In An Era of Personalized Approach to Lung Cancers-Which Type of Specimen Rules Supreme?
Swati Mehrotra, M.D.
Associate Professor
Loyola University Medical Center
Maywood, IL

Objectives
• Review of published literature on diagnosis and molecular triage of lung cancer
• Cytology vs histology
• Our data over a four year period
• Algorithm for triage
• Take home points

Disclosure
• Nothing to disclose

Introduction
What is personalized/ precision medicine?
• Personalized/ Precision medicine is “an emerging approach for disease treatment and prevention that takes into account individual variability in genes, environment, and lifestyle for each person.”
• Precision medicine is redefining the role of pathologist and has become a reality in the field of lung cancer.
Introduction

- Lung cancer is the most commonly diagnosed malignancy in the world
- It is the leading cause of death in both men and women in the United States
- Most patients (>50%) are diagnosed at Stage IV
- The overall 5 year survival rate is 17.4%
- Patients with localized tumors have a 5 year survival rate of 54.8% and those with distant disease having a dismal 4.2% 5 year survival
- Lack of an effective screening modality

Lung cancer - stage at diagnosis

- Localized (16%)
  Confined to Primary Site
- Regional (22%)
  Spread to Regional Lymph Nodes
- Distant (57%)
  Cancer Has Metastasized
- Unknown (5%)
  Unstaged

Since a significant percentage of tumors are diagnosed at an advanced stage cytology/ core biopsy may be the only specimen procured in these patients.

Demands on the procured tissue

- Diagnosis (Smear/ Touch Preparation/ H&E stain)
- Immunohistochemistry
- Molecular studies
- Array of new and evolving tests (latest approval of PD1 inhibitors linked to PDL1 testing)

Diagnosis

First and most important step in the process
IASLC/ATS/ERS multidisciplinary classification provides clear guidelines for subclassification of lung cancers

- NSCLC be further classified into a more specific histologic type, such as adenocarcinoma or squamous cell carcinoma, whenever possible.
- When a diagnosis is made in a small biopsy or cytology specimen in conjunction with special studies, it should be clarified whether the diagnosis was established based on light microscopic slides or whether special stains were required.
- If light microscopic diagnosis is clearly adenocarcinoma or squamous cell carcinoma, use these WHO diagnostic terms.
- Use minimal stains to diagnose NSCLC, favor Adenocarcinoma, or favor Squamous Cell Carcinoma.
- Interpret morphologic and staining patterns to maximize patient eligibility for therapies.
- The term NSCLC-NOS be used as little as possible, and be applied only when a more specific diagnosis is not possible by morphology and/or special stains.

Tissue specimens should be managed not only for diagnosis but also to maximize the amount of tissue available for molecular studies.

Cytology is a useful diagnostic method, especially when correlated with histology.

When paired cytology and biopsy specimens exist, they should be reviewed together to achieve the most specific and nondiscordant diagnoses.

Preserve cell blocks from cytology aspirates or effusions for molecular studies.
Immunohistochemistry for diagnosis of NSCLC

- Immunohistochemistry (IHC) not mandatory for diagnosis; use IHC judiciously

- **Recommended panel:**
  - TTF1+ / p63- : Adenocarcinoma
  - TTF1- / p63+ : Squamous cell carcinoma
  - TTF1+ / p63+ (in different cell population) : Adenosquamous carcinoma
  - TTF1- / p63- : NSCLC nos

Cocktails containing these antibodies (TTF1/NapsinA, P63/CK5/6) could alternatively be used; p40 can be used in place of p63

Molecular classification of lung cancer

- The changing landscape in oncology with availability of targeted therapies for specific mutations and increase in disease-free survival has lead to sub classification of lung cancer at molecular level

Evolving classification of Lung cancer

EGFR Mutations: afatinib, erlotinib, or gefitinib

ALK Fusion (3-6%): Crizotinib, Alectinib, Ceritinib

ALK break-apart FISH

Molecular Testing Guideline for Selection of Lung Cancer Patients for EGFR and ALK Tyrosine Kinase Inhibitors

Expert consensus opinion: Cytologic samples are also suitable for EGFR and ALK testing, with cell blocks being preferred over smear preparations.
EGFR Mutation Testing

- Testing for EGFR should be prioritized over other molecular markers in lung adenocarcinoma.
- Laboratories may use any validated EGFR testing method with sufficient performance characteristics.
- Any routine EGFR assay used in clinical practice should be able to detect the common EGFR TKI sensitizing mutations (exon 19 deletions and L858R) and mutations that confer decreased sensitivity to EGFR TKI (T790M, exon 20 insertions).
- Immunohistochemistry for total EGFR is not recommended for selection of EGFR TKI therapy.
- EGFR mutation test EGFR copy number analysis (i.e., FISH or CISH) is not recommended for selection of EGFR TKI therapy.
- Our reference laboratory uses Sanger sequencing (gold standard) for EGFR mutation detection.

