Role of Routine Histology and Special Testing in Managing Patients with Non-Small Cell Lung Carcinoma

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Role of Pathology in Managing Patients with NSCLC

• apply the results of lung biopsies to triaging patients with advanced stage nonsmall cell lung carcinoma to appropriate treatment strategies,
• apply the results of special studies to classification of nonsmall lung carcinomas, and
• integrate the results of molecular testing to managing patients with adenocarcinoma of the lung.

Classification of Lung Carcinoma
Important Clinical Inflection Points

- non-small cell carcinoma
  - squamous cell carcinoma
  - adenocarcinoma
  - large cell carcinoma

- squamous cell carcinoma
- adenocarcinoma
- large cell carcinoma
- small cell carcinoma

- small cell carcinoma
Historically, histologic subtype has not reliably been shown to have prognostic importance in advanced NSLC."


Squamous Cell Carcinoma

General Features

- cigarette smoking
- 25% of incident cases
- men > women (~ 3:1)
- central > peripheral

Squamous Cell Carcinoma

Definition

malignant epithelial tumor showing,

keratinization &/or intercellular bridges
Classification of Lung Carcinoma

Important Clinical Inflection Points

- non-small cell carcinoma
- squamous cell carcinoma
- adenocarcinoma
- large cell carcinoma
- adenocarcinoma

Adenocarcinoma

Definition

Malignant epithelial tumor with glandular differentiation and/or mucin production

... showing acinar, or solid with mucin growth patterns

Adenocarcinomas with a mixture of histologic patterns are the most frequent subtype, representing ≥ 90% of resected adenocarcinomas.

bronchioloalveolar, papillary,
Non-Small Cell Lung Carcinoma - Myers

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

William D. Travis, MD, Elisabeth Brambilla, MD, Masaaki Noguchi, MD, Andrew G. Nicholson, MD, Kim R. Goette, MD, Tomoko Sato, MD, David G. Berry, PhD, Charles A. Powell, MD, Gregory J. Risch, MD, Paul E. Tom Schell, MD, Kevin Goo, MD, John H. M. Austin, MD, Hideo Joaguma, MD, Valerie W. Rusch, MD, Fred R. Hirsch, MD, Giorgio Scagliotti, MD, Terunori Miyawatani, MD, Roderick M. Phillips, MD, Yuichi Ikeda, MD, James Ait, MD, Montserrat Sanchez-Cespedes, PhD, Jean-Paul Sculier, MD, Takeshi Takashita, MD, Massimiliano Tarallo, MD, Akio Yamazawa, MD, Spourlos Wintrobe, MD, Pin-Chyi Yang, MD, Denise Boeckx, MD, Christian Brambilla, MD, Douglas Flieder, MD, Wilber Franklin, MD, Ali Gamsar, MD, Michael Gould, MD, NS, Philip Hadjistavropoulos, MD, Douglas Henderson, MD, Bruce Johnson, MD, David Johnson, MD, Keith Kerr, MD, Koji Kuriyama, MD, Jin Seo Lee, MD, Vincent A. Miller, MD, Iver Pettersen, MD, PhD, Victor Ruggieri, MD, Rafael Rosell, MD, Nagakira Sajo, MD, Erik Thomsen, MD, Ming Tsao, MD, and David Yankenson, MD

Travis et al. J Thorac Oncol 2011; 6: 244-85

Lung Adenocarcinoma
IASLC/ATS/ERS Consensus Classification

Preinvasive lesions
atypical adenomatous hyperplasia
adenocarcinoma in situ, non-mucinous and/or mucinous

Minimally invasive adenocarcinoma, non-mucinous and/or mucinous

Invasive adenocarcinoma
lepidic predominant
acinar predominant
papillary predominant
micropapillary predominant
solid predominant

BAC

Bronchioloalveolar Carcinoma
1999/2004 WHO Definition

“Growth of neoplastic cells along pre-existing alveolar structures without evidence of stromal, vascular, or pleural invasion. Non-mucinous, mucinous, and mixed cell subtypes are recognized”
Bronchioloalveolar Carcinoma
2004 WHO Definition

non-mucinous subtype
“A non-mucinous adenocarcinoma with Clara cells and/or type II pneumocytes growing along alveolar walls without stromal invasion.”
Non-Small Cell Lung Carcinoma

Bronchioloalveolar Carcinoma
2004 WHO Definition

**non-mucinous subtype**

“A non-mucinous adenocarcinoma with Clara cells and/or type II pneumocytes growing along alveolar walls without stromal invasion.”

