of the adenocarcinoma: primary versus secondary.

If the final diagnosis was a malignancy consistent with NSCLC, favor adenocarcinoma, primary lung adenocarcinoma, or adenosquamous carcinoma, reflex molecular analysis was performed by use of polymerase chain reaction, sequencing and fluorescence in situ hybridization (FISH). EGFR mutation testing was performed on all samples unless the EGFR status of the tumor was known from prior sampling. Given the mutual exclusivity of the testable mutations, no further testing was performed on samples that were positive for an EGFR or Kras mutation. EGFR-negative samples were reflexively tested for Kras mutation. ALK molecular testing was completed if the results of both EGFR and Kras tests were negative or were known to be negative from prior testing. ALK testing was attempted in some samples deemed inadequate for Kras testing at the discretion of the cytopathologist, given the need for fewer tumor cells for ALK testing by FISH than for the techniques used for Kras testing.
Results

A total of 205 patients underwent an EBUS procedure during the study period; 56 of them had a diagnosis of NSCLC, favor adenocarcinoma, primary lung adenocarcinoma, or adenosquamous carcinoma based on immunohistochemistry. The median age of the patients in this subset was 71 years (interquartile range [IQR], 63 to 80 years), and 32 were women (57%) (Table 1).

All patients had sampling of at least one nodal station, the most common site being the lower paratracheal nodes (n = 28 [50%]) and the second most common being the subcarinal nodes (n = 24 [43%]). A total of 15 (27%) patients had two nodal stations sampled. Six patients had sampling of three nodal stations. Two patients had a transbronchial needle aspiration of a parenchymal lung mass. A median of three individual aspirates (IQR, 2 to 4) were obtained for patients having only one nodal station sampled. A median of two aspirates per station (IQR, 1 to 3) were obtained for patients having two nodal stations sampled, and a median of 2.5 aspirates per station (IQR, 1.25 to 3) were obtained in patients having three nodal stations sampled (Table 2).

The reflex testing protocol was strictly followed in 71% of the cases. In five cases, the results of EGFR testing, Kras testing, or both were known from a prior biopsy of the primary tumor and testing was limited to the remaining markers. In five cases, ALK testing was performed despite a positive finding with either EGFR or Kras testing. The remainder of the deviations from the reflex testing protocol consisted of failure to perform Kras testing, ALK testing, or both after a negative finding on EGFR testing despite sufficient material available.

A total of 52 of 56 (93%) patients had sufficient cytologic material for either complete or partial molecular testing, with partial testing defined as samples adequate for EGFR testing but insufficient for Kras and ALK testing, or samples adequate for EGFR and Kras testing but insufficient for ALK testing. Forty-six of 56 (82%) of patients had sufficient material for all clinically indicated testing.

Table 1. Overall Patient Characteristics

<table>
<thead>
<tr>
<th>Total No. of Patients</th>
<th>n = 56</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age *</td>
<td>71 (63–80)</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>24 (43%)</td>
</tr>
<tr>
<td>Female</td>
<td>32 (57%)</td>
</tr>
<tr>
<td>Total number of sampled sites</td>
<td>n = 77</td>
</tr>
<tr>
<td>Sampled sites b</td>
<td></td>
</tr>
<tr>
<td>Nodal stations</td>
<td></td>
</tr>
<tr>
<td>Upper paratracheal</td>
<td>3 (5%)</td>
</tr>
<tr>
<td>Lower paratracheal</td>
<td>28 (50%)</td>
</tr>
<tr>
<td>Subcarinal</td>
<td>24 (45%)</td>
</tr>
<tr>
<td>Hilar</td>
<td>14 (25%)</td>
</tr>
<tr>
<td>Interlobar</td>
<td>6 (11%)</td>
</tr>
<tr>
<td>Parenchymal lung mass</td>
<td>2 (4%)</td>
</tr>
</tbody>
</table>

* Median value with interquartile range.  
  b Percentages in parentheses represent proportion in total patient cohort, n = 56.

There was sufficient cytologic material for molecular testing for EGFR mutation in 46 of 51 (90%) samples tested (Table 3). There was sufficient cytologic material for molecular testing for Kras mutation in 30 of 40 (75%) samples tested. The five samples insufficient for EGFR were not tested for Kras mutation; in five additional samples that had been adequate for EGFR testing, there was insufficient material for Kras testing. There was sufficient cytologic material for testing for ALK gene rearrangement in 39 of 43 (91%) of samples tested, including three samples that had been deemed insufficient for Kras testing and one sample that had been deemed insufficient for both EGFR and Kras testing.

