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Clinical Stem 3

A patient with right upper lobe adenocarcinoma progressing on biomarker-directed therapy[1]

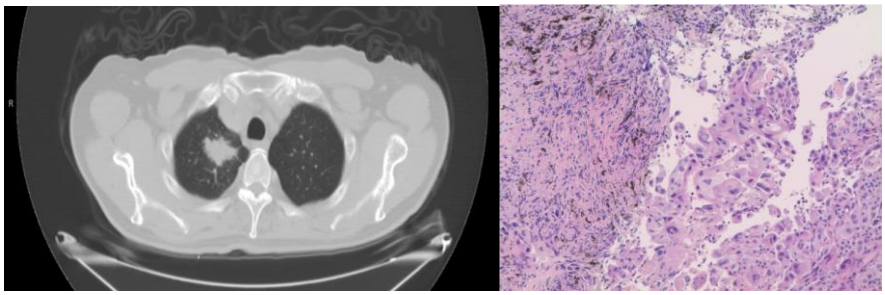
Learning objectives: the webcast participant will be able to:

1. Describe procedure-related strategy and planning for a patient with enlarging primary tumor and associated lymphadenopathy.
2. Describe techniques for overcoming challenges of obtaining and using small samples for molecular analysis.
3. Describe conservation strategies essential to providing adequate tissue for molecular analysis.
4. Describe acquisition and handling techniques to optimize specimen quality and adequacy for molecular testing.
5. Describe reasons for performing molecular analysis at time of diagnosis and disease progression.

[1] Disclaimer: This is a fictitious clinical case scenario based on a conglomerate of real patient data, modified to avoid any possibility for patient identification and to help meet educational objectives. Any resemblance to real persons, living or deceased is purely coincidental.

Case Description

A 62 year-old Asian male with a 20 pack-year smoking history presented to his primary care physician with persistent cough for 6 weeks. The chest radiograph showed a right upper lobe mass confirmed by chest computed tomography. There was no mediastinal lymphadenopathy. His FEV_1 was 36% of predicted. DLCO was 28% of predicted. Results from bronchoscopy with fluoroscopy-guided transbronchial lung biopsy showed poorly differentiated adenocarcinoma. Material was insufficient for molecular analysis due to the large number of immunostains performed in the pathology laboratory. The patient is discussed at your institution's multidisciplinary lung cancer conference.



Question 1: What would you recommend?

A. Refer the patient for lobectomy
B. Refer the patient for sub lobar resection
C. Refer the patient for stereotactic body radiation therapy (SBRT)
D. Proceed with integrated PET-CT for staging

Answer: D

Accurate staging is essential for managing patients with lung cancer because treatment options and prognosis differ significantly according to tumor stage. For this reason, patient findings are presented for discussion at multidisciplinary lung cancer meetings. If limited stage lung cancer is confirmed, treatment depends on patient operability. Systematic reviews conclude that CT scans have limited ability to rule in or exclude mediastinal metastasis. CT has a pooled sensitivity of 51% and specificity of 85% for identifying mediastinal lymph node metastasis.

PET is more accurate that Computed tomography. Pooled estimates of sensitivity and specificity for identifying mediastinal metastasis by PET scan are 74% and 85% respectively.

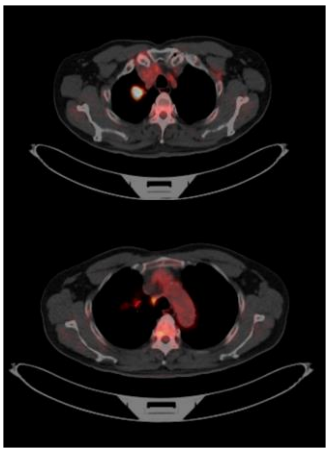
ACCP guidelines recommend PET scan to evaluate for potential mediastinal and extra-thoracic metastatic disease. PET should be considered in patients with clinical stage 1A lung cancer treated with curative intent (Grade of recommendation, 2C).

