JADPRO CE
Regional Education

From Inquiry to Investigation to Insight: Clinical Clarity in Non–Small Cell Lung Cancer

Disease Overview

Selecting Treatments Using Biomarker Testing and Molecular Profiling

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Faculty Financial Disclosures

- **Ms. Eaby-Sandy** has served as a consultant and on speakers bureaus for AstraZeneca, Helsinn, Merck, and Takeda.
- **Dr. Beardslee** has served as a consultant for AstraZeneca and Herron, and on the speakers bureau for AstraZeneca.
- **Dr. Davies** has served on speakers bureaus for AstraZeneca, Bristol-Myers Squibb, Genentech, and Merck.
- **Ms. Gilbert** has no conflicts of interest to disclose.
- **Ms. Persinger** has served on speakers bureaus for Genentech and Guardant Health, and on the advisory board for AstraZeneca.

Planning Committee Financial Disclosures

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Learning Objective

- Evaluate recent updates regarding biomarker testing and molecular profiling used to guide management decisions for metastatic NSCLC

Outline

- NSCLC: histology, common biomarkers
- Molecular testing platforms
  - NGS vs. liquid biopsy
  - Fusion panels, IHC, FISH
- Immunotherapy biomarkers
  - PD-L1 vs. TMB

NGS = next-generation sequencing; IHC = immunohistochemistry; FISH = fluorescence in situ hybridization; PD-L1 = programmed cell death ligand 1; TMB = tumor mutational burden

Audience Response Question

According to the NCCN Guidelines, oncology providers should be performing molecular testing in which types of patients with NSCLC?

A. Minimal or never smokers
B. Never smokers with adenocarcinoma
C. All nonsquamous histologies but not squamous cell carcinoma
D. All nonsquamous histologies and some squamous cell carcinoma if they are nonsmokers
E. Unsure

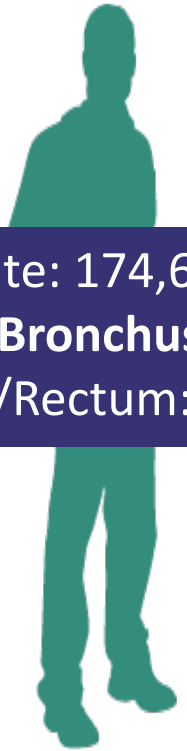
Audience Response Question

Which of the following is a true statement about molecular testing in NSCLC?

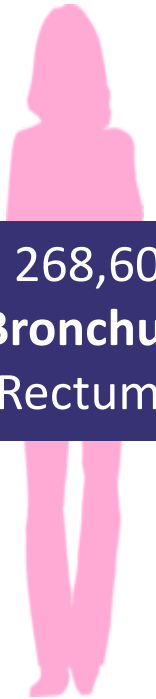
A. *EGFR* can be found on DNA sequencing and FISH, but not IHC
B. ALK can be found on DNA sequencing, FISH, or IHC
C. *NTRK* that is sensitive to treatment is best found on a DNA sequencing panel
D. Using liquid biopsy is a faster way to detect a secondary mutation in *EGFR*+ NSCLC such as a small cell transformation
E. Unsure

NSCLC: Scope of the Problem

Estimated number of new cases in US by sex: 2019



1. Prostate: 174,650
2. **Lung/Bronchus: 116,440**
3. Colon/Rectum: 78,500



1. Breast: 268,600
2. **Lung/Bronchus: 111,710**
3. Colon/Rectum: 67,100

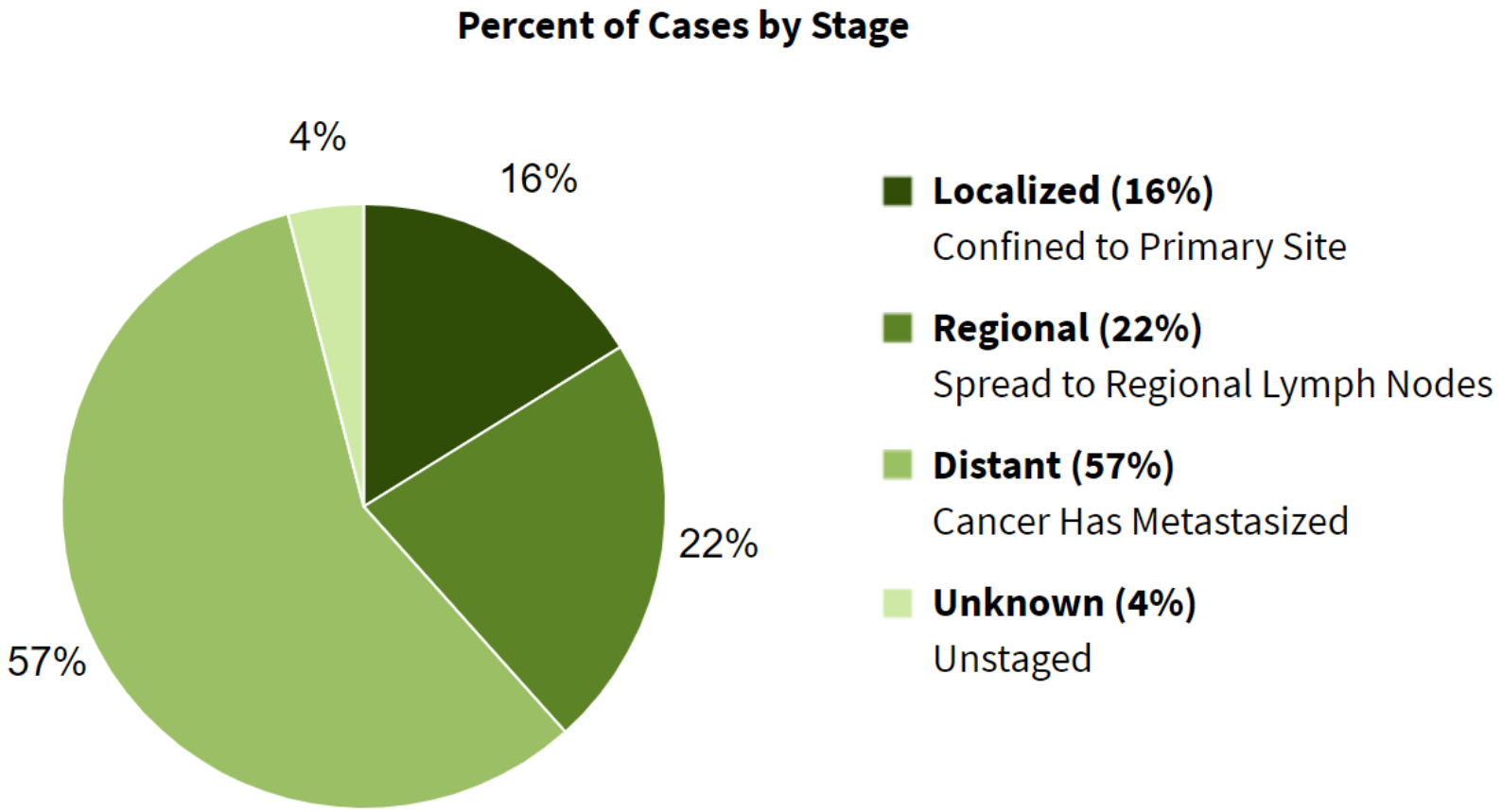
American Cancer Society. Cancer Facts and Figures 2019

