Biomarker Testing in Advanced NSCLC: Putting Guidance Into Practice

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Agenda

- The role of biomarker testing in advanced NSCLC
- Practice recommendations for biomarker testing in advanced NSCLC
  - Tissue handling
  - Considerations for EGFR testing
  - Considerations for ALK testing
- Technology advancements in biomarker testing
- Protocols to reduce testing delays
- Multidisciplinary team communication
- Case study

This material does not constitute medical advice. The decision to implement these practice suggestions should be made by each laboratory in conjunction with its clinical care team to balance resources and clinical needs with the individual healthcare setting.

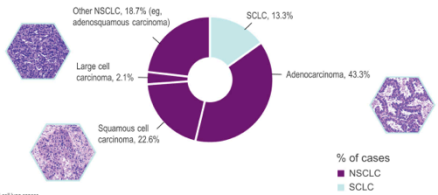
ALK=anaplastic lymphoma kinase; EGFR=epidermal growth factor receptor; NSCLC=non-small cell lung cancer.

The role of biomarker testing in advanced NSCLC

NSCLC accounts for a majority of lung cancer cases

- Lung cancer is the leading cause of cancer-related mortality in the US[1]
  - The estimated number of lung cancer deaths for 2016 is 158,080
  - ~26.5% of all cancer deaths are due to lung cancer

Lung cancer consists of 2 major types: NSCLC and SCLC[2]

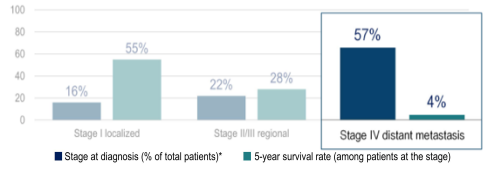


SCLC=small cell lung cancer.

1. SEER Cancer Statistics Factsheets: Lung and Bronchus Cancer. National Cancer Institute. Bethesda, MD. http://seer.cancer.gov/statfacts/html/lungb.html. Accessed May 24, 2016. 2. SEER Cancer Statistics Review (CSR) 1975-2012. http://seer.cancer.gov/csr/1975_2012/browse_csr.php?sectionSEL=15&pageSEL=sect_15_table.28.html. Accessed May 24, 2016.

Survival remains poor in lung cancer

Lung and bronchus cancer[1]



- Advanced disease involving distant metastasis (stage IV[1]) accounts for 57% of all lung cancer, with the lowest 5-year relative survival rate[1]

There is a great need to quickly identify and appropriately treat patients with advanced NSCLC.[1]

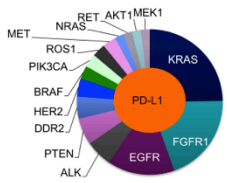
* 5% of patients are unstaged at diagnosis. †Based on the American Joint Committee on Cancer staging system for lung cancer, 7th edition.

1. SEER Cancer Statistics Factsheets: Lung and Bronchus Cancer. National Cancer Institute. Bethesda, MD. http://seer.cancer.gov/statfacts/html/lungb.html. Accessed May 24, 2016.

Biomarker profiling is integral to lung cancer characterization

- Biomarker profiling is important in the characterization of lung cancer[1]
  - ~64% of NSCLC patients have oncogenic driver mutations
- While many genetic alterations have been identified, knowing the presence of predictive biomarkers may lead to favorable patient outcomes[2,4]

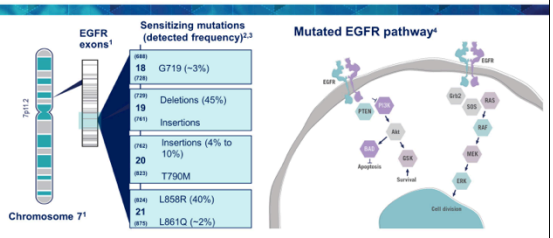
Known genetic alterations and PD-L1 expression in NSCLC[3,4]



DDR2=discoidin domain receptor 2; FGFR1=fibroblast growth factor receptor 1; HER2=human epidermal growth factor 2; KRAS=Kirsten rat sarcoma; MEK1=mitogen-activated protein kinase kinase 1; NRAS=neuroblastoma rat sarcoma; PD-L1=programmed death ligand 1; PIK3CA=phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit alpha; PTEN=phosphatase and tensin homolog; RET=rearranged during transfection; ROS1=ros proto-oncogene 1.

1. Kris MG, et al. JAMA. 2014;311:1998-2006. 2. Referenced with permission from the NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines®) for Non-small Cell Lung Cancer V.4.2016. © National Comprehensive Cancer Network, Inc 2016. All rights reserved. Accessed May 24, 2016. To view the most recent and complete version of the guideline, go online to NCCN.org. NATIONAL COMPREHENSIVE CANCER NETWORK®, NCCN®, NCCN GUIDELINES®, and all other NCCN Content are trademarks owned by the National Comprehensive Cancer Network, Inc. 3. D'Incecco A, et al. Br J Cancer. 2015;112:95-102. 4. Lovly C, et al. Molecular profiling of lung cancer. My Cancer Genome. http://www.mycancergenome.org/content/disease/lung-cancer/. Accessed June 3, 2016.

EGFR mutations play an important role in carcinogenesis

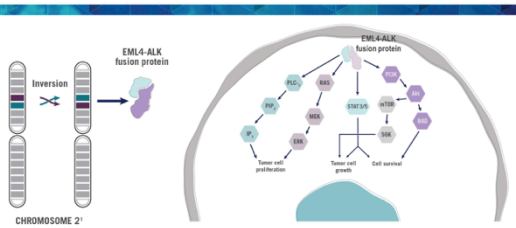


- EGFR mutation could be found in ~10% of NSCLC patients in the US[3]
- EGFR exon 19 deletions, mutant L858R (exon 21), and mutant T790M (exon 20) are predictive of therapeutic response to EGFR blockade[3]

BAD=BCL2-associated agonist of cell death; ERK=extracellular signal–regulated kinase; Grb2=growth factor receptor-bound protein 2; GSK=glycogen synthase kinase; MEK=mitogen-activated protein kinase kinase; PI3K=phosphatidylinositol 3-kinase; RAF=rapidly accelerating fibrosarcoma; RAS=rat sarcoma; SOS=son of sevenless guanine nucleotide exchange factor.