Reference

1. Silvestri GA, Gould MK, Margolis ML, et al. American College of Chest Physicians. Noninvasive staging of non-small cell lung cancer: ACCP evidenced-based clinical practice guidelines (2nd edition). Chest. 2007;Sep;132(3 Suppl):178S-201S.

The Case Continues

Whole body integrated PET–CT showed high uptake in the primary tumor and in the mediastinum; lymph node station 4R.



Question 2: What do you recommend for mediastinal staging?

A. Surgery consult for mediastinoscopy or VATS
B. EBUS-guided TBNA. If non-diagnostic perform mediastinoscopy
C. EBUS and EUS-guided TBNA. If non-diagnostic consult surgery for mediastinoscopy or VATS

Answer: C

In patients with an abnormal PET scan, evaluation of the mediastinum with sampling of abnormal lymph nodes should be performed prior to surgical resection of the primary tumor. Results from a large, randomized trial show that a combined sonographic approach has greater sensitivity for detecting nodal metastases than mediastinoscopy alone. EBUS-TBNA can access mediastinal and hilar nodes with a diagnostic yield similar to mediastinoscopy. For patients with suspected nodal involvement, staging and diagnosis can be performed in a single setting.

Non-diagnostic EBUS or EUS-guided TBNA, defined as specimens that contain neither malignant cells nor lymphocytes, occurs in as many as 20% of individual aspirates and 10% of cases overall. Mediastinoscopy is likely warranted in these cases.

References

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mediastinal nodal staging of lung cancer: a randomized trial. JAMA 2010; 304: 2245–2252.
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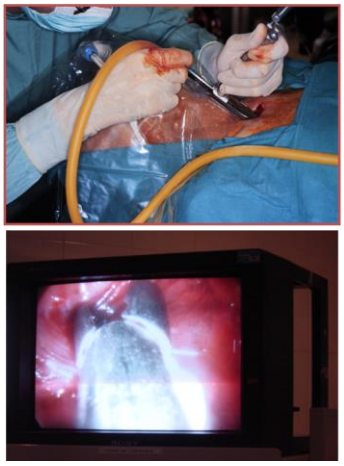
4. Alsharif M, Andrade RS, Groth SS, et al. Endobronchial ultrasound-guided transbronchial fine-needle aspiration: the University of Minnesota experience, with emphasis on usefulness, adequacy assessment, and diagnostic difficulties. Am J Clin Pathol 2008;130:434–443.

The Case Continues

Because sonography was not available, a surgical consultation was obtained and mediastinoscopy was performed. Lymph nodes at station 4R and 4L were positive for adenocarcinoma. The patient's tumor was staged T2a N3 M0 (stage IIIB).



Question 3: Would you send the lymph node specimens for molecular analysis?

A. Yes
B. No

Answer: A

Molecular analysis is warranted because this patient has locally advanced adenocarcinoma. The patient's previous primary tumor biopsy specimen was processed to the point that no material was left for molecular testing.

Studies demonstrate evidence of intra-tumoral heterogeneity with respect to mutations, and reports show discordance between the primary lesion and nodal metastases. It is not clear whether the potential for discordance should affect decisions on which specimen to test. In practice, biopsy of the most rapidly growing tumor is usually performed because it presumably contains the most biologically aggressive genetic alterations.The CAP/IASLC/AMP guideline states that either the primary or metastatic sites are acceptable for molecular testing.

Given the importance of acquiring sufficient and adequate tissue for molecular analysis, repeat bronchoscopic biopsy, or lymph node and tumor biopsies by mediastinoscopy or VATS may be warranted outside the staging setting.

