The Diagnosis of Non-Small Cell Lung Cancer in the Molecular Era

Dr. Carol Farver
Director, Pulmonary Pathology
Pathology and Laboratory Medicine Institute
Cleveland Clinic

Objectives

• Define the current required molecular testing for NSCLC

• Discuss our approach to testing NSCLC to determine cell type

• Review how we triage and optimize the handling of small specimens for diagnosis and therapy

Estimated Cancer Deaths in the US in 2014

<table>
<thead>
<tr>
<th>Site</th>
<th>Men</th>
<th>Women</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lung &amp; bronchus</td>
<td>150,010</td>
<td>279,710</td>
</tr>
<tr>
<td>Prostate</td>
<td>10%</td>
<td></td>
</tr>
<tr>
<td>Colon &amp; rectum</td>
<td>0%</td>
<td></td>
</tr>
<tr>
<td>Pancreas</td>
<td>7%</td>
<td></td>
</tr>
<tr>
<td>Liver &amp; intrahepatic bile duct</td>
<td>5%</td>
<td></td>
</tr>
<tr>
<td>Leukemia</td>
<td>6%</td>
<td></td>
</tr>
<tr>
<td>Bladder</td>
<td>4%</td>
<td></td>
</tr>
<tr>
<td>Uterine corpus</td>
<td>7%</td>
<td></td>
</tr>
<tr>
<td>Ovary</td>
<td>5%</td>
<td></td>
</tr>
<tr>
<td>Lymphoma</td>
<td>3%</td>
<td></td>
</tr>
<tr>
<td>Kidney &amp; renal pelvis</td>
<td>3%</td>
<td></td>
</tr>
<tr>
<td>All other sites</td>
<td>24%</td>
<td></td>
</tr>
</tbody>
</table>

Source: National Cancer Institute: Surveillance, Epidemiology and End Results Program (SEER)
5 Year Relative Survival (%)
SEER Program, 2004 – 2010 | Both Sexes, by Race and Cancer Site

Source: National Cancer Institute: Surveillance, Epidemiology and End Results Program (SEER)

Trends in Five-year Relative Survival (%)*, 1975-2006

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td>All sites</td>
<td>50</td>
<td>54</td>
<td>68</td>
</tr>
<tr>
<td>Breast (female)</td>
<td>75</td>
<td>79</td>
<td>90</td>
</tr>
<tr>
<td>Colon</td>
<td>52</td>
<td>59</td>
<td>66</td>
</tr>
<tr>
<td>Leukemia</td>
<td>36</td>
<td>42</td>
<td>55</td>
</tr>
<tr>
<td><strong>Lung and bronchus</strong></td>
<td>13</td>
<td>13</td>
<td>16</td>
</tr>
<tr>
<td>Melanoma</td>
<td>83</td>
<td>87</td>
<td>93</td>
</tr>
<tr>
<td>Non-Hodgkin lymphoma</td>
<td>48</td>
<td>53</td>
<td>69</td>
</tr>
<tr>
<td>Ovary</td>
<td>37</td>
<td>40</td>
<td>45</td>
</tr>
<tr>
<td>Pancreas</td>
<td>3</td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td>Prostate</td>
<td>69</td>
<td>76</td>
<td>100</td>
</tr>
<tr>
<td>Rectum</td>
<td>49</td>
<td>57</td>
<td>69</td>
</tr>
<tr>
<td>Urinary bladder</td>
<td>74</td>
<td>78</td>
<td>81</td>
</tr>
</tbody>
</table>

*Five-year relative survival rates based on follow up of patients through 2007.
Source: Surveillance, Epidemiology, and End Results Program, 1975-2007, Division of Cancer Control and Prevention.

Lung Cancer

Source: National Cancer Institute: Surveillance, Epidemiology and End Results Program (SEER)
Comprehensive Genomic Mutations in Adenocarcinoma

Evolution of Lung Cancer Classification

SPECIAL ARTICLE

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

Physicians must use EGFR and ALK molecular testing for lung adenocarcinoma patients at the time of diagnosis for patients presenting with advanced stage disease or at progression in patients who originally presented with lower stage disease but were not previously tested.

Strong Recommendation

Physicians must use EGFR molecular testing and ALK testing to select lung adenocarcinoma patients for EGFR and/or ALK targeted therapies, irrespective of clinical characteristics or when adenocarcinoma cannot be excluded.

Strong Recommendation

• Physicians may use EGFR and ALK testing in tumors with histologies other than adenocarcinoma when clinical features indicate a higher probability of an oncogenic drive.
The 2015 WHO Classification for Lung Tumors

- New emphasis on genetic / molecular studies
- Use of immunohistochemistry
- New classification for small biopsies and cytology

Questions to Answer

- Can small biopsies be used to type tumors accurately?
- What antibodies are best to use?
- Does this pathologic screening method find all of the tumors with targetable mutations?