**mucinous subtype**

“Tall columnar cells, with varying amounts of cytoplasmic mucin, which typically displace the nucleus to the base of the cell, growing along alveolar walls and without stromal invasion.”
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Pathology Recommendation 1

“We recommend discontinuing the use of the term ‘BAC.’ Strong recommendation, low-quality evidence.”

†Travis et al. J Thorac Oncol 2011; 6: 244-85
**Lung Adenocarcinoma**  
IASLC/ATS/ERS Consensus Classification

**Pathology Recommendation 2**

“For small (≤ 3 cm), solitary adenocarcinomas with pure lepidic growth, we recommend the term ‘Adenocarcinoma in situ’ that defines patients who should have 100% disease-specific survival if the lesion is completely resected (strong recommendation, moderate quality evidence).”

†Travis et al. J Thorac Oncol 2011; 6: 244-85

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**Significance of Invasion in Small (< 2.0 cms) Adenocarcinomas of the Lung**


**Grade 0** – pure BAC growth pattern

**Grade 1** – stromal invasion in area of BAC growth

**Grade 2** – invasion localized to periphery of fibrotic focus

**Grade 3** – invasion in center of fibrotic focus
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Significance of Invasion in Small (< 2.0 cms) Adenocarcinomas of the Lung

Survival (Year)

Histologic Prognostic Indicators in Early Stage Adenocarcinoma of the Lung
Yim et al. Mod Pathol 2007; 20: 233-41

n = 141 consecutive surgical resections with curative intent
AJCC stage I and II

<table>
<thead>
<tr>
<th>Group</th>
<th>BAC</th>
<th>invasive adca</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>100%</td>
<td>none</td>
</tr>
<tr>
<td>Group II</td>
<td>present ≤ 5 mm</td>
<td></td>
</tr>
<tr>
<td>Group IV</td>
<td>none</td>
<td>100%</td>
</tr>
</tbody>
</table>

Histologic Prognostic Indicators in Early Stage Adenocarcinoma of the Lung
Yim et al. Mod Pathol 2007; 20: 233-41

no deaths in Groups I & II

Dead of Disease

<table>
<thead>
<tr>
<th>Group</th>
<th>No Deaths</th>
<th>Deaths (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I (8)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Group II (21)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Group III (46)</td>
<td>9 (20%)</td>
<td>12 (18%)</td>
</tr>
<tr>
<td>Group IV (66)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Among lung adenocarcinomas, histologic assessment of invasive growth may provide valuable prognostic information, and tumors with invasion < 6 mm have a more indolent course after resection.

The presence of a small (≤ 5 mm) focus of invasive carcinoma does not change the survival advantage in low stage (≤ 3 cm) BAC. ("minimally invasive adca")

For small (≤ 3 cm), solitary adenocarcinomas with predominant lepidic growth and small foci of invasion measuring ≤0.5 cm, we recommend a new concept of Minimally invasive adenocarcinoma to define patients who should have near 100% disease-specific survival if completely resected (strong recommendation, low quality evidence).

†Travis et al. J Thorac Oncol 2011; 6: 244-85
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**Bronchioloalveolar Carcinoma (BAC) Invasion?**

“distinction between sclerosing BAC and early invasive adenocarcinoma may be difficult.”

2004 WHO

How would you diagnose (be specific) this 0.8 cm solitary nodule?

Would you classify this as “in-situ”, “minimally invasive”, or “invasive”? Test Case (N = 1)
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Non-Small Cell Lung Carcinoma - Myers

LUNG CARCINOMA SURVEY (N = 1)
Results (15 respondents)

<table>
<thead>
<tr>
<th>AIS</th>
<th>MIA</th>
<th>invasive adeno</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>3</td>
<td>9</td>
</tr>
</tbody>
</table>

“Differential diagnosis - adenocarcinoma in situ (nonmucinous) (previous term was BAC). However, it looks less bland than I would like and the cells are crowded . . . I think I can see some micropapillary areas, (which don’t exclude AIS), but would ask a trusted colleagues opinion.”