References

1. Chen ZY, Zhong WZ, Zhang XC, et al. EGFR mutation heterogeneity and the mixed response to EGFR tyrosine kinase inhibitors of lung adenocarcinomas. Oncologist. 2012;17(7):978-85.
2. Gerlinger M, Rowan AJ, Horswell S et al. Intratumor heterogeneity and branched evolution revealed by multiregion sequencing. N Engl J Med. 2012; 366(10):883-92.
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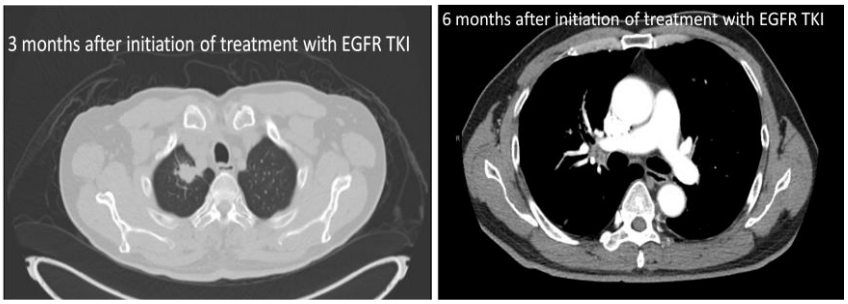
4. Kalikaki A, Koutsopoulos A, Trypaki M, et al. Comparison of EGFR and K-RAS gene status between primary tumours and corresponding metastases in NSCLC. Br J Cancer. 2008;99:923–929.
5. Han HS, Eom DW, Kim JH, et al. EGFR mutation status in primary lung adenocarcinomas and corresponding metastatic lesions: discordance in pleural metastases. Clin Lung Cancer. 2011;12:380–386.

The Case Continues

EGFR status was positive in the patient's lymph node specimens. The risks and benefits of first line chemoradiotherapy versus biomarker-driven therapy were discussed with the patient. Considering his poor lung function, poor performance status, and risk for treatment-related pulmonary toxicity, a shared decision was made to begin treatment with an EGFR TKI.Three months later, a repeat chest CT scan showed decrease in size of the right upper lobe mass and a small interlobar lymph node at station 11R superior, but no mediastinal or other hilar adenopathy. A total of six months after initiation of EGFR TKI therapy, another chest CT scan showed an increase in size of the right upper lobe mass and a small increase in size of the 11R s adenopathy. The patient is discussed again in the multidisciplinary lung cancer conference.



Question 4: What would you recommend next?

A. Switch treatment to conventional chemotherapy
B. Continue current EGFR TKI therapy
C. Re-biopsy the right upper lobe mass
D. TBNA of the right interlobar node

Answer: C

Significant clinical or radiographic response to biomarker-driven therapy is seen initially in many patients with EGFR mutations or ALK rearrangements, but disease commonly progresses as a result of acquired resistance against EGFR and ALK inhibitors.

In patients with documented prior actionable genetic alterations in whom disease has progressed during therapy, retesting should be done exclusively on re-biopsy specimens of a progressing lesion; specifically the lesion that had become clinically relevant. Clinicians should communicate this information with their pathologist to assure that requests for testing on patients with prior history of biopsy are made only for the most recently obtained specimen.

This patient's imaging studies reveal probable disease progression. The benefits of potentially improved outcomes by re-biopsy should be balanced against the risks associated with the use of invasive procedures for tissue acquisition. The location of the tumor and the level of risk involved in the procedure are relevant to deciding whether repeat biopsy is recommended.

For this patient, progression of disease was likely in both the right interlobar node and the primary tumor, but is most obvious in the primary tumor. The primary lesion is therefore the preferred site for rebiopsy.

About 50% of resistant tumors show the T790M mutation, 5% show MET overexpression and PIK3CA mutations. Transformation into small cell lung cancer occurs in about 15%. Targeted treatments against T790M mutations, MET- and PIK3CA are currently available in clinical trials.

While rebiopsy may allow detection of resistance mechanisms and alter therapy, indications for rebiopsy depend on treatment setting and availability of clinical trials and other cancer-related research.

References

1. Carter CA, Giaccone G. Treatment of nonsmall cell lung cancer: overcoming the resistance to epidermal growth factor receptor inhibitors. Curr Opin Oncol. 2012;24:123–129.
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4. Sequist LV, Waltman BA, Dias-Santagata D, et al. Genotypic and histological evolution of lung cancers acquiring resistance to EGFR inhibitors. Sci Transl Med. 2011;3(75):75ra26.