Tumor Type in Biopsy and Resection Specimen

- Paired biopsy and resection specimen
- n=1064
- TTF-1, Napsin A, p63, p40, CK5/6, CK7
- 90% accuracy of biopsy with resection specimen

## Discordance between Biopsy and Resection Specimen

### Morphology

- Additional tissue on resection specimen:
  - revealed morphologic evidence for squamous or glandular differentiation
  - morphologic evidence of pleomorphic carcinoma

### Immunohistochemistry

- Tumor heterogeneity
- Fixation of biopsy vs. resection specimen


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## Questions to Answer

- Can small biopsies be used to type tumors accurately?
- What antibodies are best to use?

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### International Association for the Study of Lung Cancer/American Thoracic Society/European Respiratory Society International Multidisciplinary Classification of Lung Adenocarcinoma

*William D. Travis, MD, Elizabeth Brambilla, MD, Masazumi Negoro, MD, Andrew G. Nicholson, MD, Kim R. Gutierrez, MD, Yasushi Yatabe, MD, David E. Bear, PhD, Charles A. Powell, MD, Gregory J. Reely, MD, Paul E. Van Saar, MD, Karin Gany, MD, John E. M. Banks, MD, Hiroshi Numamoto, MD, Valerie W. Busch, MD, Fred B. Hirsch, MD, Giorgio Scagliotti, MD, Tetsuya Misek, MD, Rahul M. Abbruzzese, MD, Yasics Okulues, MD, James Art, MD, Montserrat Sanchez-Cespedes, PhD, Jean-Paul Sotello, MD, Takashi Takahashi, MD, Maudiero Ziad, MD, Johann Vassiliadis, MD, Ignacio Wainrich, MD, Pan-Cher Tang, MD, Dentic Sheer, MD, Christian Brambilla, MD, Douglas Fletcher, MD, Wilber Francia, MD, Ash Gaidatz, MD, Michael Gould, MD, MS, Philip Haddow, MD, Douglas Henderson, MD, Bruce Johnson, MD, David Johnson, MD, Keith Kerr, MD, Krko Kuri, MD, M. S. Lee, MD, Vincent S. Miller, MD, Jose Pires, MD, PhD, Victor Reig, MD, Rafael Rosell, MD, Nagahiro Saji, MD, Erik Trumppen, MD, Ming Du, MD, and David S. Travis, MD*

One antibody for adenocarcinoma, one antibody for squamous cell carcinoma and one mucin stain.

**Antibodies for Lung Carcinomas**

<table>
<thead>
<tr>
<th>Adenocarcinoma</th>
<th>Squamous cell carcinoma</th>
</tr>
</thead>
<tbody>
<tr>
<td>TTF-1</td>
<td>p63</td>
</tr>
<tr>
<td>Napsin A</td>
<td>p40</td>
</tr>
<tr>
<td>Carcinoembryonic antigen</td>
<td>CK5/6</td>
</tr>
<tr>
<td>CK7</td>
<td></td>
</tr>
<tr>
<td>MOC-31</td>
<td></td>
</tr>
</tbody>
</table>

**Sensitivity versus Specificity**


**Adenocarcinoma antibodies**

- **TTF-1**
  - Sensitive
  - Specific
  - BG7G3/1
  - Non-specific clones
    - SPT24
    - SP141
  - Nuclear antibody
  - Labels type 2 pneumocytes

- **Napsin A**
  - Not as sensitive as TTF-1
  - Monoclonal is more specific
  - Cytoplasmic antibody
  - Labels type 2 pneumocytes and intra-alveolar macrophages

### Squamous cell carcinoma antibodies

**p63**
- Very sensitive
- Less specific
- Nuclear antibody
- Also found in other tumors
  - Sarcomatoid carcinomas
  - Large cell carcinomas
  - High grade NE carcinomas
  - Adenocarcinomas (weakly)
  - Adenosquamous cell carcinomas

**p40**
- Monoclonal is more sensitive
- Very sensitive
- Very specific
- No p40 → Not Squamous cell
- Nuclear antibody


### TTF-1 and p40 IHC in NSCLC

<table>
<thead>
<tr>
<th>TTF-1</th>
<th>p40</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative Predictive Value (NPV)</td>
<td>62-81%</td>
</tr>
<tr>
<td>Positive Predictive Value (PPV)</td>
<td>89-100%</td>
</tr>
</tbody>
</table>