1. Cheng L, et al. *Mod Pathol*. 2012;25:347-369. 2. Yasuda H, et al. *Sci Transl Med*. 2013;5:216ra177. 3. Referenced with permission from the NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines®) for Non-small Cell Lung Cancer V.4.2016. © National Comprehensive Cancer Network, Inc 2016. All rights reserved. Accessed May 24, 2016. To view the most recent and complete version of the guideline, go online to NCCN.org. NATIONAL COMPREHENSIVE CANCER NETWORK®, NCCN®, NCCN GUIDELINES®, and all other NCCN Content are trademarks owned by the National Comprehensive Cancer Network, Inc. 4. McCubrey JA, et al. *Biochim Biophys Acta*. 2007;1773:1263-1284.

ALK gene rearrangements are the primary drivers of disease in ALK-positive NSCLC



- ALK rearrangement could be found in approximately 2% to 7% of NSCLC patients in the US[2]
- EML4-ALK is the most common rearrangement[1]

EML4=echinoderm microtubule-associated protein-like 4; IP3=inositol triphosphate; mTOR=mechanistic target of rapamycin; PIP2=phosphatidylinositol bisphosphate; PLC-γ=phospholipase C-γ; S6K=S6 kinase; STAT=signal transducer and activator of transcription.

1. Solomon B, et al. *Clin Pharmacol Ther*. 2014;96:15-23. 2. Referenced with permission from the NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines®) for Non-small Cell Lung Cancer V.4.2016. © National Comprehensive Cancer Network, Inc 2016. All rights reserved. Accessed May 24, 2016. To view the most recent and complete version of the guideline, go online to NCCN.org. NATIONAL COMPREHENSIVE CANCER NETWORK®, NCCN®, NCCN GUIDELINES®, and all other NCCN Content are trademarks owned by the National Comprehensive Cancer Network, Inc.

Practice recommendations for biomarker testing in advanced NSCLC

ASCO-endorsed CAP/IASLC/AMP recommendations: Highlights from the 2013 guidelines[1,2]

Who should be tested for EGFR mutations and ALK rearrangements?[2]	
In the setting of resection specimens	• Adenocarcinomas and mixed lung cancers with an adenocarcinoma component, regardless of histologic grade
In the setting of more limited specimens	• Squamous or small cell carcinomas*
When should you test patients for EGFR mutations and ALK rearrangements?[2]	
At time of diagnosis	• Stage IV† disease‡ • Testing at earlier stages (I, II, or III) is encouraged, but the decision should be made with the MDT§
At time of recurrence or progression	• Previously diagnosed with lower-stage disease but not tested‡

AMP=Association for Molecular Pathology; ASCO=American Society of Clinical Oncology; CAP=College of American Pathologists; IASLC=International Association for the Study of Lung Cancer; MDT=multidisciplinary team; TNM=tumor, node, metastasis.

* In the setting of more limited specimens where an adenocarcinoma component cannot be completely excluded. Clinical criteria such as age and smoking history may help to determine appropriate patients. †According to the 7th edition TNM staging system. ‡Suggestion (Grade C evidence; for ALK only). §Expert consensus opinion (Grade D evidence). Recommendations are supported by Grade A or B evidence unless otherwise specified.

1. Leighl NB, et al. *J Clin Oncol*. 2014;32:3673-3679. 2. Lindeman NI, et al. *J Mol Diagn*. 2013;15:415-453.

ASCO-endorsed CAP/IASLC/AMP recommendations: Highlights from the 2013 guidelines (cont'd)[1,2]

Where do you obtain tissue for testing EGFR mutations and ALK rearrangements?[2]	
• Primary tumors or metastatic lesions • Test each tumor for patients with multiple, apparently separate, primary lung adenocarcinomas* • Testing of multiple areas within a single tumor is not necessary*	
How do you prioritize tissue for biomarker testing?[2]	
Histologic diagnosis	• Be judicious in the use of tissue sections for histopathological diagnosis* • Retain sufficient tissue for biomarker testing*
Biomarker testing	• Prioritize EGFR testing, followed by ALK testing, over other proposed biomarkers • KRAS mutation testing is not recommended as a sole determinant of EGFR TKI therapy

TKI=tyrosine kinase inhibitor.

* Expert consensus opinion (Grade D evidence). Recommendations are supported by Grade A or B evidence unless otherwise specified.

1. Leighl NB, et al. *J Clin Oncol*. 2014;32:3673-3679. 2. Lindeman NI, et al. *J Mol Diagn*. 2013;15:415-453.

ASCO-endorsed CAP/IASLC/AMP recommendations: Highlights from the 2013 guidelines (cont'd)[1,2]

How do you perform EGFR testing?[2]	
Tissue processing	• Larger tumor specimens (eg, resections) are preferred* • Use FFPE, fresh, frozen, or alcohol-fixed specimens for PCR-based tests* • Avoid acidic or heavy metal fixatives, or decalcifying solutions*
Specimen requirement	• Each lab should establish the minimum proportion and number of cancer cells required* • Assess tumor content and perform microdissection for tumor cell enrichment as needed*
Testing method	• Any validated EGFR testing method that can detect* • Mutations in specimens with ≥50% cancer cell content (as little as 10% cancer cells is strongly encouraged) • All individual EGFR mutations that have been reported with a frequency of ≥1% • IHC and copy number analysis (FISH/CISH) are not recommended

CISH=chromogenic in situ hybridization; FFPE=formalin-fixed paraffin-embedded; FISH=fluorescence in situ hybridization; IHC=immunohistochemistry; PCR=polymerase chain reaction.

* Expert consensus opinion (Grade D evidence). Recommendations are supported by Grade A or B evidence unless otherwise specified.

1. Leighl NB, et al. *J Clin Oncol*. 2014;32:3673-3679. 2. Lindeman NI, et al. *J Mol Diagn*. 2013;15:415-453.

ASCO-endorsed CAP/IASLC/AMP recommendations: Highlights from the 2013 guidelines (cont'd)[1,2]

How do you perform ALK testing?[2]	
Tissue processing for FISH	• Generally similar to those for EGFR mutation testing with the following exceptions* • Recently cut section is preferred • Avoid alcohol-fixed samples
Specimen requirements for FISH	• ≥50% of all nuclei should be easily analyzable* • Avoid areas of overlapping tumor cells*
Testing method	• FISH assay using dual-labeled break-apart probes • Validated IHC may be considered; positive results should be confirmed by FISH • RT-PCR is not recommended
How do you interpret results from ALK testing?[2]	
FISH	• FISH positive: 1 separate orange/red and 1 separate green signal with a gap of >2 signal diameters or loss of green probe* • Positivity is required in ≥15% of 50 nuclei

RT-PCR=reverse transcription-polymerase chain reaction.
* Expert consensus opinion (Grade D evidence). Recommendations are supported by Grade A or B evidence unless otherwise specified.
1. Leigh NB, et al. J Clin Oncol. 2014;32:3673-3679. 2. Lindeman NI, et al. J Mol Diagn. 2013;15:415-453.