The Case Continues

The patient was referred to thoracic surgery for possible surgical biopsy to obtain adequate specimens for molecular testing. Based on the patient's tumor anatomy and given the high probability of significant loss of lung function, a surgical resection was not recommended for molecular testing alone in this patient. The patient was re-discussed at the multidisciplinary lung cancer conference.

International guidelines recommend a multidisciplinary approach to patients with limited stage and advanced stage lung cancer

•**British Thoracic Society** Guidelines on the selection of patients with lung cancer for surgery. Thorax 2001; 56:89-108.

•**European Society for Medical Oncology** Clinical Practice Guidelines for diagnosis, treatment and follow-up (Metastatic non-small-cell lung cancer working group); Ann Oncol. 20 12;23 Suppl 7:vii56-64).

Question 5: To optimize the specimen submitted for molecular testing, which of the following items should you discuss with your pathology and oncology colleagues prior to further interventions?

A. Size and quality of the required tumor sample
B. Specific histologic sampling for molecular analysis testing
C. Specific markers needed in this patient
D. All of the above

Answer: D

Attention to the quantity, quality and processing of tissues helps optimize molecular testing. Tumor cellularity appears to be the most significant factor for test success regardless of whether a cytology or pathology specimen is used. The presence of non-malignant tissue within specimens can lead to decreased accuracy of molecular testing, especially when testing is based on nucleic acids extracted from the entire sample.

Molecular testing is currently indicated in non-small cell lung carcinoma showing an adenocarcinoma component regardless of histologic grade or subtype, in small biopsies or incomplete excision specimens showing only squamous or small cell histology, and in poorly differentiated tumors or tumors that otherwise cannot be classified as pure squamous, pure small cell, or pure neuroendocrine carcinomas. Given the emergence of new molecular markers for other histologic types, need for specific tests should be clarified with the referring oncologist prior to tissue acquisition.

Each molecular laboratory has a minimal amount and concentration of tumor cells required for accurate detection of molecular alterations based on specific tumor enrichment protocols available and assay platforms used for testing. The treating team and the pathologist should be aware of these requirements so that the number of specimens rejected by the molecular laboratory is minimized.

References

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The Case Continues

At the lung cancer conference, it was suggested that rebiopsy be performed. Both the oncologist and pathologist suggested that samples for histology and cytology be obtained.

Question 6: Which procedure would you recommend?

A. CT-guided FNA and biopsy of the right upper lobe mass
B. Bronchoscopy with transbronchial biopsy, brushings and lavage from the right upper lobe mass
C. EBUS-guided TBNA from the right interlobar node
D. EBUS –TBNA from the right interlobar node and transbronchial biopsy, brushings, and lavage from the right upper lobe mass.

Answer: D

Empiric evidence suggests patient management is optimized by the use of multidisciplinary lung cancer conferences. Interaction among key specialists avoids a system of serial and autonomous referrals that may delay and fracture care. The active involvement of key specialists, including those most knowledgeable of the optimal use of small specimens for molecular analysis is increasingly relevant. The impact of compliance or discordance with decisions and recommendations made at multidisciplinary conferences warrants further study.

At this conference, pathologists noted that tissue requirements for molecular analysis may exceed those for diagnostic cytologic or histologic examination. The pulmonologist noted that in peripheral parenchymal lesions, a systematic review showed an overall sensitivity of 69% for conventional bronchoscopy with transbronchial biopsy, brushings and lavage, but without electromagnetic navigation or radial ultrasound assistance. Brushing alone had a sensitivity of 52 %, whereas transbronchial biopsy had a sensitivity of only 46%. BAL and washing had a sensitivity of 43%. The size of the lesion impacts yield. The sensitivity of all modalities for peripheral lesions less than 2 cm was 0.33 compared to 0.62 for lesions greater than 2 cm. At least 4-5 biopsies should probably be performed to obtain sufficient material for molecular testing.