### TTF-1 versus p40 IHC in NSCLC

<table>
<thead>
<tr>
<th>TTF-1</th>
<th>p40</th>
</tr>
</thead>
<tbody>
<tr>
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</tr>
</tbody>
</table>

Rules to Follows

- p40 has a very strong NPV
  - If it is negative, it is NOT a squamous cell carcinoma
- TTF-1 has a strong PPV
  - If it is positive, it is an ADN
- Diffuse and strong p40 expression is SQC
  - If TTF-1 +: think ADN-SQC
- If both are negative, it favors AND based on molecular testing, but (on a small biopsy) should be signed out as NSCLC, not otherwise specified

Questions to Answer

- Can small biopsies be used to type tumors accurately?
- What antibodies are best to use?
- Does this pathologic screening method find all of the tumors with targetable mutations?

Cases with Targetable Mutations
2004 - 2010

- n=336 NSCLC
- 70% kRAS mutations
- 30% EGFR mutations
- 12 Squamous cell carcinomas
  - 5 Squamous cell carcinomas by p40+
  - 2 EGFR mutations
    - 1 targetable mutation
  - 3 kRAS mutations
  - Using current diagnostic criteria, 1 targetable mutation was missed

2016 IASLC / CAP / AMP
Guidelines for the Molecular Testing of Lung Cancer Patients for EGFR and ALK Tyrosine Kinase Inhibitors
Draft Recommendations

Strong Recommendation
Physicians may use EGFR and ALK testing in tumors with histologies other than adenocarcinoma when clinical features indicate a higher probability of an oncogenic driver.

When to Molecular Test Biopsies with Squamous Cell Carcinoma Immunophenotype

- Adenosquamous cell carcinoma
  - genomic profiling shows more association of adenosquamous with adenocarcinoma
  - Small biopsies in non-smokers or light smokers with the diagnosis of squamous cell carcinoma
  - misrepresented sampling of squamous cell
  - some may have targetable mutations

Determining Tumor Type in Small Biopsy Specimens

Non-small cell carcinoma
- NSCLC, NOS
  - TTF-1, p40

Squamous cell carcinoma
- NSCLC
  - TTF-1
  - p40

Adenocarcinoma
- NSCLC
  - TTF-1
  - p40

NSCLC, not otherwise specified (NOS)
- TTF-1
  - p40 + CYTOKERATIN
Tissue for Ancillary Studies

**Immunohistochemistry**
- p40
- TTF-1
- Pancytokeratin

**cDNA and Cells for Molecular Testing**
- EGFR
- EML4-Alk
- ROS-1
- RET

**Tissue for Ancillary Studies**

**Immunohistochemistry**
- Bronchoscopic biopsy
- Cell Block for Bronchoalveolar Lavage Fluid (BALF)
- Needle Core Biopsy
- FNAB
- Cell Block from FNAB

**Bronchoscopic Tissue Biopsy**
Bronchoalveolar Lavage Cell Block

Needle Core Biopsy

Fine Needle Aspiration Biopsy
Residual Tissue
Fine Needle Cell Block

Tissue for Ancillary Studies

**Immunohistochemistry**
- p40
- TTF-1
- Pancytokeratin

**cDNA and Cells for Molecular Testing**
- EGFR
- EML4-Alk
- ROS-1
- RET

Detection of molecular abnormalities

**DNA insertion/deletion**
- EGFR ~20%
- KRAS ~25%

**Oncogenic Fusion**
- EML4-ALK ~5%
- ROS ~2%
- RET ~1%
- Breakapart FISH probes

All of which must be done on the cell block, needle biopsy, residual cell pellet
Next Generation Sequencing

**EGFR**

- Next generation sequencing platforms can rapidly sequence DNA from small samples of tumor and provide “hotspot” testing to specific molecular targets (EGFR, KRAS, MET, etc).
- DNA extracted from FFPET (biopsies, resections, cell block).
- Cytology specimens (cell free DNA, residual cell pellet, sacrificed smears).

**DNA Extraction**

- DNA extraction may be performed on the cell pellet, the cell block or the supernatant, which contains cell free cDNA.
- Cell block involves cutting of sections, melting and cleansing of paraffin.

Oncogenic fusion testing

**EML4-ALK, ROS-1, RET**

- Currently, there are limited technologies that can detect DNA fusions.
- Cytogenetics
- FISH
- Newer sequencing assays are appearing.
DNA extraction may be performed on the cell pellet, the cell block or the supernatant, which contains cell free cDNA. Cell block involves cutting of sections, melting and cleansing of paraffin.