ASCO-endorsed CAP/IASLC/AMP recommendations: Highlights from the 2013 guidelines (cont'd)[1,2]

How is technical testing validated?[2]	
• Technical validation is required in the US under CLIA '88* • Validate all types of samples used for testing and all relevant mutations or rearrangements*	
EGFR	• Assess analytic sensitivity in mutated specimens with low tumor content to determine the minimal tumor cell content required for testing* • Perform sensitivity studies with >1 specimen; the least sensitive result should be the overall test sensitivity* • Confirm specificity by clinically validated Sanger sequencing or traceable methods*
ALK	• Test benign tissue and wild-type tumors to establish the minimum frequency and 2-probe-diameter-distance of split signals* • Confirm specificity by clinically validated Abbott Vysis ALK Break Apart FISH Probe Kit or traceable methods*

CLIA=Clinical Laboratory Improvement Amendments of 1988.
* Expert consensus opinion (Grade D evidence). Recommendations are supported by Grade A or B evidence unless otherwise specified.
1. Leigh NB, et al. J Clin Oncol. 2014;32:3673-3679. 2. Lindeman NI, et al. J Mol Diagn. 2013;15:415-453.

ASCO-endorsed CAP/IASLC/AMP recommendations: Highlights from the 2013 guidelines (cont'd)[1,2]

How rapidly should test results be available?[2]	
Time from receiving requests to sending specimen	• Outside molecular pathology labs: 3 working days* • Intramural labs: 24 hours*
Time from receiving specimen to obtaining results	• Goal: 1 week (5 working days) to 2 weeks (10 working days)*
How should testing be implemented and reported?[2]	
Implementation	• Implement testing algorithms to enhance efficiency* • Reflex testing may help to ensure expedited and consistent routing of specimens • Enroll in proficiency testing, if available*
Reporting	• Include a results and interpretation section readily understandable by the MDT*

* Expert consensus opinion (Grade D evidence). Recommendations are supported by Grade A or B evidence unless otherwise specified.
1. Leigh NB, et al. J Clin Oncol. 2014;32:3673-3679. 2. Lindeman NI, et al. J Mol Diagn. 2013;15:415-453.

National Comprehensive Cancer Network® (NCCN®) recommendations

NCCN recommendations for EGFR mutation and ALK rearrangement testing (category 1 for nonsquamous NSCLC and NSCLC NOS) are similar to the ASCO-endorsed CAP/IASLC/AMP recommendations.[1,2]

How do you test EGFR mutations and ALK rearrangements?[1]	
EGFR	• DNA mutational analysis (methods that are more sensitive than direct sequencing are available) • Validated broad molecular profiling systems (eg, multiplex PCR or NGS)
ALK	• FISH • IHC; FISH can be used to confirm positivity • Validated broad molecular profiling systems (eg, NGS) designed to detect gene fusions

NGS=next-generation sequencing.
* All recommendations are category 2A unless otherwise specified.
1. Referenced with permission from the NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines®) for Non-small Cell Lung Cancer V.4.2016. © National Comprehensive Cancer Network, Inc 2016. All rights reserved. Accessed May 24, 2016. To view the most recent and complete version of the guideline, go online to NCCN.org. NATIONAL COMPREHENSIVE CANCER NETWORK®, NCCN®, NCCN GUIDELINES®, and all other NCCN Content are trademarks owned by the National Comprehensive Cancer Network, Inc. 2. Lindeman NI, et al. J Mol Diagn. 2013;15:415-453.

Tissue handling

Optimize tissue quantity and quality at acquisition

- In a recent survey from the American College of Chest Physicians on challenges regarding biomarker testing for pathologists[1]

>70% found insufficient size of tissue sample to be the biggest issue

>30% had difficulty obtaining quality tissue

Adequate quality and quantity of DNA in specimens is necessary for biomarker profiling of NSCLC tumors.[2]

1. ACCP. Survey: how pulmonologists use biomarker testing. http://www.chestnet.org/~/media/chestnet/org/Publications/Documents/CHEST-Physician/Survey/HowPulmonologistsUse.ash. Updated May 2013. Accessed May 24, 2016. 2. Lindeman NI, et al. J Mol Diagn. 2013;15:415-453.

Tumor content determines a specimen's suitability for biomarker testing

- Sample adequacy is critical to help prevent false negatives and assay failures[1]
- The ASCO-endorsed CAP/IASLC/AMP guidelines state that larger tumor specimens (eg, resections) are preferred for mutation assays[1,2]
  - Small specimens may produce false-negative results

Any specimen that meets the laboratory's requirements for tumor content, fixation, and quality, as established during validation, may be chosen for analysis.[1]

EBUS-TBNA=endobronchial ultrasound-guided transbronchial needle aspiration; EUS-FNA=endoscopic ultrasound-guided fine needle aspiration; TTNBx=transthoracic needle biopsy.

1. Lindeman NI, et al. *J Mol Diagn*. 2013;15:415-453. 2. Leighl NB, et al. *J Clin Oncol*. 2014;32:3673-3679. 3. Rivera MP, et al. *Chest*. 2013;143(suppl 5):e142S-e165S. 4. Thunnissen E, et al. *Lung Cancer*. 2012;76:1-18. 5. Muley TR, et al. *Transl Lung Cancer Res*. 2012;1:111-121.

Ensure sufficient tissue quantity is acquired for testing

- Evaluation procedures may be used during biopsy to improve adequacy rates[1]
  - ROSE is an on-site assessment that optimizes diagnostic accuracy and ensures adequate sample acquisition
- In order to capture 200 to 400 cells per section, this typically requires[2,3]
  - 4 FNA passes
  - 4 to 5 transbronchial biopsies
  - 2 to 3 CT-guided core-needle biopsies

A sufficient amount of tissue should be acquired to allow for current and future biomarker testing needs.[4]

FNA=fine needle aspiration; ROSE=rapid, on-site evaluation.

1. Pusztaszeri M, et al. *J Pulm Respir Med*. 2012;S5:002. doi:10.4172/2161-105X.S5-002. 2. Cooper WA, et al. *Pathology*. 2011;43:103-115. 3. Pirker R, et al. *J Thorac Oncol*. 2010;5:1756-1713. 4. Travis WD, et al. *Arch Pathol Lab Med*. 2013;137:668-684.