The Interventional radiologist said that diagnostic yield for transthoracic CT-guided biopsy and needle aspiration is reportedly greater than 90%. She argued that CT-guided biopsy was the optimal procedure in this patient because adequate quantity and quality of lung tissue can be obtained for molecular testing if multiple passes are performed. Complications include hemorrhage and pneumothorax, which, in high-risk patients might prompt a preference for a bronchoscopic approach. The pulmonologist responded that EBUS-TBNA from the interlobar node could be performed at the time of bronchoscopy with transbronchial biopsy, brushings and lavage from the right upper lobe mass. Using EBUS-TBNA, cytology and tissue cores for histology are obtained that are adequate for molecular testing.

The rates of successful molecular testing with small volume biopsies and cytology specimens obtained by transthoracic and bronchoscopy needle aspiration-biopsy probably vary depending on the *fixation method* and the *extent of analysis* performed. In one study, molecular analysis was successful in only 48% of fresh specimens submitted for gene expression profiling. On the other hand, fresh frozen biopsies from TTNA submitted for EGFR testing alone was successful in 100% of cases.

References

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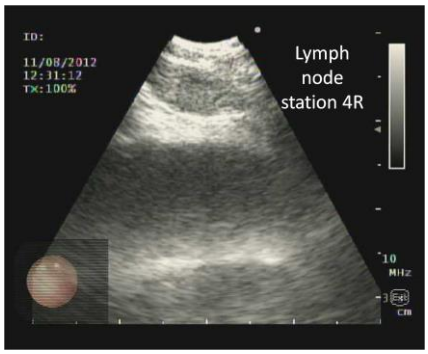
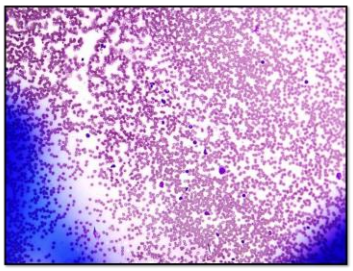
The Case Continues

The multidisciplinary team chose to proceed with bronchoscopy and EBUS-TBNA. White light bronchoscopy showed normal airways. EBUS exploration of the mediastinum showed lymph nodes at stations 4R and 11R superior. EBUS-TBNA from both nodes was attempted. The nodes were very difficult to penetrate. A different inter-cartilaginous space was chosen for puncturing the nodes by redirecting the needle; the 4R lymph node could not be penetrated. Two aspirates of the 11R superior lymph node were obtained. On ROSE, however, only blood and a few scattered lymphocytes were visualized.



Unsatisfactory nodal aspiration: scanty lymphocytes are present on a background of red blood cells. No malignant cells are seen



<u>Question 7</u>: What would you do next?

A. Abort the EBUS procedure and proceed with transbronchial biopsy, brushings and lavage from the right upper lobe mass
B. Change the puncture site again and reattempt EBUS-TBNA from station 4R and 11Rs
C. Perform conventional TBNA of region 4R using a 19 gauge histology needle
D. Abort bronchoscopy and consult interventional radiology

Answer: A

In this case, technical difficulties were encountered while sampling nodal stations 4R and 11R. ROSE confirmed the lack of representative tissues, prompting a change in strategy. Selecting the correct biopsy site for molecular testing may have implications on patient morbidity. When it is difficult to obtain enough tissue because of technical problems or intra-procedural complications, it is important to prioritize acquisition and use of tissue based on which information can be obtained with the limited specimen.

Performing TBNA at all cost by changing the puncture site again, prolonging the procedure, or switching to conventional TBNA may not be in the patient's best interest for at least two reasons: tissue can be obtained from another site of likely disease progression; in this case the enlarging right upper lobe mass, and multiple repeated needle aspirations are likely to result in a bloodier specimen that will be difficult to analyze.