NSCLC: Scope of the Problem (cont.)

Estimated number of deaths in US: 2019

PROSPECTIVE (deaths)
Lung/Bronchus = 142,670 (24%)
Breast + Prostate + Colon/Rectum = 124,400

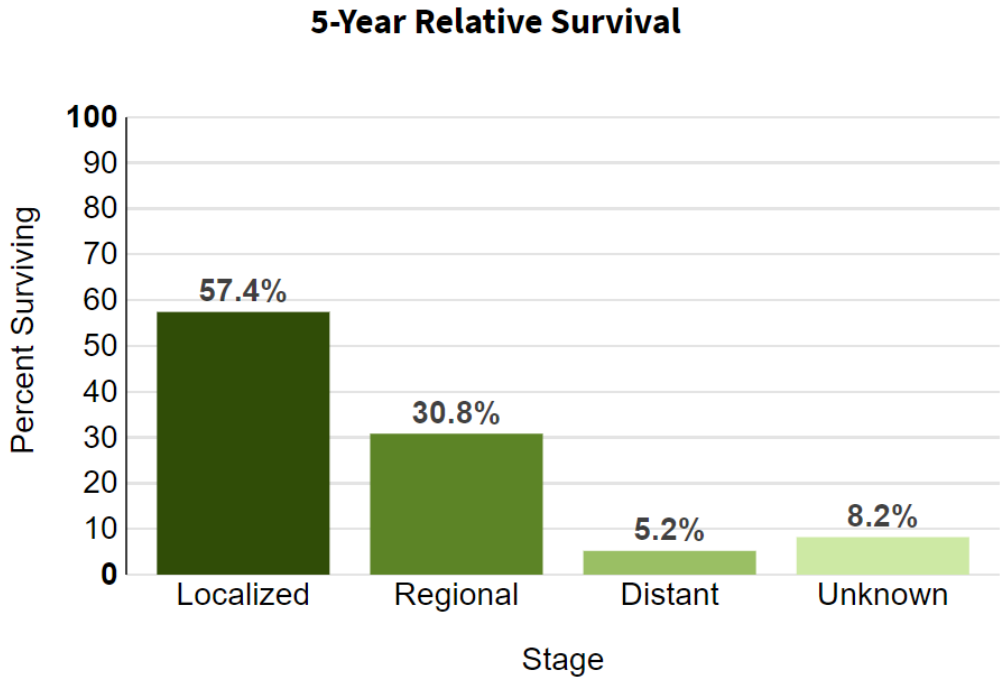
Lung Cancer



https://seer.cancer.gov/statfacts/html/lungb.html

Lung Cancer 5-year Survival Rates

Lung cancer



VS.

Breast cancer

5-Year Relative Survival Percent, Female Breast Subtypes by SEER Summary Stage 2000

Subtype	Localized	Regional	Distant
HR+/HER2-	100.0%	89.7%	29.8%
HR-/HER2-	91.0%	64.9%	11.2%
HR+/HER2+	98.3%	89.0%	41.8%
HR-/HER2+	95.8%	81.6%	36.3%
Unknown	95.6%	77.1%	15.3%
Total	98.8%	85.5%	27.3%

https://seer.cancer.gov/statfacts/html/lungb.html
https://seer.cancer.gov/statfacts/html/breast-subtypes.html

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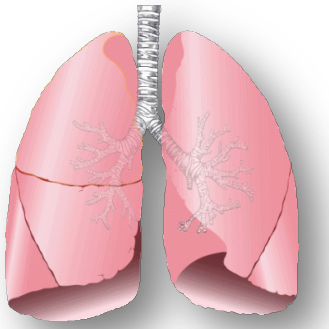
Lung Cancer: Main Pathologic Subtypes

Non–Small Cell Lung Cancer

- Most common type (84%)
- Smoking still main risk factor
- 10%–15% of cases in never smokers
- 2 main histologic subtypes
  - Adenocarcinoma
  - Squamous cell carcinoma