The EGFR analysis yielded a positive result in five (10%) of samples tested. Kras was positive in ten (25%) samples, and ALK was positive in five (12%) samples tested. For the entire cohort of 56 patients, including the 5 patients for whom molecular testing results were available from prior biopsy or sampling of other sites, the positive rates for EGFR, Kras, and ALK were 9%, 20%, and 9% respectively. No complications were associated with EBUS-TBNA.

Comment

Until recently, all NSCLC patients were treated without regard for histologic subtype, and treatment involved platinum-based therapies [18]. The current standard of care for advanced NSCLC treatment follows an algorithm for therapy based on NSCLC histologic subtype [6, 19]. With the emergence of targeted therapies for lung adenocarcinoma, molecular testing to identify key driver mutations is required for appropriate treatment of patients with adenocarcinoma. These ancillary tests require an adequate amount of tissue. The need to subclassify NSCLCs and identify mutations has coincided with the increasing use of EBUS-TBNA, a safe alternative to cervical mediastinoscopy in the initial assessment and staging of NSCLC. Relative to specimens acquired traditionally by mediastinoscopy, video-assisted thoracoscopic surgery, and thoracotomy, the sample size of EBUS-TBNA specimens is much smaller. If EBUS-TBNA is to be an effective tool in the diagnosis and staging of NSCLC, it is essential that the small cytologic sample be sufficient for all the necessary testing.

In this study, EBUS-TBNA performed with patients under moderate sedation was shown to provide sufficient tissue for molecular testing of lung adenocarcinomas in the majority of patients. A strength of this study was that molecular analysis was attempted in all diagnoses of lung adenocarcinoma in reflexive testing protocol. This stands in contrast to other studies based on cases in which the testing was not performed in all cases but was requested by the treating physician in selected cases or deemed adequate for molecular studies by the pathologist.

Our results are similar to those published by Navani and colleagues [20], who were able to conduct EGFR mutation analysis on 107 of 119 (90%) patients in whom the testing was requested. Seventy-eight percent (14/18) of lung adenocarcinomas analyzed by Yung and colleagues [21]...
had satisfactory results; in that study, only cell blocks with more than 40% tumor cells were sent for molecular testing. Sakairi and colleagues [22] detected ALK positivity by immunohistochemistry and FISH analysis in 7 of 109 (6.4%) cases diagnosed by EBUS-TBNA.

Factors that contributed to the success rate in this study include the availability of ROSE and the use of a protocol designed to optimize the amount of aspirated material triaged to cell block. Studies have demonstrated that ROSE increases the overall diagnostic yield [23]. ROSE must be used for more than confirmation that an aspirate is diagnostic, however. Proper use of ROSE and close collaboration between the bronchoscopist and cytologist are critical to obtaining adequate material for necessary ancillary testing, including both immunohistochemical staining and molecular analysis. A common pitfall with EBUS-TBNA is the “wasting” of tumor material on smears; if excessive aspirate is used to make a smear beyond what is needed for ROSE, an otherwise adequate sample may have too little material in the cell block for ancillary testing. The goal must be to limit the amount of material used for on-site review and to maximize the amount of material from diagnostic aspirates triaged for cell block so that there is sufficient material for ancillary studies. This is best accomplished, on the basis of our experience, by using ROSE to quantitatively assess the amount of tumor in each aspirated sample and by performing additional passes in diagnostic sites if needed for cell block if the amount of material is thought possibly to be insufficient.

Like histologic specimens, formalin-fixed, paraffin-embedded cell blocks do not require additional validation and are incorporated in the daily workflow of the pathology laboratory. Alcohol-based fixatives may better preserve nucleic acids, though [24]. Although tissue can be dissected from smears and other liquid-based preparations successfully for molecular testing, these techniques have significant disadvantages [4, 25–28]. First, they involve removing cells from diagnostic slides, which may have medicolegal implications. Second, they are not part of the usual sample processing in laboratories that perform testing on paraffin-embedded tissue. Other techniques have been used for optimizing the yield for molecular testing, including microdissection, either manual or by laser capture, which increases the number of cells available for testing [28, 29].