Assessing Invasion in Adenocarcinomas
Rates of Agreement Among Experts

Photomicrographs – 2 per case
“typical invasion” (n = 20)
“no invasion” (n = 24)
“problem cases” (n = 24)

Scored by 28 international experts
(including authors of 2012 IASLC/ATS/ERS classification)
1 – definite invasion
2 – probably invasion
3 – undetermined
4 – probable no invasion
5 – no invasion

†Thunnissen et al. Mod Pathol 2012; 25: 1574.

Assessing Invasion in Adenocarcinomas
Rates of Agreement Among Experts

Kappa Statistics

<table>
<thead>
<tr>
<th># of Categories</th>
<th>5</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>&quot;Easy&quot;</td>
<td>0.55</td>
<td>0.55</td>
</tr>
<tr>
<td>&quot;Hard&quot;</td>
<td>0.08</td>
<td>0.15</td>
</tr>
</tbody>
</table>

†Thunnissen et al. Mod Pathol 2012; 25: 1574.
The presence of a small (≤ 5 mm) focus of invasive carcinoma does not change the survival advantage in low stage (≤ 3 cm) BAC . . .

. . . but it can be really hard for even expert reviewers to agree on presence or absence – much less the size – of invasion!

“There is strong evidence that histologic subtype is predictive of treatment efficacy and/or toxicity.”
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Pathology Recommendation 9†

“For small biopsies and cytology, we recommend that NSCLC be further classified into a more specific histologic type, such as adenocarcinoma or squamous cell carcinoma, whenever possible (strong recommendation, moderate quality evidence).”

†Travis et al. J Thorac Oncol 2011; 6: 244-85

SqiCC vs Adca on Small Specimens
A Practical Approach for 2014

typically stained slides

SCLC

SqiCC

Adca

PD

NSCLC

Squamous vs Non-Squamous Cell Carcinomas
Role of Special Studies

Percent Positive Staining

<table>
<thead>
<tr>
<th></th>
<th>SqCC</th>
<th>Adca</th>
</tr>
</thead>
<tbody>
<tr>
<td>CK5/6</td>
<td>≈73%</td>
<td>≈30%</td>
</tr>
<tr>
<td>p63</td>
<td>92 - 100%</td>
<td>18 - 66%</td>
</tr>
<tr>
<td>TTF-1</td>
<td>4 - 8%</td>
<td>65 - 92%</td>
</tr>
</tbody>
</table>
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**SqCC vs Adca on Small Specimens**

A Practical Approach for 2014

- Routinely stained slides
- CK5/6
- P63/p40
- NSCLC

**SqCC vs Adca on Small Specimens**

A Practical Approach for 2014

- Routinely stained slides
- CK5/6
- P63
- TTF1

- Poorly diff adenocarcinoma

- CK5/6

- P63

- TTF1
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**34βE12 vs CK5/6**

- CK5/6 + P63 + TTF1 = SqCC
- CK5/6 – P63 ± TTF1 = Adca
- IHC ambiguous = PD NSCLC

**RTK vs Adca on Small Specimens**

- SCLC
- SqCC
- Adca
- PD NSCLC

**A Practical Approach for 2014**

- Routinely stained slides

**Non-Small Cell Lung Carcinoma**

- CK5/6 + napsin + TTF-1 = poorly differentiated adenocarcinoma

**SCLC vs Adca**

- CK5/6 + P63 + TTF1 = SqCC
- CK5/6 – P63 ± TTF1 = Adca
- IHC ambiguous = PD NSCLC
“It is unknown whether there is any added value provided by refining NSCLC-NOS via immunohistochemistry on small biopsies or cytology samples. This requires assessment in future trials using systemic therapy.”

†Travis et al. J Thorac Oncol 2011; 6: 244-85

††Travis et al. J Thorac Oncol 2011; 6: 85

EGFR Mutations in Lung Cancer: Correlation with Clinical Response to Gefitinib Therapy

EGF receptor gene mutations are common in lung cancers from ‘never smokers’ and are associated with sensitivity to gefitinib and erlotinib.

JNCI 2005; 97: 643
Science 2004; 304: 1497
PNAS 2004; 101: 13306

Lung Adenocarcinoma
Frequency of Mutations For Targeted Therapy!

419 patients (1JUL09 – 1AUG10)
- mean age 61 (24-95)
- never smokers 23%
- adenoc 87%
- adenosq ca 3%
- sq cell ca 2%
- LCC 1%
- NSCLC, NOS 33%

Patient's lung cancer should be tested for EGFR mutation/ALK fusion gene before targeted therapy is given.