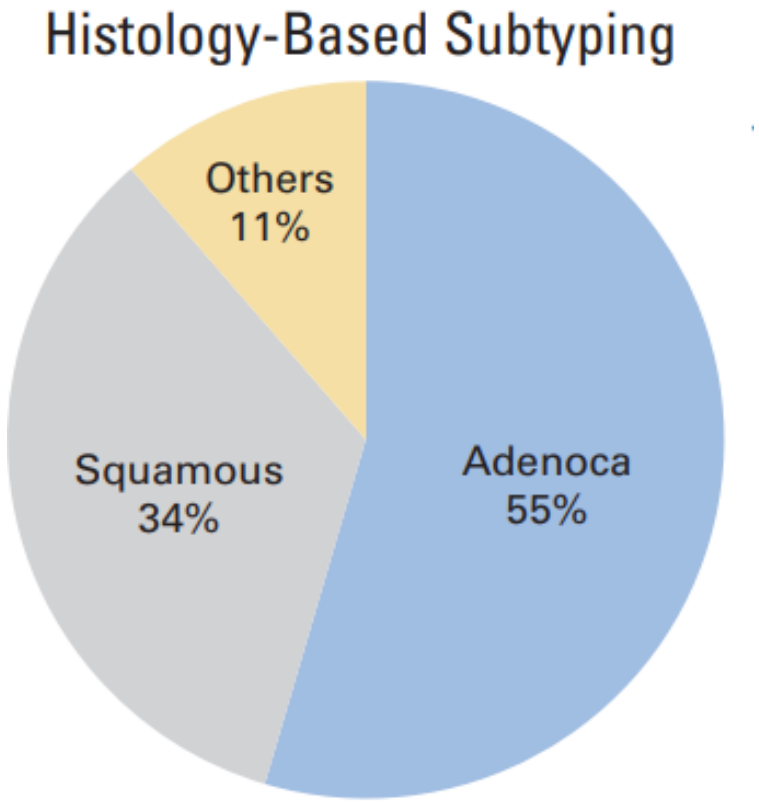
Small Cell Lung Cancer

- 99% of cases associated with cigarette smoking
- About 13% of all lung cancers
- Very fast growing, although very sensitive to initial chemotherapy
- Poor prognosis



American Cancer Society. Cancer Facts and Figures 2019

Histologic Subtypes of NSCLC



- Adenocarcinoma
  - Most likely to harbor a molecular biomarker
  - Most common type in nonsmokers
- Squamous cell
  - Generally more centrally located
- Other/large cell
  - Large cell NSCLC: associated with neuroendocrine features, but not a small cell
  - Others: mixed histologies, carcinoid, NOS

NOS: not otherwise specified

Li T et al, *J Clin Oncol.* 2013;31(8):1039–1049.

Biomarker Testing

Who should we test?
And how?

Molecular Testing: NCCN Guidelines Nonsquamous Histology

Primarily found in adenocarcinoma, but should test all nonsquamous

1. *EGFR*[a], *ALK*[a], *ROS1*, *BRAF*, PD-L1[a]
2. Testing should be part of a broad molecular profile[b]

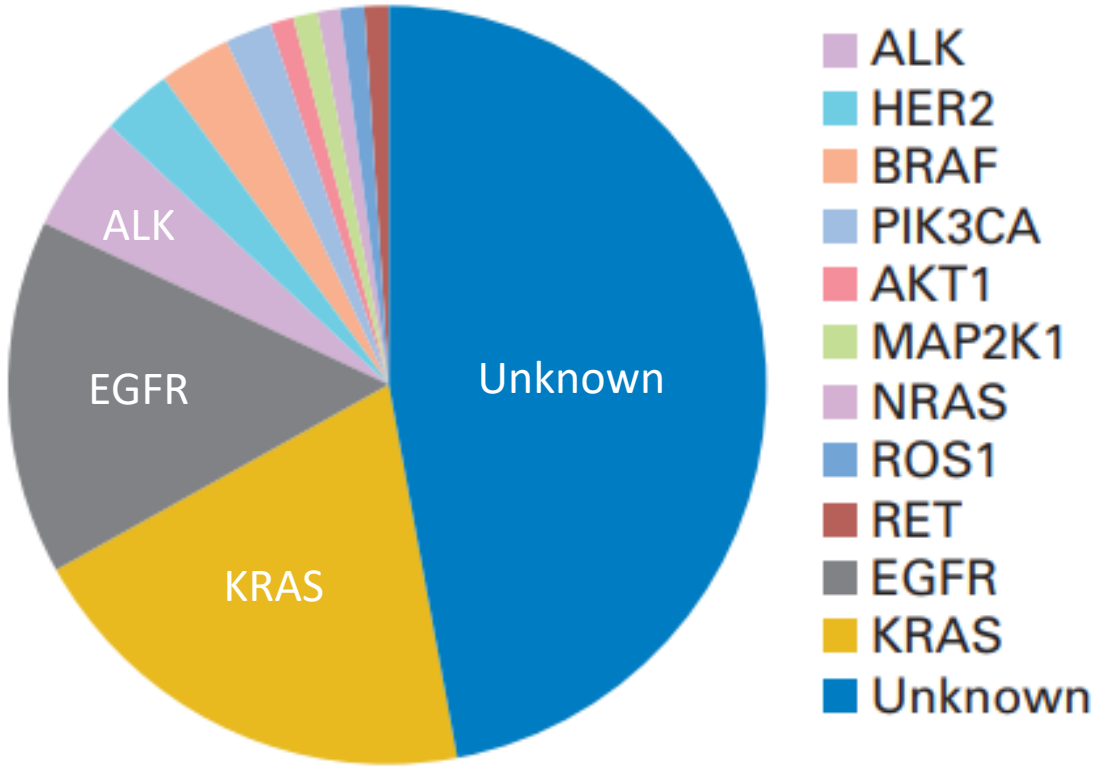
[a]Category 1 recommendation
[b]Goal is to find rare driver mutations for which drugs may be already approved or to consider enrollment in clinical trial. Broad panel should include *NTRK*.