### Table 2. Lymph Node Stations Sampled

<table>
<thead>
<tr>
<th>Nodal Stations</th>
<th>Total No. of Patients</th>
<th>Mean No. of Passes</th>
<th>Median No. of Passes*</th>
<th>IQR*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Nodal station</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Upper paratracheal</td>
<td>2</td>
<td>2.5</td>
<td>2.5</td>
<td>–</td>
</tr>
<tr>
<td>Lower paratracheal</td>
<td>24</td>
<td>3</td>
<td>3</td>
<td>2-4</td>
</tr>
<tr>
<td>Subcarinal</td>
<td>18</td>
<td>3.5</td>
<td>3</td>
<td>2-4</td>
</tr>
<tr>
<td>Hilar</td>
<td>10</td>
<td>2.7</td>
<td>3</td>
<td>2-3</td>
</tr>
<tr>
<td>Interlobar</td>
<td>4</td>
<td>3.8</td>
<td>3</td>
<td>2.5–5</td>
</tr>
<tr>
<td>2 Nodal stations</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Upper paratracheal</td>
<td>1</td>
<td>3</td>
<td>3</td>
<td>–</td>
</tr>
<tr>
<td>Lower paratracheal</td>
<td>7</td>
<td>2.7</td>
<td>3</td>
<td>2-3</td>
</tr>
<tr>
<td>Subcarinal</td>
<td>3</td>
<td>2.3</td>
<td>1</td>
<td>1-5</td>
</tr>
<tr>
<td>Hilar</td>
<td>5</td>
<td>2.4</td>
<td>2</td>
<td>2-3</td>
</tr>
<tr>
<td>Interlobar</td>
<td>2</td>
<td>1.5</td>
<td>1.5</td>
<td>–</td>
</tr>
<tr>
<td>3 Nodal stations</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Upper paratracheal</td>
<td>1</td>
<td>3</td>
<td>3</td>
<td>–</td>
</tr>
<tr>
<td>Lower paratracheal</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>–</td>
</tr>
<tr>
<td>Subcarinal</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>–</td>
</tr>
<tr>
<td>Hilar</td>
<td>1</td>
<td>3</td>
<td>3</td>
<td>–</td>
</tr>
<tr>
<td>Interlobar</td>
<td>2</td>
<td>3</td>
<td>3</td>
<td>–</td>
</tr>
</tbody>
</table>

* Median value with interquartile range [IQR].

### Table 3. Molecular Analysis of Tested Samples

<table>
<thead>
<tr>
<th>Sample Type</th>
<th>No. of Samples Tested</th>
<th>Positive (%)</th>
<th>Negative (%)</th>
<th>Insufficient (%)</th>
<th>Sufficient for Testing</th>
</tr>
</thead>
<tbody>
<tr>
<td>EGFR</td>
<td>51</td>
<td>5 (10%)</td>
<td>41 (80%)</td>
<td>5 (10%)</td>
<td>46 (90%)</td>
</tr>
<tr>
<td>ALK</td>
<td>43</td>
<td>5 (12%)</td>
<td>34 (79%)</td>
<td>4 (9%)</td>
<td>39 (91%)</td>
</tr>
<tr>
<td>Kras</td>
<td>40</td>
<td>10 (25%)</td>
<td>20 (50%)</td>
<td>10 (25%)</td>
<td>30 (75%)</td>
</tr>
</tbody>
</table>

Numbers in parentheses represent proportion in total number of samples tested.

ALK = anaplastic lymphoma; EGFR = epidermal growth factor receptor; Kras = Kirsten rat sarcoma.
Although the success rates for testing for EGFR mutation and ALK gene rearrangement exceeded 90% in this study, 18% of patients did not have sufficient material for complete sequential molecular testing. In one case, the patient underwent a second EBUS-TBNA for resampling, and sufficient material was obtained. The reasons why a sample could be adequate for diagnosis but insufficient for molecular analysis include small sample size, a sparsely cellular sample resulting from tumor necrosis or sampling of nodal micrometastasis, contamination of the samples with blood or benign bronchial cells, or insufficient material triaged to cell block. Small sample size contributed to the partial results for some patients. Kras analysis was not possible in 25% of the samples intended for testing, but half of those insufficient samples were specimens that were depleted in the course of EGFR testing. Another reason for failure is related to the techniques used to identify mutations. EGFR and Kras testing require DNA extraction; a sample could be sufficiently cellular but fail to produce sufficient DNA for testing, for a variety of reasons. This explains why ALK testing, which uses FISH and requires fewer cells, might be successful in samples deemed insufficient for EGFR and Kras testing. All these issues further highlight the need for strategies for optimizing EBUS-TBNA sampling and optimizing specimen processing to reduce the likelihood of an inadequate specimen.