EGFR Mutation Testing

- Mutation analysis recommended for EGFR
  - FISH and traditional IHC not recommended
  - IHC using antibodies to specific EGFR mutations under investigation

EML4-ALK – inv(2)(p21p23)

Multiple EML4 Fusion Partners

Schematic diagram of EML4 and ALK genes, as well as representative fusion variants listed by EML4 exon breakpoints. Multiple breakpoints within a given exon have been reported, for instance exons 2a and 2b, and 6a and 6b (14,15).

Encoded EML4 domains
- CC=coiled coil
- HELP=hydrophobic echinoderm microtubule-associated protein-like domain
- WD=WD repeats

Encoded ALK domains
- TM=transmembrane
- Kinase=intracellular tyrosine kinase
- Exon 20 breakpoint (dashed line).
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### EML4-ALK Positive Adenocarcinoma
#### Younger, Never Smokers

|                | Consecutive Surgical Resection | Referred to Oncology
<table>
<thead>
<tr>
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<tbody>
<tr>
<td></td>
<td>BWH (116)</td>
<td>UP (111)</td>
</tr>
<tr>
<td>ALK rearranged (%)</td>
<td>1 (0.9%)</td>
<td>0</td>
</tr>
<tr>
<td>Women (%)</td>
<td>63%</td>
<td>57%</td>
</tr>
<tr>
<td>Age (median)</td>
<td>67</td>
<td>70</td>
</tr>
<tr>
<td>Never smoker (%)</td>
<td>12%</td>
<td>17%</td>
</tr>
</tbody>
</table>

*p value

BWH (116) UP (111) MGH (131)


### EML4-ALK Positive Adenocarcinoma
#### Higher stage

- ALK-POS
  - LN NEG (%): 20%
  - Stage I - II: 52%
  - Stage III - IV: 85%

- ALK-NEG
  - LN NEG (%): 15%
  - Stage I - II: 58%
  - Stage III - IV: 43%


### Case Presentation

- 59-year-old woman, never smoker
- progressive shortness of breath and cough x 5 months
- 10 lb weight loss
- bilateral crackles on auscultation
- hypoxemic on room air (89%); worse with exercise (82%)

**Chest CT:** miliary bilateral nodules, right middle lobe and left lower lobe consolidation, right lower lobe infiltrate, and bilateral septal thickening
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TBBx: Well differentiated adenocarcinoma with predominantly bronchioloalveolar growth pattern.

CK7, TTF-1 positive

10/26/2011 erlotinib

ALK Rearrangement Testing by FISH at UM
Vysis ALK Break Apart FISH Probe Kit (FDA-approved)

ALK Rearrangement

Negative

ALK Rearrangement

Split Probes
ALK Rearrangement Positive

ALK Testing
CAP-IASLC-AMP Lung Cancer Biomarkers Guideline Committee†

- Best assessed by molecular cytogenetic techniques such as FISH
- Due to many variants and other translocations, PCR is not recommended
- ALK immunohistochemistry, if carefully validated, may be considered as a screening methodology to select specimens for ALK FISH testing

†Lindeman et al. Arch Pathol Lab Med 2013; 137: 828-60

ALK Testing
Immunohistochemistry as Screening Tool†

IHC

Negative 0  Equivocal 1+ or 2+  Positive 3+

ALK NEGATIVE  FISH  ALK POSITIVE

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**Non-Small Cell Lung Carcinoma - Myers**

**EML4-ALK Positive NSCLC**

Crizotinib Resistance

“two secondary mutations within the kinase domain of EML4-ALK in tumor cells isolated from a patient during the relapse phase of treatment with [crizotinib].”

“Each mutation developed independently in subclones of the tumor and conferred marked resistance to two different ALK inhibitors.”


**Squamous Cell Carcinoma**

Targeted Therapy?

“we are sort of where we were four or five years ago with adenocarcinoma.”

Dr. Bruce Evan Johnson, Dana-Farber
Role of Pathology in Managing Patients with NSCLC

• apply the results of lung biopsies to triaging patients with advanced stage nonsmall cell lung carcinoma to appropriate treatment strategies,
• apply the results of special studies to classification of nonsmall lung carcinomas, and
• integrate the results of molecular testing to managing patients with adenocarcinoma of the lung.