**FNAB ThinPrep Slides**

FISH for *EML4-ALK*
Tissue for Ancillary Studies

**Immunohistochemistry**
- Bronchoscopic biopsy
- Cell Block for BALF
- Needle Core Biopsy
- FNAB
- Cell Block from FNAB

**cDNA and Cells for Molecular Testing**
- Bronchial biopsy
  - cDNA for EGFR (NGS)
- Needle core biopsy
  - cDNA for EGFR (NGS)
- Cell pellet supernatant
  - cDNA for EGFR (NGS)
- Cell block
  - Alk-D5F3 IHC
  - FISH for EML4-ALK
- ThinPrep
  - FISH for EML4-ALK

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**Cleveland Clinic Molecular Testing Algorithm**

**EGFR**
- Mutational analysis (Next Gen Sequence)

**Cytopathology**
- Cell Pellet/Supernatant
  - Thin prep FISH

**Surgical pathology**
- Specimens (if + for tumor)
  - EML4-ALK

**DNA isolation**
- Insufficient
- Report as insufficient

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**Developmental Phases of Available Drugs Against Oncogenic Proteins**

<table>
<thead>
<tr>
<th>Mutation</th>
<th>Status</th>
<th>Drugs</th>
</tr>
</thead>
<tbody>
<tr>
<td>EGFR</td>
<td>Approved</td>
<td>Gefitinib, Erlotinib, Afatinib, Osimertinib, Olmutinib</td>
</tr>
<tr>
<td>ALK</td>
<td>Approved</td>
<td>Crizotinib, Alectinib, Ceritinib</td>
</tr>
<tr>
<td>ROS1</td>
<td>Approved</td>
<td>Crizotinib</td>
</tr>
<tr>
<td>MET</td>
<td>Phase 2</td>
<td>Crizotinib, Cabozantinib, INC280, MGCD265</td>
</tr>
<tr>
<td>HER2</td>
<td>Phase 2</td>
<td>Trastuzumab, Aratinib, Dacomitinib</td>
</tr>
<tr>
<td>BRAF</td>
<td>Phase 2</td>
<td>Vemurafenib, Debrafenib</td>
</tr>
<tr>
<td>RET</td>
<td>Phase 2</td>
<td>Cabozantinib, Alectinib, Apatinib</td>
</tr>
<tr>
<td>NTRK1</td>
<td>Phase 2</td>
<td>Entretinib, LOX-10, Cabozantinib</td>
</tr>
<tr>
<td>PTK2661</td>
<td>Phase 3</td>
<td>UX007149, PQR309</td>
</tr>
<tr>
<td>MAP2K1</td>
<td>Phase 3</td>
<td>Selumetinib</td>
</tr>
</tbody>
</table>
*PD-L1*

PD-L1 is expressed on Cytotoxic T cells and can kill cells via cell mediated death.

In cancer, PD-L1 is expressed on tumor cells and immune cells and it may help in evasion of the immune response.

Targeted chemotherapy drugs (atezolizumab) may inhibit PD-L1 and enable activation of cytotoxic T-cells.

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**PD-L1 Antibodies**

<table>
<thead>
<tr>
<th>PD-L1 Antibody</th>
<th>26.4</th>
<th>2403</th>
<th>PF-L1</th>
<th>SF383</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drug</td>
<td>Nivolumab (BMS)</td>
<td>Pembrolizumab (Merck)</td>
<td>Atezolizumab (Roche)</td>
<td>Durvalumab (AstraZeneca)</td>
</tr>
<tr>
<td>FDA Class II diagnostic partner</td>
<td>Dako</td>
<td>Dako</td>
<td>Ventana</td>
<td>Ventana</td>
</tr>
<tr>
<td>Scoring method for PD-L1 IHC</td>
<td>% tumor cells</td>
<td>% tumor cells</td>
<td>% tumor cells or immune cells</td>
<td>% tumor cells</td>
</tr>
<tr>
<td>FDA/PD Status</td>
<td>Complementary (RSO, RSO, melanoma)</td>
<td>All</td>
<td>Comparison (NOSC)</td>
<td>All</td>
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<tr>
<td>PD-L1 thresholds under evaluation</td>
<td>12%, 15%, or 20%</td>
<td>12%, 25%</td>
<td>TC12/23 (11%, 15%, 20%)</td>
<td>IC12/23 (11%, 15%, 20%)</td>
</tr>
</tbody>
</table>

Rimn D. et al. IASLC, Chicago September 2016

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**PD-L1**

**Lung Cancer Grading**

- Less than 1% (<1%)
- 1% to 50% (≥1%≤50%)
- More than 50% (>50%)
Summary

• Targeted therapies for lung cancer therapy have proven effective and continue to evolve.

• Immunohistochemical studies to define the cell type are essential.

• Tissue utilization for molecular testing on small biopsies must be optimized using biopsy and cytology specimens.