Molecular Testing: NCCN Guidelines Squamous Cell Carcinoma

1. Consider *EGFR* and *ALK* testing in never smokers, mixed histology specimens, or small biopsy specimens
2. Consider *ROS1*, *BRAF*, broad molecular profile
3. PD-L1 testing recommended

https://www.nccn.org/professionals/physician_gls/pdf/nscl.pdf; version 4.2019

Biomarkers in NSCLC: Adenocarcinoma



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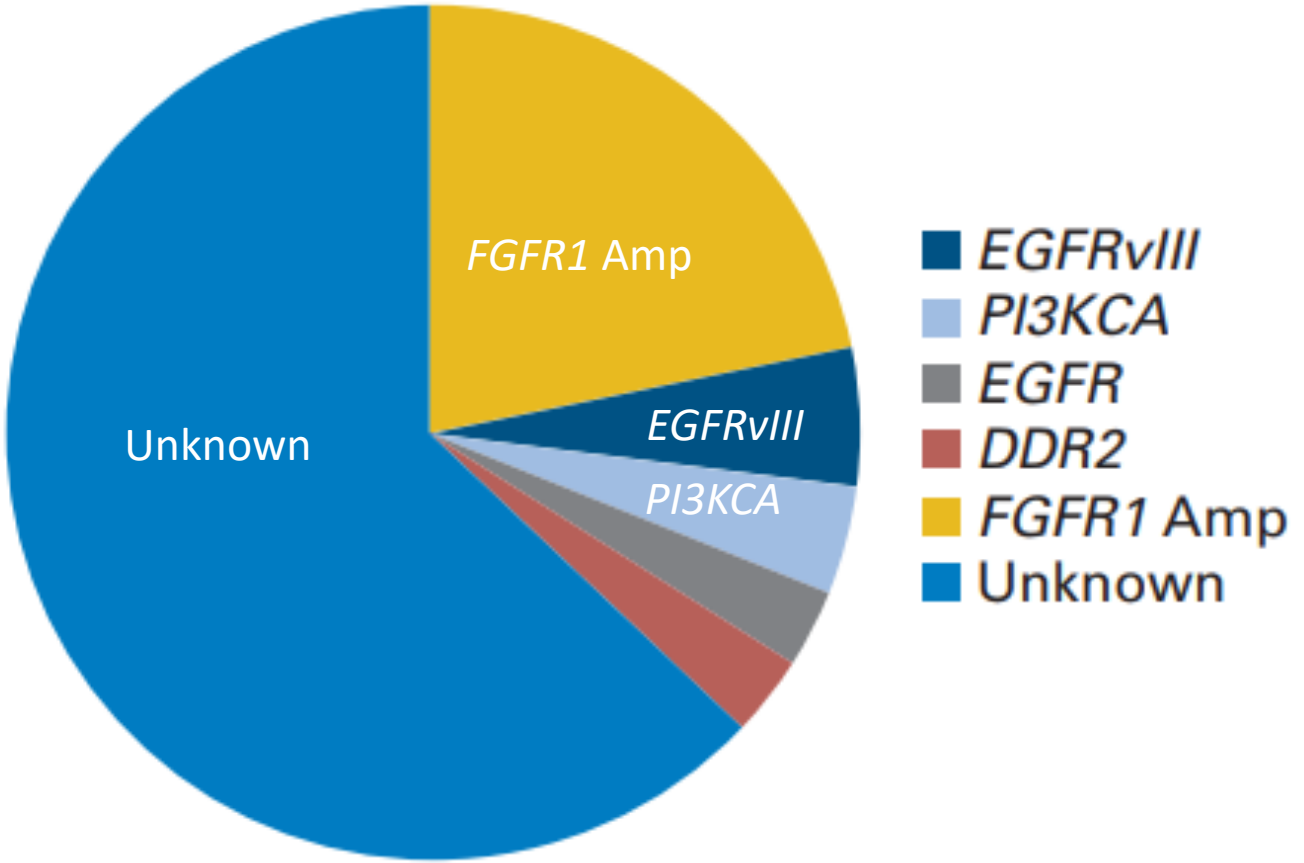
Biomarkers in NSCLC: Squamous Cell Carcinoma



Li T et al, *J Clin Oncol*. 2013;31(8):1039–1049.

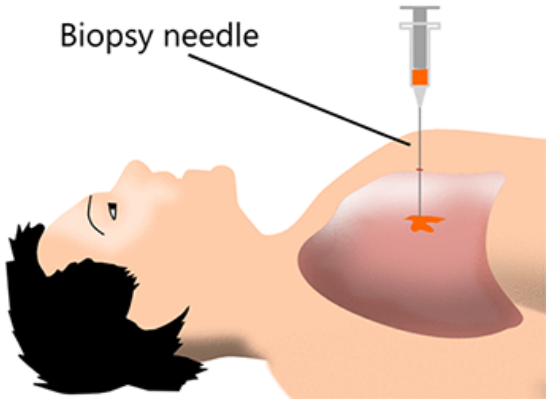
Recent Updates to the Molecular Testing Guidelines

- IHC is not an acceptable test for *EGFR* mutation and should not be used to treat with EGFR TKIs
- In *ALK* testing, IHC is an equivalent alternative to FISH testing
- Multiplex, broad sequencing panels are preferred over single-gene testing
- Oncologists may use molecular testing in patients with histologies other than adenocarcinoma who exhibit clinical features that predict for an oncogenic driver.

IHC = immunohistochemistry; TKI = tyrosine kinase inhibitor; FISH = fluorescence in situ hybridization.

Lindeman N, et al. 2018. J Mol Diagn. 20:129-159

Sampling Challenges



- Lung cancer biopsies are less cellular than other solid tumors
- Bone biopsies usually not good due to decalcification to read for pathology first
- Quality assurance of genomic medicine (multiple platforms, validation?)
- Logistical: timing of DNA sequencing can take weeks. Centralized vs. send out to distant laboratory.
- QNS: quantity/quality not sufficient (need 10%–20% of viable cancer cells in sample for reliable results)

Vanderlaan PA, et al. *Lung Cancer*. 2014;84:39-44.; Hiley, CT et al. *Lancet.* 2016;388:1002-1011.