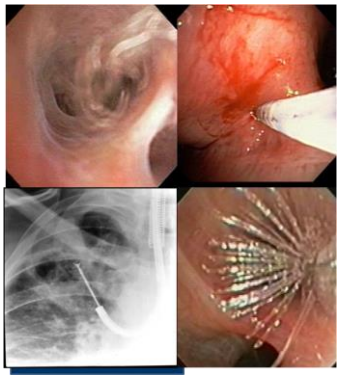
The safety of obtaining a biopsy must be weighed against the need to select biomarker-driven therapy in fragile patients with lung cancer who often have significant cardiac and pulmonary comorbidities.

References

1. Maheswaran S, Sequist LV, Nagrath S, et al. Detection of mutations in EGFR in circulating lung-cancer cells. N Engl J Med. 2008 Jul 24;359(4):366-77.

The Case Continues

A conventional TBNA was not performed. The EBUS scope was removed and transbronchial biopsy, brushings and BAL were performed using fluoroscopic guidance.



Question 8: Had TBNA been successful, what should be the major focus of specimen handling in the procedure suite?

A. Prepare as many slides as possible for ROSE
B. Prepare 1-3 slides for histologic diagnosis and save the rest for cell block
C. Perform aspirations until a tissue core is obtained

Answer: B

Slides can be prepared and preliminarily interpreted by cytotechnologists to assure an adequate specimen for molecular testing. This may reduce time, effort, and complications. Physicians and their staff should be familiar with the process of smear preparation. Cytologic sampling is limited by the amount of tissue obtained because a small number of passes are made through the lesion. Often, all the aspirated material is expressed onto a single or small number of slides. Any type of cytologic sample and preparation may be used, including stained or unstained smears, automated Thin Prep slides and cytospin. Cell-block is commonly used for molecular analysis. The proportion of unsatisfactory cytology specimens for molecular analyses varies significantly with the type of cytological specimen. Obtaining core tissue should be attempted but is not always successful.

When cell blocks are planned, only a few slides might be needed for Diff-Quick and Papanicolau smear. The rest of the specimen is saved for cell-block preparation. Material obtained from additional sampling of the lesion or lymph node is expulsed from the needle into a solution such as Cytolyt. This is rendered into a pellet which is embedded in paraffin. This preparation may not represent the underlying tissue architecture, but is adequate for special stains and molecular analysis. Because there is some debate whether alcohol fixation is appropriate for FISH testing, techniques should be discussed with the institution's pathologist.

EBUS-TBNA and pleural fluid are more suitable than BAL because a larger number of tumor cells are usually retrieved, and there is greater possibility to make a cell-block.

Molecular testing for EGFR, K-RAS, BRAF, ALK and PIK3CA on cytology specimens provides results that are equivalent to those obtained from histology specimens in about 80% of cases.

References

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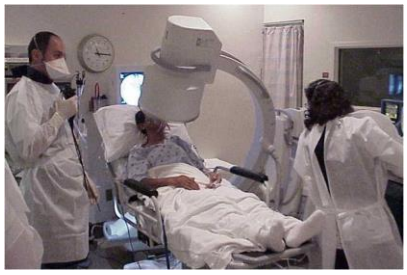
The Case Continues

Using fluoroscopic guidance, two brushings and five transbronchial biopsies were performed, followed by BAL with a total fluid instilled of 120 ml and return of 60 ml of bloody fluid. Results from the biopsy showed adenocarcinoma. The treating oncologist considered reflex molecular testing to obtain a complete molecular profile as soon **a**s possible.



Question 9: At this point, what would you do?

A. Defer molecular testing to the treating oncologist
B. Defer molecular testing to the pathologist
C. Order the tests based on the previous discussions with the oncologist and the pathologist
D. Do not order any tests until after lung cancer tissue acquisition, handling and processing are streamlined at your institution

Answer: C

Reflex testing is also known as automatic testing. It is often done by pathologists at the time of histologic diagnosis to ensure that complete histologic and molecular profiles of the tumor are available to the oncologist as early in the treatment algorithm as possible.

There are reports of discordance in *molecular* alterations between the primary tumor and nodal metastasis with similar histology. In addition, *histologic* discrepancy between the primary tumor and associated lymph nodes with similar molecular alterations has also been reported. Discussions with the referring oncologists and the pathologists are important prior to ordering molecular tests. Streamlining the institution's workflow for molecular markers is desirable.