Considerations for biopsy methods

BIOPSY METHOD	POTENTIAL ADVANTAGES[1-7]	POTENTIAL LIMITATIONS[1,2,4,6-8]
EBUS-TBNA EUS-FNA	• High yield for endoscopically visible bronchial lesions • High predictive value • Good reproducibility • Outpatient procedure • Real-time image guidance • Multiple biopsies with high-quality histologic cores	• Limited access
Thoracentesis	• Minimally invasive • Outpatient procedure	• Cytology examination only
CT-guided core-needle biopsy	• Outpatient procedure • High diagnostic accuracy • Better for larger and more outlying lesions	• Potential for higher rate of complications for lesions ≤4 cm
CT-guided FNA	• Outpatient procedure • Safe • High diagnostic accuracy • Better for smaller, deeper, and malignant lesions	• Limited tissue yield • Lacks specific diagnosis
Standard bronchoscopy	• Relatively safe	• Limited access to peripheral tissue • Lacks target visualization • Variable tissue yield
Conventional surgical resection	• High tissue yield	• Not possible for all patients • Requires longer processing time • General anesthesia and warm ischemia time may impact gene expression • Increased morbidity and length of hospital stay
VATS	• High tissue yield • High accuracy and specificity	• Increased morbidity and length of hospital stay

CT=computed tomography; VATS=video-assisted thoracoscopic surgery.

1. Muley TR, et al. *Transl Lung Cancer Res*. 2012;1:111-121. 2. Manhire A, et al. *Thorax*. 2003;58:920-936. 3. Nakajima T, et al. *Chest*. 2007;132:597-602. 4. Rivera MP, et al. *Chest*. 2013;143(suppl 5):e142S-e165S. 5. Duquoi R Jr, et al. *J Am Coll Radiol*. 2012;7:859-864. 6. Greif J, et al. *Acta Cytol*. 1999;43:756-763. 7. Pruyss LS, et al. *Acta Radiol*. 2013;54:640-645. 8. Yasufuku K, et al. *Chest*. 2006;130:710-716.

Preserve sample quality through careful sample processing

- The quality of extracted tissue and the method of preserving it for testing affect the accuracy and sensitivity of biomarker analyses[1]
- Avoid tissue treatments that may compromise sample quality[1]
  - Heavy metal competes with cofactors and enzymes from assays
  - Acid solutions fragment DNA extensively
  - Nonacidic chelating decalcifying solution may better preserve DNA for bone metastasis

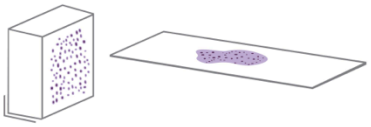
Recommendations	DNA mutation analysis[1]	FISH[1]	IHC[2]
Fixative	• FFPE (10% NBF) • Fresh • Frozen • Alcohol	• FFPE (10% NBF) • Fresh • Frozen	• FFPE (10% NBF, 1:10 ratio)
Time of fixation	• Small biopsy: 6 h-12 h • Large surgical samples: 8 h-18 h	• 6 h-48 h	• 6 h-48 h

NBF=neutral buffered formalin.

1. Lindeman NI, et al. *J Mol Diagn*. 2013;15:415-453. 2. Tsao MS, et al. *IASLC Atlas of ALK Testing in Lung Cancer*. Aurora, Colorado: International Association for the Study of Lung Cancer; 2013. https://www.iaslc.org/publications/iaslc-atlas-alk-testing-lung-cancer. Accessed June 5, 2016.

Cytological preparations for lung biomarker testing

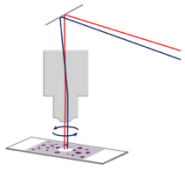
- The ASCO-endorsed CAP/IASLC/AMP guidelines state that cytologic samples may be used for EGFR and ALK testing[1]
  - The use of cell blocks is preferred over smear preparations
  - The cell pellet should be fixed in 10% NBF for 6 to 48 hours before processing
    - Fixation in 70% ethanol is also acceptable with appropriate validation
- ALK IHC is equally promising with cytologic specimens, including cell blocks and conventional or liquid-based cytologic preparations[2]



1. Lindeman NI, et al. *J Mol Diagn*. 2013;15:415-453. 2. Tsao MS, et al. *IASLC Atlas of ALK Testing in Lung Cancer*. Aurora, Colorado: International Association for the Study of Lung Cancer; 2013. https://www.iaslc.org/publications/iaslc-atlas-alk-testing/lung-cancer. Accessed March 8, 2016.

Establish minimum proportion and number of cancer cells needed for testing

- Appropriate assessment of the specimen is critical for accurate results[1]
- Minimum tumor requirement varies depending on the methods used[1]
- An enrichment strategy may be used to isolate cells from the more concentrated area, including[1]
  - Gross macrodissection or coring
  - Microdissection from glass slides
  - Flow cytometric sorting
  - Laser capture microdissection



1. Lindeman NI, et al. *J Mol Diagn*. 2013;15:415-453.

Consider a multidisciplinary plan to prioritize and protect small specimens for biomarker testing

Plan a well-thought-out strategy to manage tissue[1,2]

- Think beyond initial diagnosis and through the course of disease management

Prioritize the proper sequence of pathology tests[2]

- Continue open communication with the oncologist about the patient

Protect valuable tissue with precautionary steps[3]

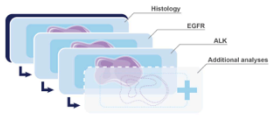
- Consider approaches to preserving tumor specimens

1. Davidson MR, et al. *J Thorac Dis.* 2013;5:S483-S478. 2. Travis WD, et al. *Arch Pathol Lab Med.* 2013;137:668-684. 3. Lindeman NI, et al. *J Mol Diagn.* 2013;15:415-453.

25

Prioritize tissue samples for EGFR and ALK testing

- The increasing number of biomarkers to be tested is constrained by the limited availability of tissue for analysis[1]
- A well-thought-out strategy in coordination with the histology and immunohistochemistry laboratories is important to conserve tissue[2]



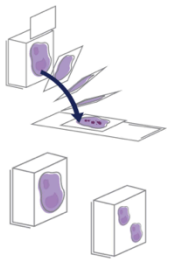
- **EGFR and ALK testing are the most important uses of the tissue sample after a diagnosis of adenocarcinoma is established[3]**
- **Prioritize EGFR testing, followed by ALK testing[3]**

1. Davidson MR, et al. *J Thorac Dis.* 2013;5:S483-S478. 2. Travis WD, et al. *Arch Pathol Lab Med.* 2013;137:668-684. 3. Lindeman NI, et al. *J Mol Diagn.* 2013;15:415-453.