We rely heavily on ROSE and close collaboration between the bronchoscopist and the cytologist in our efforts to optimize EBUS-TBNA yield. If ROSE is not available, a minimum of three aspirations per site has been shown to optimize yield [30]. When sampling nodal metastases, concurrent bronchoscopic sampling of the primary tumor can also be considered to acquire additional material. For patients who ultimately do not have sufficient material for molecular analysis, repeat sampling or subsequent surgical sampling should be considered, given the prognostic and therapeutic importance of these tests.

Limitations of this study include a small sample size and the lack of specific comparison groups. In samples with low tumor volume or those that are sparsely cellular, mutations could be present but below the level of detection of individual tests, resulting in false negative findings. A direct comparison of the yield of testing in matched samples obtained from mediastinoscopy or thoracoscopy in individual patients would further validate the efficacy of EBUS-TBNA. However, an insufficient number of patients in the present study had surgical specimens.

Increasingly, EBUS-TBNA is being used in clinical practice for the diagnosis and staging of lung carcinoma as an alternative to cervical mediastinoscopy. In an era of targeted therapy for adenocarcinoma, it is essential that the specimen obtained be adequate not just for diagnosis but also for complete mutational testing. Our study demonstrates that EBUS-TBNA done with the patient under moderate sedation can be effectively used for these purposes. As additional mutations in NSCLCs are identified, which may represent new opportunities for therapeutic intervention, the demands that we place on the tumor specimen will increase in kind, highlighting the need for careful attention to the techniques of specimen acquisition. Future improvements in the procedures for mutation testing, including increasing test sensitivity and batching of tests, may allow for more extensive testing in small samples. Prioritization may also become important. The sequential testing used in this study is no longer used at our institution; testing for EGFR and ALK, which hold the promise of therapy, is performed concurrently in all samples of lung adenocarcinoma, followed by Kras mutation testing if the results of EGFR and ALK testing are negative.

References


**DISCUSSION**

**DR MITCHELL MAGEE (Dallas, TX): Did the surgery group do this, or was it the pulmonologists?**

**DR JURADO:** The pulmonologists performed the bronchoscopies.

**DR JOHN HOWINGTON (Evanston, IL): You noted that you have gone from three tests now to multiple tests as we get more and more biological markers, and, as you can see, it’s only a minority of patients that have a specific genetic defect. Do you think it’s still going to hold up that the amount of material that you will get from a needle will allow you to do five, six, seven, eight, nine tests, or is this just a blip on the radar in a period of time and in the future we will need more tissue to do an array of tests for patients? Thank you.

**DR JURADO:** Thank you. Agreed, it is important. Right now we are testing for five molecules, and as the molecular markers continue to increase, we can start batching the samples. Also at our institution we manually micro dissect, as well as perform laser micro dissections, we can increase the tumor population of the samples themselves, therefore making sure that it is not diluted with non-tumor cells. So as more molecular markers come into play, we can definitely use batching as well as micro dissection to continue to increase our samples.

**DR ROBERT CERFOLIO (Birmingham, AL): Congratulations. I want to remind the voting audience that she is in the resident competition and for us to take note how well she answered that last question. You are doing great. Keep it up. Dr Sonnet and I are honest, and he told me the other day I was full of garbage, so I get to see honestly that this is really important. This is an incredibly important paper. We have come up with an algorithm at UAB because we have such little tissue, to which tests to run first. I’m now back doing more mediastinoscopies than I was before, because we haven’t quite figured out how to do these tests on EBUS samples. Do you have an algorithm? Our pathologist is looking at EGFR first, and if it’s negative, then KRAS or ALK or ROS because that requires a little bit more, he is then going to looking at the ROS. Now, you didn’t mention ROS, but, of course, ROS is our next thing. So I think we need to get together with our pathologists to have an actual algorithm to use the assay a little more carefully. Do you have any algorithm as to which test you do first, and if negative, you can then avoid a couple of other tests, because there is a predictive value; if one is negative, we cannot use the tissue for two others. Do you have any information on that to give us?