Experts propose developing an institutional multidisciplinary lung cancer tissue management committee to decide which patients should undergo molecular testing and which tests should be performed. Guidelines from The European Society of Medical Oncologists as well as CAP/IASLC/AMP the guideline do not currently recommend routine testing for KRAS, BRAF, ERBB2, PIK3CA somatic gene mutations outside of clinical trials. Routine testing for mRNA levels of ERCC1, RRM1, TS and BRCA1 is not currently recommended outside of clinical trials.

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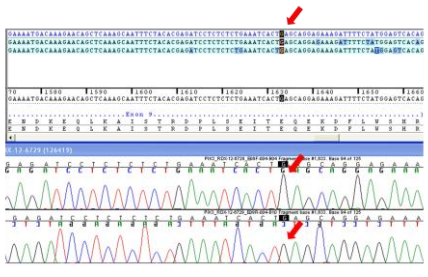
The Case Continues

Reflex testing in this institution applied only to EGFR and KRAS mutations and ALK rearrangement. In this patient, additional studies were desired by the oncologist. The tumor was negative for KRAS and for secondary EGFR mutations, and for PIK3CA mutation but showed c-MET amplification. No ALK rearrangement was noted.

Because currently used molecular markers may be requested even though their impact on clinical outcomes is unclear, the issue of standardizing the molecular testing process was discussed in the multidisciplinary lung cancer conference. The team created a form to accompany all lung cancer patients to their appointments with oncologists, surgeons, interventional radiologists and pulmonologists. The molecular markers included on this form were debated. Other elements included on the form were whether cytology or histology specimens were obtained, the quality of the specimen, and the quantity of adequate specimen for testing. The team decided to individualize the choice of referral molecular laboratories.

Wild type PIK3 by sequencing



Courtesy Response Genetics

Question 10: What elements are critical to help assure that sufficient amount of representative small samples can be processed for molecular analysis?

A. Minimize the number of immunostains for cytohistologic diagnosis
B. Use ROSE whenever possible for FNA procedures
C. Try to obtain tissue cores for histology, obtain specimens for cell block, and document collection time as well as time to fixation.
D. All of the above

Answer: D

Results of molecular tests can alter therapy. In this case, the patient could be enrolled in a clinical trial using a monoclonal antibody against c-MET. An immunohistochemistry panel can aid in sub-classifying NSCLC. Multiple IHC stains, however, may reduce the quantity of tissue available for molecular analysis. Tissue conservation is crucial so that tissue is available for potential molecular testing.

Pathologists should minimize the number of IHC preparations used to sub-classify a lesion. When morphology is equivocal and there is no clear glandular or squamous differentiation, IHC using a panel of antibodies; typically TTF-1, p63 and p40, cytokeratin 7 and cytokeratin 5/6 may be used on previously stained smears, automated slides, and cell-blocks.

Rapid On-Site Evaluation (ROSE), while not available everywhere, is an important complement to needle aspiration. ROSE helps improve the accuracy of the procedure, but does not necessarily improve yield. By using ROSE, the cytopathologist can confirm that the specimen is adequate and representative of the targeted lesion. For example, a significant number of lymphocytes should be found when a lymph node is targeted. ROSE also helps confirm that sufficient material is obtained for a definitive final diagnosis *and* molecular testing. ROSE thus optimizes future allocation and processing of specimens for specific analyses according to suspected or known diagnosis.

Results of IHC usually favor a single diagnosis. Adenocarcinomas of pulmonary origin are typically reactive for TTF-1 and cytokeratin 7 and non-reactive for p63 and cytokeratin 5/6. Squamous cell carcinomas are typically reactive for p63 and cytokeratin 5/6 but non-reactive for TTF-1 and cytokeratin 7. The concordance between needle aspirate and biopsy in NSCLC subtyping is very high (96%), especially when analysis of material in cell-blocks can be performed.

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Credits

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