Methods of Molecular Testing in NSCLC: DNA Sequencing

- Looking for an activating somatic mutation
  - Insertions, point substitutions, in-frame deletions
- DNA sequencing will detect mutations in *EGFR*, T790M, *KRAS*, *BRAF*, *HER2*, *MET*
- Methods: Direct sequencing, PCR, NGS (commercial kits)
  - Direct sequencing: only one mutation at a time
  - NGS kits can do broad panels

PCR = polymerase chain reaction; NGS = next-generation sequencing

Vanderlaan PA, et al. *Lung Cancer*. 2014;84:39-44.; Hiley CT et al. *Lancet*. 2016;388:1002-1011; Shim HS, et al. *J Path Trans Med.* 2017;51:242-254

Sample DNA Sequencing Panel at University of Pennsylvania

Solid Tumor NGS Report

Center for Personalized Diagnostics- Corrected
Perelman School of Medicine at the University of Pennsylvania
Solid Tumor Genomic Sequencing Panel Report

Demographics:
Case Number: PD-17-0004222
Specimen Type: Cytology
Collection Date: 11/28/2017 16:59
Received Date: 12/5/2017 14:28

Tissue Source: Pleural fluid, left
Specimen Identifier: NG-17-11042

Estimated Tumor Percentage: 11-25%
Specimen Quality Acceptable: Yes
DNA Quality Acceptable: Yes
Indication for Study: Adenocarcinoma present

Abnormal Report

DISEASE ASSOCIATED VARIANTS (see interpretation and comments):

GENE	PROTEIN CHANGE	cDNA CHANGE
SETD2	p.S1988Kfs*23	c.5948_5961dup
BRAF	p.V600E	c.1799T>A

VARIANTS OF UNCERTAIN SIGNIFICANCE (see interpretation and comments):

GENE	PROTEIN CHANGE	cDNA CHANGE
AKT1	p.R121P	c.362G>C
ATM	p.D1563H	c.4687G>C
CDH1	p.Y296C	c.887A>G
EZH2	p.K48E	c.142A>G
KMT2C	p.P2493L	c.7478C>T
PBRM1	p.N972S	c.2915A>G
PTPN11	p.H8Y	c.22C>T

Methods of Molecular Testing in NSCLC: RNA Fusions Panels (Gene Rearrangements)

- FISH and IHC fall in this category
- RNA fusions can detect fusion abnormalities
  - *ALK*, *ROS1*, *RET*, *NTRK*, *FGFR*
  - Our report at Penn: RNA fusion panel also reports EGFR, however, it is NOT an *EGFR* mutation, only a fusion—not clinically useful in NSCLC

Vanderlaan PA, et al. *Lung Cancer*. 2014;84:39-44.; Hiley CT et al. *Lancet*. 2016;388:1002-1011. Shim HS, et al. *J Path Trans Med.* 2017;51:242-254

Sample RNA Fusion Panel at University of Pennsylvania

Fusion Transcript Panel Report

Center for Personalized Diagnostics
Perelman School of Medicine at the University of Pennsylvania
Fusion Transcript Panel Report

Demographics:
Case Number: PD-18-0003188
Specimen Type: Paraffin Embedded Tissue
Collection Date: 8/20/2018 15:16
Received Date: 8/27/2018 08:34

Tissue Source: RUL
Specimen Identifier: CC-18-1013 (MF-18-321)
Block: B1 (A1)
Estimated Tumor Percentage: 10-25%
Specimen Quality Acceptable: Yes
RNA Quality Acceptable: Yes
Indication for Study: Malignant neoplasm of unspecified part of right bronchus or lung

Negative Report
Abnormal Transcripts: NOT DETECTED

INTERPRETATION AND COMMENTS:

This is a NEGATIVE sequencing study that did not detect abnormal transcripts of the targets included in the panel (see Test Description section, below).

No fusion transcripts involving ALK, ROS, RET, NTRK1, NTRK2, or NTRK3 or aberrant spliceforms of MET were identified in the current study. This assay does not detect all possible genomic variants, and additional testing using other methodologies may be informative. Correlation with all available clinical, histopathologic, and laboratory data is recommended.

Methods of Molecular Testing in NSCLC: IHC or FISH

- ALK, ROS1 can be performed by FISH
  - Highly specialized equipment, specially trained staff
- ALK, ROS1 can be done by IHC as well
  - Identifies a protein expression
  - Easier, just as good as FISH, and faster

Vanderlaan PA, et al. *Lung Cancer*. 2014;84:39-44.; Hiley CT et al. *Lancet*. 2016;388:1002-1011. Shim HS, et al. *J Path Trans Med.* 2017;51:242-254

Sample FISH Analysis at University of Pennsylvania

Cytogenetics Report

Cytogenetics Report
FISH Analysis

Case Demographics:
Case Number: C-13-0001982 Indication for Study: Lung Cancer
Specimen Received: Cells Preliminary Report: 10/10/2013
Date of Procedure: 10/04/2013 13:19 Final Report: 10/15/2013 15:10
Date Received: 10/08/2013 16:20

Fluorescence In-Situ Hybridization (FISH):

Requested Study: ALK split - Dual Color, Break Apart
Number of Cells Analyzed: 150
Probe (not FDA-approved*): LSI ALK (2p23)

SPECIMEN SOURCE: Lung, right upper lobe
SPECIMEN TYPE: Paraffin-embedded tissue
SPECIMEN IDENTIFIER: HS-13-24909 Block: 1B
COLLECTION DATE: 9/16/2013
DIAGNOSIS: Invasive moderately differentiated adenocarcinoma
INDICATION FOR TESTING: Determination of responsiveness to targeted therapy

ISCN FISH Nomenclature:

POSITIVE FOR ALK REARRANGEMENT

nuc ish(5'ALKx1,3'ALKx2),(5'ALK con 3'ALKx1)[53/150]/(ALKx2)(5'ALK sep 3'ALKx1)[7/150]