26

Preserve tissue for biomarker testing

- Be judicious in the use of tissue to establish a histopathologic diagnosis[1]
  - Use a limited IHC workup only if needed to further classify tumor histology
- Consider cutting extra unstained slides from the paraffin block "up front" when the sample is first processed to reduce the number of times a block is refaced[1]
- If there is sufficient tumor tissue in the biopsy samples, consider separating the specimen into 2 separate blocks[2]
  - One block can be used to confirm histology and the other for biomarker testing
- Obtain additional tissue cores during biopsy procedures, implement rapid on-site cytology evaluation, and store frozen tissues for maximal yield of genomic material[3]



1. Lindeman NI, et al. *J Mol Diagn.* 2013;15:415-453. 2. Travis WD, et al. *Arch Pathol Lab Med.* 2013;137:668-684. 3. Tan DS, et al. *J Thorac Oncol.* 2016;11:946-963.

27

Considerations for EGFR testing

28

EGFR testing should be sensitive and specific

- Recommended methods should be able to detect mutations in specimens with at least 50% cancer cells[1]
  - Detection with as little as 10% cancer cells is ideal
- Analysis methods that are more sensitive than Sanger sequencing should be available[1]
- The ASCO-endorsed CAP/IASLC/AMP guidelines recommend the following testing methods for tissue specimens[1,2]:
  - The amplification refractory mutation system
  - Length analysis
  - Restriction fragment length polymorphism
  - Real-time PCR
  - High-resolution melting curve analysis
  - Single-base extension genotyping (including mass spectrometry-based genotyping)
  - Denaturing high-performance liquid chromatography
  - Massively parallel sequencing (also known as next-generation sequencing)

The ASCO-endorsed CAP/IASLC/AMP guidelines recommend using any validated EGFR testing method with sufficient performance characteristics for testing.[1,2]

1. Lindeman NI, et al. *J Mol Diagn.* 2013;15:415-453. 2. Leighl NB, et al. *J Clin Oncol.* 2014;32:3673-3679.

29

Ideal testing methods should cover a broad spectrum of EGFR mutations

- Clinical EGFR mutation testing should be able to detect all individual mutations that have been reported in at least 1% of EGFR-mutated lung adenocarcinomas[1]

Exon 18 mutations	Exon 19 deletions	Exon 20 mutations	Exon 21 mutations
• E709 • G719	Bp deletions: • 15, 18, 9, 12, 24, 27 Bp insertions • 15, 18	• S768 • T790M • Insertions	• L858R • T854 • L861Q

1. Lindeman NI, et al. *J Mol Diagn.* 2013;15:415-453.

30

Tissue biopsy may not be possible for all patients

- Biopsies are highly invasive procedures and tissue samples cannot be obtained from all patients[1-3]
- Initial biopsies do not always give definitive results and re-biopsy is not possible in all patients[1]

Approximately 10% to 20% of advanced NSCLC patients present with tissue biopsy–related challenges, including insufficient tissue, poor tissue quality, unwillingness to be tested, and comorbidities.[1-3]

Patients unsuitable for tissue biopsy, or for whom a biopsy did not yield a definitive result, may be treated with chemotherapy.[1]

1. Chouaid C, et al. *Lung Cancer*. 2014;86:170-173. 2. Westwood M, et al. *Health Technol Assess*. 2014;18:1-166. 3. Yoon HJ, et al. *Radiology*. 2012;265:939-948.

31

Blood-based cftDNA testing is approved to detect EGFR mutations

- The FDA has approved a plasma-based test using cell-free tumor DNA in the blood to detect EGFR mutations in NSCLC[1]
- With blood-based cftDNA testing, the MDT has a new option for ordering EGFR mutation testing for patients with tissue biopsy–related challenges[2]
  - Patients with tissue biopsy-related challenges harboring EGFR mutations may be treated with chemotherapy if they were not tested[3]
  - Consider requesting blood–based cftDNA testing on pathology reports for patients with tissue biopsy-related challenges[2,4]

cftDNA tests may complement traditional tissue biopsy for biomarker detection in NSCLC patients with tissue biopsy–related challenges.[2]

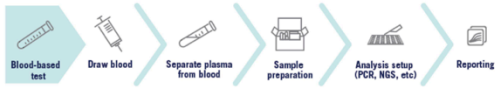
cftDNA=cell-free tumor DNA.

1. US Food and Drug Administration. FDA approves first blood test to detect gene mutation associated with non-small cell lung cancer. http://www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/ucm504468.htm. Updated June 1, 2016. Accessed June 1, 2016. 2. Bordi P, et al. *Transl Lung Cancer Res*. 2015;4:584-597. 3. Chouaid C, et al. *Lung Cancer*. 2014;86:170-173. 4. Lindeman NI, et al. *J Mol Diagn*. 2013;15:415-453.

32

Sample workflow for blood-based cftDNA test

Blood-based cftDNA test workflow[1,2]



- Specific sample preparation may vary by detection methods; it is important to pay attention to an individual laboratory's testing requirements[2,3]

1. Bordi P, et al. *Transl Lung Cancer Res*. 2015;4:584-597. 2. Thress KS, et al. *Lung Cancer*. 2015;90:509-515. 3. Lindeman NI, et al. *J Mol Diagn*. 2013;15:415-453.

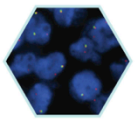
33

Considerations for ALK testing

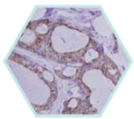
34

ALK rearrangements may be detected by FISH and IHC

- Laboratories should use an ALK FISH assay with dual-labeled break-apart probes to select patients for ALK[1]
- ALK IHC assay has been approved as a diagnostic to detect patients with ALK rearrangements[2]
- RT-PCR is not recommended as an alternative to FISH for selecting patients for ALK inhibitor therapy[1]



FISH



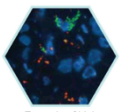
IHC

1. Lindeman NI, et al. *J Mol Diagn*. 2013;15:415-453. 2. US Food and Drug Administration. List of cleared or approved companion diagnostic devices (in vitro and imaging tools). http://www.fda.gov/medicaldevices/productsandmedicalprocedures/invitrodiagnostics/ucm301431.htm. Updated December 21, 2015. Accessed May 24, 2016.