Increase in the number of core biopsies

- Recent years have witnessed a dramatic increase in the number of core biopsies being performed so much so that in organs like liver and breast, FNA has been largely replaced by cores.
- Several years ago core biopsies were rather uncommon in lungs, however with the advent of small gauge needles pathology practices are seeing a rise (exponential) in lung core biopsies as well.

Molecular methods/assays for ALK and ROS1

- FISH is used to detect ALK and ROS1 fusions using dual-labeled break-apart probes.
- ALK and ROS1 IHC, if carefully validated, may be considered as a screening methodology to select specimens for ALK FISH testing.

<table>
<thead>
<tr>
<th>Table 1: Trend of Specimen Type according to Year and Organ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Specimen type</td>
</tr>
<tr>
<td>-------------------</td>
</tr>
<tr>
<td>Liver</td>
</tr>
<tr>
<td>FNA only</td>
</tr>
<tr>
<td>TKI only</td>
</tr>
<tr>
<td>Both</td>
</tr>
<tr>
<td>Lung</td>
</tr>
<tr>
<td>FNA only</td>
</tr>
<tr>
<td>TKI only</td>
</tr>
<tr>
<td>Both</td>
</tr>
<tr>
<td>Kidney</td>
</tr>
<tr>
<td>FNA only</td>
</tr>
<tr>
<td>TKI only</td>
</tr>
<tr>
<td>Both</td>
</tr>
<tr>
<td>Pancreas</td>
</tr>
<tr>
<td>FNA only</td>
</tr>
<tr>
<td>TKI only</td>
</tr>
<tr>
<td>Both</td>
</tr>
<tr>
<td>Total (excluding liver, lung, kidney, and pancreas)</td>
</tr>
<tr>
<td>FNA only</td>
</tr>
<tr>
<td>TKI only</td>
</tr>
<tr>
<td>Both</td>
</tr>
<tr>
<td>Total</td>
</tr>
<tr>
<td>Targeted cores accessed in surgical pathology division</td>
</tr>
</tbody>
</table>

Is core biopsy superior to cytology?

- General belief that core biopsy yields more material and has a higher diagnostic yield
- Traditionally core biopsy has been used for ancillary and molecular testing probably because of greater “confidence” in achieving optimal tissue for workup
- Familiarity with the morphology (architecture) for general surgical pathologist

Core biopsy

<table>
<thead>
<tr>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>• High diagnostic accuracy for malignancy</td>
<td>• Transfer of diagnostic cells onto touch prep - ***</td>
</tr>
<tr>
<td>• Provide preliminary diagnosis</td>
<td>• Sample single area with single pass (FNA samples wider area)</td>
</tr>
<tr>
<td>• Adequate sampling</td>
<td>• Diagnostic clues different from smears</td>
</tr>
<tr>
<td>• Appropriate triage</td>
<td>• Drying artifact for alcohol fixation</td>
</tr>
<tr>
<td></td>
<td>• Crush artifact</td>
</tr>
<tr>
<td></td>
<td>• ? Higher Complication rate</td>
</tr>
</tbody>
</table>

Depletion of core biopsies after touch imprint cytology is a well recognized complication. The largest study to date states “Biopsies from distinct sites might be affected differently by TP and biopsies from lung were the ones most frequently lacking diagnostic cells when a TP was performed.”


Is cytology comparable to core biopsy?

- Several studies in the literature conclude that cytology preparations are better if not as good as core biopsies for diagnosis of lung cancer and ancillary triage
Cytology

**Advantages**
- Ability to provide ROSE
- Pure population of tumor cells with little contaminating stromal cells
- Smears (stained / unstained can be used) - cell transfer techniques
- Alcohol a better fixative (DNA better preserved)
- “Smaller caliber needle” less risk to the patient

**Disadvantages**
- Cell block - variable yield of cellularity
- Non uniform cellularity - section cut for FISH may not have enough tumor cells
- Nuclear truncation artifacts of the cell block may lead to spurious results for FISH assays

Problems which may affect core biopsies and cytology cell blocks both

- Are those associated with formalin fixation
- Formalin can lead to DNA degradation and fragmentation by cross-linking proteins and interacting with DNA directly
- up to 30% of nucleic acids may be lost during fixation
- Heat of melted wax may promote DNA degradation
- De-paraffinization may result in DNA degradation
- Nuclear truncation artifacts during block sectioning may lead to erroneous FISH results

“The evidence is currently insufficient to support a difference between FNAB and CNB in identifying lung malignancies in patients with lung lesions. Compared with FNAB, CNB might have a higher specificity to diagnose specific benign lesions. Well-designed, good-quality studies comparing FNAB with CNB for diagnostic characteristics and yields in diagnosing lung cancer should be encouraged.”


“The results suggest that FNA, CBx, and B are comparable for arriving at a specific diagnosis and having sufficient tissue for molecular studies; they specifically attained the diagnostic and prognostic goals of minimally invasive procedures for lung carcinoma.”