Interpretation and Comments:

Liquid Biopsy for Molecular Testing

- Blood test to detect these mutations (some are starting to also identify PD-L1)
- Often referred to as a “liquid biopsy”
- Can be done without invasive procedure and usually requires only about 2 tubes of blood done as routine phlebotomy
- Particularly an attractive option when looking for the T790M mutation or any resistance mutations
  - It cannot detect histology, thus cannot detect a “small cell transformation” from EGFR+ NSCLC
- Liquid biopsy relies on DNA shedding from the tumor into the bloodstream, and this can vary, so the sensitivity of liquid biopsies may vary

Sample Liquid Biopsy Report

Summary of Somatic Alterations & Associated Treatment Options

KEY ✓ Approved in indication ~ Approved in other indication ⊗ Lack of response

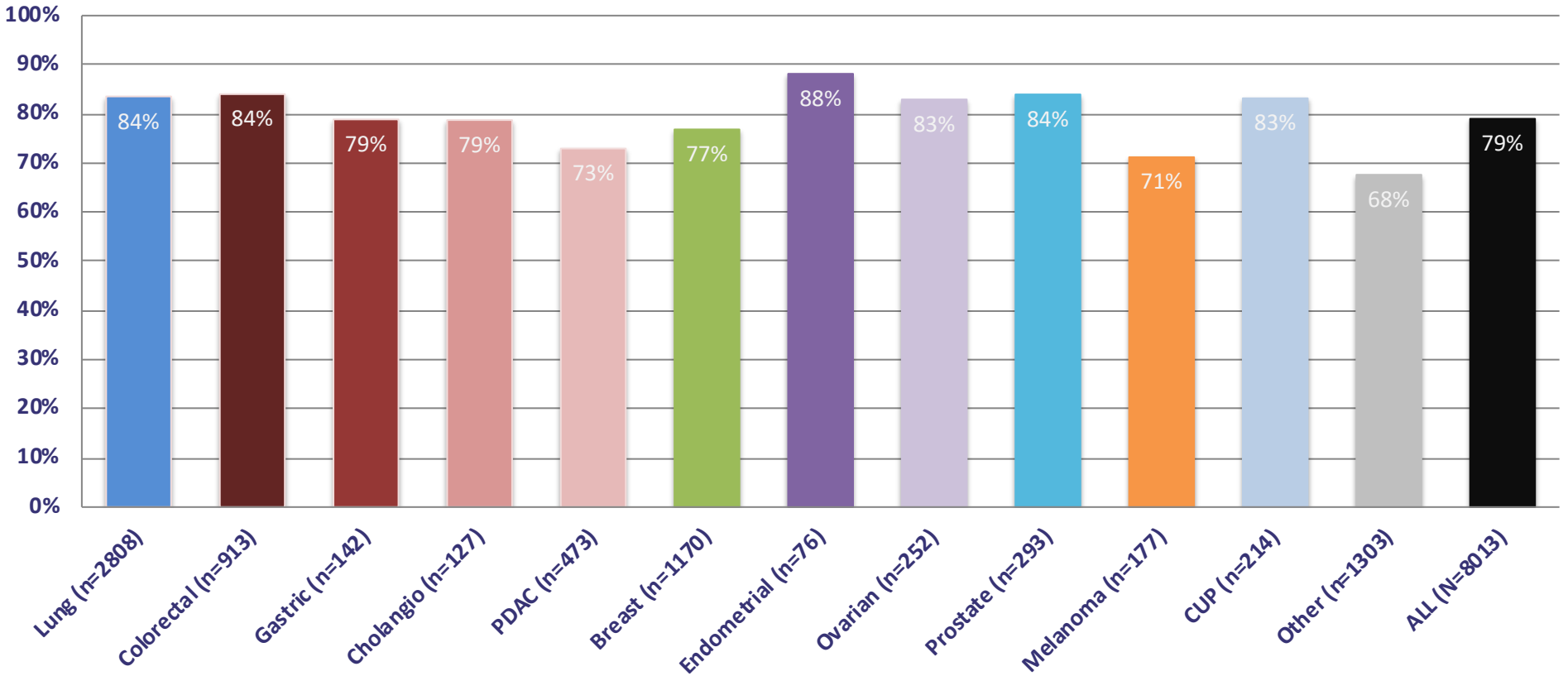
Alteration	% cfDNA or Amplification	Associated FDA-approved therapies	Clinical trial availability (see page 3)
EGFR E746_T751delinsI (Exon 19 deletion)	9.1%	✓ Afatinib, Erlotinib, Gefitinib, Osimertinib ~ Neratinib	Yes
TP53 G244D	15.7%	None	Yes
MYC Amplification	Medium (++)	None	Yes
MET R412fs	1.0%	None	No

Variants of Uncertain Significance

RET A692T (11.9%)

The functional consequences and clinical significance of alterations are unknown. Relevance of therapies targeting these alterations is uncertain.

ctDNA Identified in Majority of Advanced-Stage Solid Tumor Cases (n = 9,000)



ctDNA = circulating tumor DNA.

Banks, et al. Poster B410; AACR/NCI/EORTC Annual Meeting, Nov 2015 Boston, MA.

Liquid Biopsy: Sensitivity and Specificity

- 2 meta-analyses were conducted looking at the sensitivity and specificity of *EGFR* detection on liquid biopsy

Study	Sensitivity [95% CI]	Specificity [95% CI]
Luo (2014)	67.4% [51.7–80]	93.5% [88.8–96.3]
Qiu (2015)	62% [51.3–71.6]	95.9% [92.9–97.7]

Luo, J, Shen, Li, & Zheng, Di. *Scientific Reports.* 2014; 4(6269)
Qiu M, et al. *Cancer Epidemiol Biomarkers Prev.* 2015 Jan;24(1): 206-212.