35

Assess tumor architecture, cytology, and specimen quality for ALK FISH testing

- A minimum of 50 assessable tumor cells are required for FISH testing[1]
- Choose slides or regions of slides in which the tumor cells can be readily distinguished from admixed normal cells under fluorescence[2]
  - ≥50% of all nuclei should be easily analyzable, with minimal background or nuclear fluorescent noise
  - Avoid overlapping areas



Example of high background noise

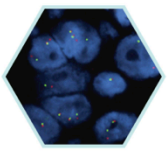
1. Tsao MS, et al. *IASLC Atlas of ALK Testing in Lung Cancer*. Aurora, Colorado: International Association for the Study of Lung Cancer; 2013. https://www.iaslc.org/publications/iaslc-atlas-alk-testing-lung-cancer. Accessed June 15, 2016. 2. Lindeman NI, et al. *J Mol Diagn*. 2013;15:415-453.

36

Interpretation of ALK FISH results

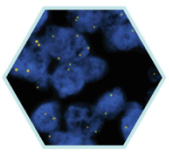
- Rearranged tumors: FISH positive cell rate ≥15% of cells[1]

Positive[1]



1 separate orange/red and 1 separate green signal with a gap of >2 signal diameters or loss of green probe

Negative[1]



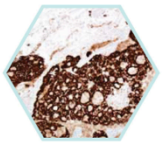
Yellow fusion signal or 2 narrowly split orange/red and green signals

1. Lindeman NI, et al. *J Mol Diagn.* 2013;15:415-453.

An IHC assay has been approved by the FDA to detect ALK rearrangement

- An approved IHC assay has demonstrated high sensitivity, specificity, and reproducibility for ALK rearrangement testing[1,2]
- A single uniform technique, or comparator, has not been evaluated in the studies on ALK IHC in NSCLC[3]
  - Specific requirements for testing may be different for different assays; it is important to refer to an individual test's label for testing requirements

ALK IHC with the FDA-approved diagnostic test

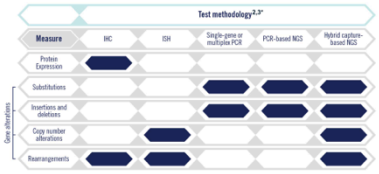


1. Lindeman NI, et al. *J Mol Diagn.* 2013;15:415-453. 2. US Food and Drug Administration. VENTANA ALK (D5F3) CDx Assay - P140025. http://www.fda.gov/MedicalDevices/ProductsandMedicalProcedures/DeviceApprovalsandClearances/RecentlyApprovedDevices/ucm454476.htm. Updated July 10, 2015. Accessed July 29, 2016. 3. Tsao MS, et al. *IASLC Atlas of ALK Testing in Lung Cancer.* Aurora, Colorado: International Association for the Study of Lung Cancer; 2013. https://www.iaslc.org/publications/iaslc-atlas-alk-testing-lung-cancer. Accessed March 8, 2016.

Technology advancements in biomarker testing

Innovations in multigene testing methodologies may hold significant promise in clinical testing

- NGS is a new clinical diagnostic technology that allows for[1]:
  - Sequencing of multiple genes in parallel
  - Detection of multiple types of gene alterations in a single test



ISH=in situ hybridization.
* Includes all laboratory-developed assays to date.

1. Ross JS, Cronin M. *Am J Clin Pathol.* 2011;136:527-539. 2. Lindeman NI, et al. *J Mol Diagn.* 2013;15:415-453. 3. Vnencak-Jones C, et al. My Cancer Genome. https://www.mycancergenome.org/content/molecular-medicine/types-of-molecular-tumor-testing/. Updated February 9, 2016. Accessed June 15, 2016.

Considerations for an NGS testing program

- An NGS program is composed of 3 main processes[1]
  - Pre-analytics include sample preparation
  - Analytics include DNA extraction and library preparation
  - Bioinformatics include sequence generation, sequence mapping and alignment, identifying variants, annotating variants, and clinical interpretation

1. Aziz N, et al. *Arch Pathol Lab Med.* 2015;139:481-493. 2. Dillon A, et al. *Clin Cancer Res.* 2015;21:3631-3639.

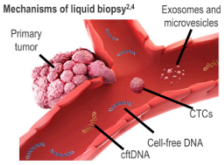
Liquid biopsy is an emerging method of cancer biomarker testing

- Liquid biopsy is a diagnostic technique that measures either cfDNA, circulating tumor cells, or tumor exosomes from bodily fluids such as blood, urine, or saliva[1,2]
- There are various oncology clinical applications for liquid biopsy, including screening, diagnosis, therapy selection, and monitoring[2]

Liquid biopsy approaches[2]

cfDNA	Detects mutations from small fragments of tumor DNA released into the bloodstream
CTC	Correlates disease activity with number of cells released from the primary tumor mass into the bloodstream
Tumor exosomes	Extracts molecular information from actively released vesicles derived from tumor cells

Mechanisms of liquid biopsy[2,4]



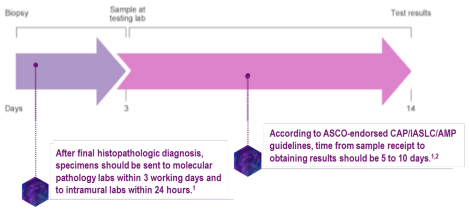
cftDNA tests can measure cancer biomarkers that may drive therapy selection.[3]

CTC=circulating tumor cell.

1. Ebrahimi Yl, et al. *Int J Mol Sci.* 2013;14:18925-18958. 2. Gold B, et al. *J Mol Diagn.* 2015;17:209-224. 3. US Food and Drug Administration. FDA approves first blood test to detect gene mutation associated with non-small cell lung cancer. http://www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/ucm504488.htm. Updated June 1, 2016. Accessed July 29, 2016. 4. Diaz LA, et al. *J Clin Oncol.* 2014;32:579-586.