**DR JURADO:** The protocol at the time of the study was that we reflexively tested EGFR first, then KRAS and ALK. Our current protocol consists of triaging the cells, especially for EGFR and ALK, because they are the most clinically relevant at the time. At the moment, KRAS is the one that requires DNA and which is a little bit harder to amplify compared to ALK, for example, that only requires 50 cells versus the KRAS and EGFR, which require more tissue, therefore triaging the cells is an effective method of improving our cytological samples.

**DR CERFOLIO:** And finally, I think this is the so called “triple negative lung cancer” like there is a triple negative breast cancer” which is ER, PR, and HER2 negative; ours is EGFR, KRAS, ALK4, and/or ORS negative. And we need these papers on markers to get up to the general surgeons. We are behind them in lung cancer. We have got to get up there by doing this. Congratulations.

**DR JURADO:** Thank you.

**DR CHADRICK DENLINGER (Charleston, SC): You have shown very nicely that you obtained adequate tissue with an EBUS biopsy to get an answer. What ways do we have of making sure
those answers are actually representative of the entire tumor or at least that lymph node? What is the gold standard to compare to?

DR JURADO: The gold standard is still mediastinoscopy which we use to confirm our false negative results which are approximately 5%.

DR DENLINGER: Thank you.

DR SONETT: I will just add to Dr Cerfolio. I think sequential testing about what you think is important is, in practicality, the best way to go, and we try to do that in our institution. Basically we used to do KRAS first, because if KRAS is positive, you are pretty much done. All the other markers are, for all practical purposes, mutually exclusive. However, the public demands everything be sent for everything, at least in New York. That's what I'm coming down to is that even if we're rational and we say once you get a positive of one, you pretty much don't have to test for the others. But the fact of the matter is a lot of the patients are demanding it. In the future we may not know what we need. They are looking for that whole panel. And even if you had a KRAS positive, they're saying, well, you didn't test me for the rest of the panel, and we all know they're going to be negative, but the patients are demanding it. So that's why we're trying to go away from what we thought was logical and money saving to more of batch testing. As we get more and more samples, it's going to be more and more challenging to get it through EBUS, and we may have to do more ROSE and more biopsies, or try it that way and then do a mead or another methodology afterwards. But given that we can do a pretty darn good yield that way just like an EBUS first, then we go to mediastinoscopy if it's negative, the same way with molecular testing. So just go to the next step if we have to, but make it the first minimally invasive test at first.

DR MAGEE: Do you know whether the 21-gauge needle made a bigger difference than the 22, for instance, or once you got a diagnosis, could you just go back and make a second pass to get more tissue?

DR SONETT: You can make a second pass. The thing about the second pass and the benefit of having the ROSE—and the real key to this is not the endoscopist and not the needle. It's having a cytology and pathology department that's on board with you, because we have translated this experience to our radiologists who do perc needles, fine needles of small nodules, who basically used to get tumor, yes or no. And now we tell them, here's what you've got to do with the slides. You've got to get a cyto tech there showing you how to save and scrimp all the tissue, because a lot of times you get these things and you spray it onto the slide and lose valuable specimen. Practicality.

DR BILL PUTNAM (Nashville, TN): Very interesting information. The question I had was, was this information used clinically, and if so, what happened?

DR JURADO: There is data that demonstrates the importance of targeted therapies for EGFR and ALK. Crizotinib and Erlotinib are two examples that have shown success with EGFR and ALK gene rearrangement respectively with improved survival so we can use the information from this study clinically.

DR PUTNAM: The question was, in this patient population that you studied through the information that you gathered, was that used to change therapy for this population of patients?

DR JURADO: Yes.

DR CERFOLIO: But not all oncologists buy into it, and so that's the next step.

DR SONETT: These were not all de novo lung cancer patients. Some of these were patients that had been treated and didn't have proper molecular testing at first, and they came back looking for a marker to target after they were staged.

DR PUTNAM: So I think the important point here is that this information was clinically useful and it did change therapy in the population tested.

DR CERFOLIO: As opposed to ERCC1 or RRM1 and some of the other tests that the oncologists don't buy into.

DR SONETT: Correct.