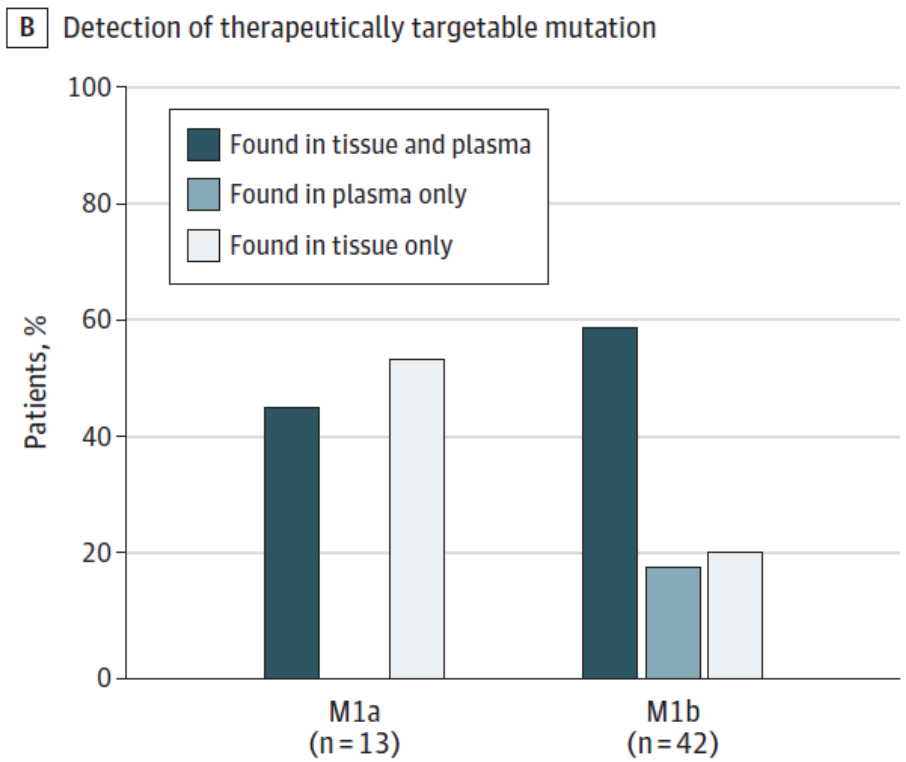
Accuracy of Tissue vs. Liquid Biopsy

- When evaluating for T790M, one study found that sensitivity for detection in plasma was 70% of patients with confirmed tissue diagnosis of T790M
  - In patients with T790M-negative tumors, T790M was detected in plasma of 31% of these patients
- Results
  - Tumors are heterogeneous and results may be missed on tissue biopsy
  - If there is insufficient DNA shedding into the plasma, liquid biopsy may be falsely negative as well.
- **Therefore, in the second-line setting looking for resistance mutations, it is reasonable to start with the least invasive test: liquid biopsy. However, if the results are negative, proceed with biopsy.**

Oxnard GR, et al. *J Clin Oncol.* 2016 Oct 1; 34(28):3375-3382

Tissue and Plasma Testing? Or Both?

- 55 patients who had both blood and tissue testing reported
  - M1a: 13 patients with locally advanced disease in chest
  - M1b: 32 patients with metastatic disease outside of chest
- 85% of patients with plasma only targetable mutations achieved CR/PR or stable disease on the targeted therapy.