Protocols to reduce testing delays

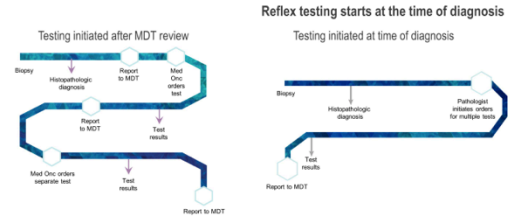
Testing delays may impact patient care because of the rapid clinical course of advanced NSCLC



Stage IV lung cancer patients have a median untreated life expectancy of 16 weeks; patients should not spend a significant portion of this time waiting for test results.[1]

1. Lindeman NI, et al. *J Mol Diagn*. 2013;15:415-453. 2. Leighl NB, et al. *J Clin Oncol*. 2014;32:3673-3679.

Establishing a testing procedure from the start can minimize overall testing time

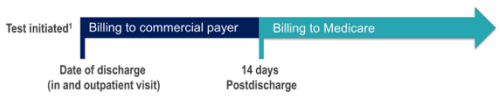


According to the ASCO-endorsed CAP/IASLC/AMP guidelines, establishing a procedure for automatic, or reflex, testing in appropriate patients at the time of histologic diagnosis may reduce testing time.[1,2]

1. Lindeman NI, et al. *J Mol Diagn*. 2013;15:415-453. 2. Leighl NB, et al. *J Clin Oncol*. 2014;32:3673-3679.

Timely processing of biomarker testing is important for patient care

- The CMS date-of-service rule for laboratory services establishes structure for direct billing to Medicare[1]
  - CMS date-of-service rules include 14 days postdischarge before direct billing to Medicare[1]
  - The ASCO-endorsed CAP/IASLC/AMP guidelines, however, recommend at most 13 working days postdiagnosis to obtain test results[2,3]



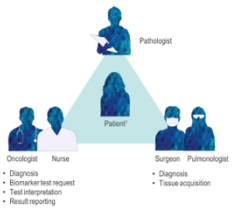
Delaying ordering of biomarker testing may impact timely report of results and compromise patient care.[4*]

CMS=Centers for Medicare & Medicaid Services.
* Billing policy may differ between institutions. It is important to consult individual institutions for specific billing considerations.

1. Centers for Medicare & Medicaid Services. HHS. §.414.510 Laboratory date of service for clinical laboratory and pathology specimens. www.gpo.gov/fdsys/pkg/CFR-2011-title42-vol3/pdf/CFR-2011-title42-vol3-sec414-510.pdf. Accessed May 24, 2016. 2. Leighl NB, et al. *J Clin Oncol*. 2014;32:3673-3679. 3. Lindeman NI, et al. *J Mol Diagn*. 2013;15:415-453. 4. Yohnar KE, et al. *Arch Pathol Lab Med*. 2015;139:1115-1124.

Maintain MDT communication

Efficient lung cancer treatment involves multidisciplinary team communication



- Consider MDT communication up front because future treatment options can affect diagnostic strategy[2]
- Examples of communication points between MDT members include[1]:
  - Consulting on biopsy to determine sufficient tissue and tumor content
  - Ordering biomarker testing
  - Reporting test results
  - Nurse navigators streamlining patient care and facilitating consistent communication

The ACCP and ASCO-endorsed CAP/IASLC/AMP guidelines recommend MDT decision-making for biomarker testing.[2-4]

ACCP=American College of Chest Physicians.

1. Levy BP, et al. *Oncologist*. 2015;20:1175-1181. 2. Osi DE, et al. *Chest*. 2013;143(suppl 5):e121S-e141S. 3. Leighl NB, et al. *J Clin Oncol*. 2014;32:3673-3679. 4. Lindeman NI, et al. *J Mol Diagn*. 2013;15:415-453.

Considerations for potential action items for the MDT to improve testing

Challenges[1,2]	MDT considerations[1,2]*
Rapidly evolving practice standard, including emerging biomarkers and new testing methodologies	• Establish and promote communication of biomarker education
Lack of sufficient quality and quantity of tissue samples	• Schedule meeting with the MDT to review literature and discuss the optimal approach • Implement on-site assessment of tissue quantity and quality by pathology/cytology whenever possible
Testing delays	• Update process and policy to include ○ Simultaneous testing for EGFR and ALK ○ Documentation of why EGFR and ALK testing were not completed ○ Creating processes and tools for monitoring • Develop a protocol to ensure timely identification of biomarkers
Lack of communication and pathology-driven reflexive molecular testing	• Maintain electronic health records • Nurse navigators may improve communication • Develop and implement a reflexive molecular testing pathway • Implement a pathology-driven interdisciplinary task force to evaluate options and make recommendations
Lack of standardized pathology reports	• Standardize the application of the CAP lung biomarker reporting template

* Action items listed are for consideration only and should comply with individual institution policy.

1. Levy BP, et al. Oncologist 2015;20:1175-1181. 2. Kim J. Molecular testing in the community oncology setting. Association of Community Cancer Centers. 2015. https://www.accc-cancer.org/oncology_issues/articles/2F15JF10-Molecular-Testing-in-the-Community-Oncology-Setting.pdf. Accessed June 1, 2016.

49

Test reporting should be clear and complete

- Oncologists should be able to quickly glean the information needed to appropriately manage the patient[1]
- Pathologists should be able to determine from the report whether additional testing or repeat testing is appropriate[1]
- Another laboratorian should be able to understand how the testing was done[1]

EGFR ALK NGS

1. Lindeman NI, et al. J Mol Diagn. 2013;15:415-453.

50

Test reporting should be clear and complete

- Oncologists should b
- Pathologists should b
- Another laboratorian

1. Lindeman NI, et al. J Mol Diagn. 2013;15:415-453.

50

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1. Lindeman NI, et al. J Mol Diagn. 2013;15:415-453.

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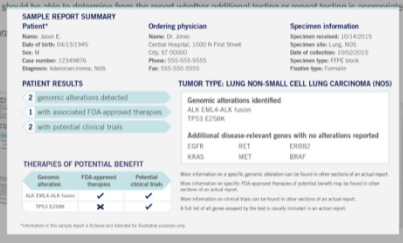


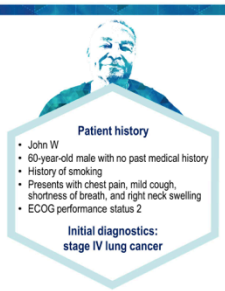
1. Lindeman NI, et al. J Mol Diagn. 2013;15:415-453.

50

Case studies

54

Case study*: tissue handling



Patient history

- John W
- 60-year-old male with no past medical history
- History of smoking
- Presents with chest pain, mild cough, shortness of breath, and right neck swelling
- ECOG performance status 2

Initial diagnostics: stage IV lung cancer

PATIENT BIOPSY HAS been SCHEDULED

ECOG=Eastern Cooperative Oncology Group.
* Information in this sample report is fictional and intended for illustrative purposes only.

Discussion questions

What biopsy procedure would you use for the case presented? Why?

- Larger tumor specimens are preferred for mutation assays because of the greater amount of material and greater capacity to enrich the malignant content by dissection[1]
- Less invasive procedures such as FNA may also be used[1]

How would you verify tissue quality and quantity of your sample?