Molecular Testing Guideline for Selection of Lung Cancer Patients for EGFR and ALK Tyrosine Kinase Inhibitors

Guideline from the College of American Pathologists, International Association for the Study of Lung Cancer, and Association for Molecular Pathology

Nadia Li, MD, PhD; Philip S. Eagle, MD; Mary Beth Buyse, MD; Dhananjay Anup Chale, MD; Dina Elasmar, MD, PhD; Gaspare Carenzi, MD, PhD; Robert Alan Jenkins, MD, PhD; Elad Kovalski, MD, PhD; Jain-Scott Abraham, MD; Jeremy Spagnuolo, PhD; John Thompson, MD, PhD; Marc Ludly, MD

Section 6: How Should EGFR Tests Be Performed?

Question 4: How Should Specimens Be Processed for EGFR Mutation Testing?

4.1: Expert consensus opinion: Pathologists should use formalin-fixed, paraffin-embedded (FFPE) specimens or fresh, frozen, or alcohol-fixed specimens for PCR-based EGFR mutation testing. Other tissue samples (e.g., ascites or pleural effusions), or decellularized solutions should be avoided in specimens destined for EGFR testing.

4.2: Expert consensus opinion: Cytologic samples are also suitable for EGFR and ALK testing, with cell blocks being preferred over smear preparations.

Question 5: What Are the Spectrum Requirements for EGFR Testing?

New cytology platforms prompting confidence in obtaining tissue for ancillary studies

- Utilization of direct smears with manual or laser capture microdissection and cell transfer technique (air-dried or alcohol-fixed smears may be used including monolayer preparation)
- Fresh frozen cytology sample – long term stability specially for RNA
- Filter paper storage - FNA material stored on a small piece of Whatman filter paper card at room temperature


Our data

- Retrieved data for 4 year period from August 2011 to August 2015
- 361 cases/specimens of lung cancer were sent for molecular studies

(Does not take into account upfront drop-off due to insufficiency as decided at the time of diagnostic evaluation)
Diagnosis distribution

- Adenocarcinoma
- SCC
- Adenosquamous carcinoma
- NSCLC NOS

Type of specimen diagnosis rendered on
- Surgical
- Cytology
- Both

Diagnosis on paired core biopsy and cytology samples

Distribution of Immunohistochemistry (IHC) performed by part type
- no IHC
- IHC on surgical
- IHC on cytolog
- IHC on both

Distribution of IHC by part type
Sample prioritization for the study of predictive biomarkers in patients with advanced lung adenocarcinoma. Route A is for cases that require immunohistochemistry (IHC), while route B is for cases that are diagnosed based on histological alone.

Proposed algorithm

- FNA
- ROSE
- Touch Imprint
- Satisfactory
- Additional passes for cell block and/or
- Additional cores for ancillary studies
So what does this mean for the practicing pathologist?

- Cytology material is optimal for diagnosis and molecular testing
- Core biopsy should be paired with cytology (preferably signed out by the same pathologist) to conserve tissue
- Commercial laboratories at the present time are not accepting cytology material other than cellblock for molecular analysis
- Folks who have in-house molecular diagnostic facilities have an opportunity to use newer cytology platforms and harvest almost pure tumor population off the smears

How much is enough?

- Unfortunately it is not so easy
- For determining the minimal percentage of tumor cells required for reliable results, the analytic sensitivity of the mutation assay used should be known.
- So for EGFR which our reference lab does by Sanger sequencing, the analytic sensitivity is 15 to 20% which translates to 30 to 40% minimum tumor cells (tumor purity)
- Other variables like amplification and loss of heterozygosity can affect the percentage of mutated slide thereby influencing tumor purity
- Additionally studies show that estimates based on % of tumor cells on H&E stained sections are inaccurate (estimates based on number of nuclei may be more accurate)
- The above is especially true for cell block sections which have variable cellularity
- So if in doubt send for molecular testing (may want to combine core and cell block and unstained slides when available)

Proposed algorithm

Accession both cytology and core to the same pathologist

> 1 block

- “Touch and go” sectioning; cut 2-4 micron ultra thin sections
- Use experienced histotechnologist
- Only 1-2 sections per slide
- Cut unstained slides upfront for IHC
- Preserve tissue for molecular analysis
- Be involved with the Reference laboratory regarding adequacy of tissue for molecular testing

It's not over yet!!

- FDA approved Keytruda (Pembrolizumab, Merck) in Oct 2015 for patients with advanced (metastatic) non-small cell lung cancer (NSCLC) whose disease has progressed after other treatments
  - IHC 22-3 C3 pharmDx test on the Autostainer Link 48 (Dako)
    - Patients with >50% of malignant cells staining showed significantly more response
- FDA also approved Opdivo (Nivolumab, Bristol-Myers Squibb) in Oct 2015 to treat patients with advanced (metastatic) non-small cell lung cancer whose disease progressed during or after platinum-based chemotherapy
  - PD-L1 IHC 28-8 pharmDx test on the Dako detection system
    - Response rates greater when >1% of tumor cells stained, overall 31-52% response rate
Take home points

• “Doing more with less”
• We cytopathologists have to be the “guardian of the tissue”
• We should be part of the multidisciplinary team comprised of surgeons, oncologists, radiologists tasked with procurement and triage of tissue
• Re biopsy - expensive and waste of health care

References