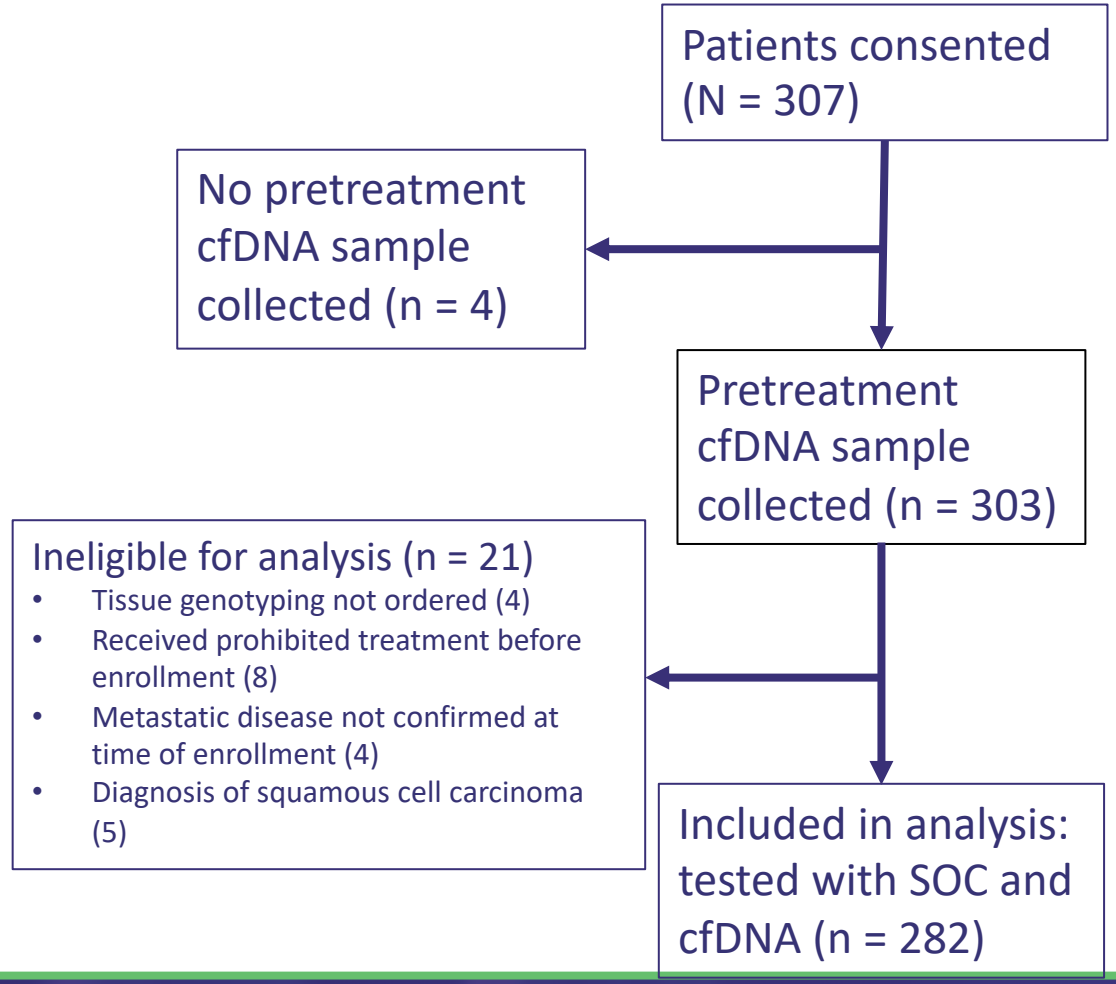


CR = complete response; PR = partial response

Aggarwal C. et al. *JAMA Oncol.* 2019;5(2):173-180. doi:10.1001/jamaoncol.2018.4305

NILE Study: Commercially Available cfDNA Test as Effective as Tissue Testing in Detecting NSCLC Biomarkers

- Clinical practice guidelines recommend genotyping for all patients with newly diagnosed metastatic NSCLC
- Study aimed to demonstrate noninferiority of comprehensive cfDNA vs. SOC tissue genotyping
  - July 2016–April 2018 from 28 North American centers



cfDNA = circulating free DNA; SOC = standard of care

Pirker, R et al. *Clinical Lung Cancer* 2018;20(1):1–6. https://doi.org/10.1016/j.cllc.2018.09.016

NILE Study Results

- Largest cfDNA study in previously untreated mNSCLC finds that cfDNA test identifies guideline-recommended biomarkers
  - At a higher rate
    - Among 282 patients, SOC identified 60 patients (21.3%) and cfDNA identified 77 patients (27.3%)
  - With high tissue concordance (80% cfDNA clinical sensitivity)
    - > 98.2% for FDA-approved targets (EGFR, ALK, ROS1, BRAF)
    - EGFR, ALK, and BRAF had 100% positive predictive value for cfDNA vs. tissue
  - With a higher turnaround time (median turnaround time 9 vs. 15 days; $p < 0.0001$)
  - Using cfDNA in addition to tissue increased detection by 48% (60 to 89 patients)
  - And more completely than tissue-based genotyping (268 vs. 51 patients)

Immuno-Oncology Biomarkers for NSCLC: PD-L1

- PD-L1 expression
  - IHC test, usually comes back in 3 days after ordered on pathology tissue.
  - Often referred to in some clinical trials as TPS% (tumor proportion score) or in other cancers CPS% (combined positive score of tumor + inflammatory cells)
  - Imperfect biomarker; cutoff points varied within clinical trials

Immuno-Oncology Biomarkers for NSCLC: PD-L1 (cont.)

- Pembrolizumab: only drug reliant on TPS PD-L1 score for certain approvals
  - If PD-L1 expression TPS score is ≥ 50% or now ≥ 1%, pembrolizumab is approved in the first-line setting for metastatic NSCLC. In second-line setting approved at ≥ 1%
- Durvalumab
  - In study by Antonia et al. (2018), used TPS of 25% to look at responses
  - Subset analysis of the PD-L1–negative patients did not have a statistically significant benefit in the stage III setting.

Antonia SJ, et al. Overall survival with durvalumab after chemoradiotherapy in stage III NSCLC. NEJM. 2018

Immuno-Oncology Biomarkers: Tumor Mutational Burden

- Tumor mutational burden (TMB): the # of mutations per DNA megabase (Mut/Mb)
  - No clinical guidelines for decision-making at this time
  - Imperfect; difficulty determining what qualifies as “high” TMB
  - The assays used to measure TMB have had varied results

Immuno-Oncology Biomarkers: Tumor Mutational Burden (cont.)

- Phase III trial: CheckMate 227 study of nivolumab + ipilimumab in NSCLC first-line setting chose TMB as biomarker (in addition to PD-L1)
  - Found PFS higher in patients with high TMB (defined as ≥ 10 mutations per megabase) irrespective of PD-L1 expression
  - However, OS data updated in 1/2019 was not different for high or low TMB
- MYSTIC trial looking at durvalumab/tremelimumab
  - Durva/Treme not better than chemo in all-comers
  - More confusion: in the TMB ≥ 20 Mut/Mb durva better than chemo, but minimally (not statistically significant) in the MB 10 ≥ Mut/Mb

Hellman MD, et al. Nivolumab plus ipilimumab in lung cancer with a high tumor mutational burden. *NEJM* 2018;378:2093-2104.
Peters S, et al. Presented at: 2019 AACR Annual Meeting; March 29-April 3, 2019; Atlanta, GA. Abstract CT074.

Clinical Pearls

- Guidelines recommend testing in certain populations
  - You cannot treat a mutation that you never found
- APPs must understand the methodology to explain to patient
  - IHC/FISH and using RNA for finding fusions
  - DNA sequencing reporting somatic mutations
- Interpreting reports
- Site of biopsy, type of mutation
- Liquid biopsy
  - Generally sensitive, less invasive, faster results

Audience Response Question

According to the NCCN Guidelines, oncology providers should be performing molecular testing in which types of patients with NSCLC?

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D. All nonsquamous histologies and some squamous cell carcinoma if they are nonsmokers
E. Unsure

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Which of the following is a true statement about molecular testing in NSCLC?

A. *EGFR* can be found on DNA sequencing and FISH, but not IHC
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C. *NTRK* that is sensitive to treatment is best found on a DNA sequencing panel
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Questions?