- Test methodology should be carefully considered by the MDT, as different tests may have different tissue requirements[1,2]
- Fixatives may vary by different testing methods; avoid fixatives that damage DNA content (acidic, heavy metal, decalcifying solution); limit fixation time; prepare cytology samples in FFPE cell blocks or as recommended by the ASCO-endorsed CAP/IASLC/AMP guidelines[1]
- Each lab should establish the minimum proportion and number of cancer cells required for biomarker testing needs, and use enrichment strategies as needed to improve tumor content[1]
- A minimum of 50 assessable tumor cells are required for FISH for the ALK gene rearrangement[2]

FNA=fine needle aspiration.

1. Lindeman NI, et al. *J Mol Diagn*. 2013;15:415-453. 2. Tsao MS, et al. *IASLC Atlas of ALK Testing in Lung Cancer*. Aurora, Colorado: International Association for the Study of Lung Cancer; 2013. https://www.iaslc.org/publications/iaslc-atlas-alk-testing-lung-cancer. Accessed June 3, 2016.

Discussion questions (cont'd)

How would you preserve tissue to meet the needs of histology and additional biomarker testing?

- Use a limited IHC workup only if necessary[1]
- If there is sufficient tissue, separate the specimen into 2 separate blocks, one for histologic confirmation and one for biomarker testing[2]
- Cut extra unstained slides from the paraffin block when the sample is first processed to reduce the number of times a block is refaced[1]
- Prioritize tissue samples for EGFR testing, followed by ALK testing[1]

What are your options if you encounter QNS?

- Options include running the test again on archival tissue, requesting a new biopsy, or cftDNA testing[1,3,4]
- cftDNA tests may complement traditional tissue biopsy for biomarker detection in NSCLC patients with tissue biopsy–related challenges[4]

QNS=quantity not sufficient.

1. Lindeman NI, et al. *J Mol Diagn*. 2013;15:415-453. 2. Travis WD, et al. *Arch Pathol Lab Med*. 2013;137:668-684. 3. Tan DS, et al. *J Thorac Oncol*. 2016;11:946-963. 4. Bordi P, et al. *Transl Lung Cancer Res*. 2015;4:584-597.

Case study*: time to test results

Sample processing

- EBUS-FNA of lymph nodes acquired, fixed in 10% NBF for 12 hours, and embedded into a paraffin block
- Sections are used for histochemical/ immunohistochemical stains to establish diagnosis
- Additional unstained sections are cut for any potential future biomarker testing

DIAGNOSIS IS ADVANCED-STAGE ADENOCARCINOMA

EBUS-FNA=endobronchial ultrasound-guided fine needle aspiration.
* Information in this sample report is fictional and intended for illustrative purposes only.

Discussion questions

Is there a standard practice or testing algorithm set up in your institution for biomarker testing? Is this guided by the stage of the patient's disease?

- Implementation of standardized testing algorithms is recommended to enhance efficiency and meet the required testing time recommended by the ASCO-endorsed CAP/IASLC/AMP guidelines[1,2]

What is your average time needed to receive test results? What is the time from biopsy to testing lab vs time from specimen receipt to obtaining results?

- Specimens should be sent to outside biomarker testing labs within 3 working days and intramural labs within 24 hours[1]
- The recommended goal for EGFR and ALK testing is 5 to 10 working days from the time between specimen receipt at the biomarker testing laboratory and reporting test results to the clinical care team[1]

1. Lindeman NI, et al. *J Mol Diagn*. 2013;15:415-453. 2. Leighl NB, et al. *J Clin Oncol*. 2014;32:3673-3679.

Discussion questions (cont'd)

How would you handle testing in this situation (sequential, concurrent)?

- Concurrent testing for multiple markers may take on increased importance as more clinically relevant biomarkers are identified[1]
- Although sequential testing may save on resources and labor, concurrent testing has been shown to reduce testing delays[2,3]

Does your institution have a reflex testing policy?

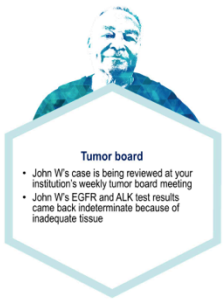
If yes, how is it implemented to accommodate specific cases?

If not, what policies are implemented to ensure efficient specimen routing?

- Automatic, or reflex, testing in appropriate patients at the time of histologic diagnosis may reduce time to testing results[1]

1. Lindeman NI, et al. *J Mol Diagn*. 2013;15:415-453. 2. Levy BP, et al. *Oncologist*. 2015;20:1175-1181. 3. Schrink JC, et al. 2013 ASCO Annual Meeting Abstract. 2013;e22093.

Case study*: MDT communication



Tumor board

- John W's case is being reviewed at your institution's weekly tumor board meeting
- John W's EGFR and ALK test results came back indeterminate because of inadequate tissue

* Information in this sample report is fictional and intended for illustrative purposes only.

61

Discussion questions

Which MDT members are involved in the biomarker testing process and what are their roles?

- Members involved may include medical oncologists, respiratory physicians, radiologists, pathologists, and nurses[1]
- Examples of roles of the MDT members include collecting tissue, ordering biomarker testing, and reporting test results[1]

How are test results communicated within your MDT? What are some specific challenges that you experience at your institution?

- Common communication channels for the MDT include tumor board/case conferences and informal face-to-face meetings, as well as communication over the phone, health record documentation, or e-mail[2]
- Some common challenges experienced by the MDT include rapidly evolving practice standards, lack of sufficient quality and quantity of tissue samples, testing delays, lack of communication within the MDT, and lack of standardized pathology reports[1,3]

1. Levy BP, *et al. Oncologist.* 2015;20:1175-1181. 2. Rowlands S, *et al. Eur J Cancer Care.* 2013;22:20-31. 3. Kim J. Molecular testing in the community oncology setting. Oncology Issues. 2015;30:28-32. http://www.nxtbook.com/nxtbooks/aocz/oncologyissues_20150102/index.php?startid=28. Accessed June 13, 2016.

62

Summary of biomarker testing considerations

Evaluate your institution's current biomarker testing processes

Identify addressable gaps across the entire testing procedure

- Small biopsy specimens or samples with low tumor content
- Testing delays or inefficient specimen routing
- Inconsistent communication among MDT members

Optimize your institution's testing accuracy and efficiency

- Consider consulting with pathologists at tissue biopsy or preparing samples for future biomarker testing during initial specimen processing
- Explore whether reflex testing is appropriate for your institution
- Encourage greater multidisciplinary communication among your colleagues
- Refer to current guideline recommendations

63