Diagnostic & Predictive Immunohistochemistry in Lung Carcinomas

Lynette M. Sholl, M.D.
Associate Pathologist, Brigham and Women’s Hospital
Associate Professor, Harvard Medical School
Boston, MA
Disclosures

• Consultant for Foghorn Therapeutics, AstraZeneca, LOXO Oncology
Objectives

- Identify best practices for use of immunohistochemistry in the diagnosis of lung carcinomas
- Recognize pitfalls of commonly used IHC markers in the diagnosis of lung cancers
- Examine the appropriate use and interpretation of predictive IHC markers for selection of patients for targeted therapies
Small cell carcinoma

Very aggressive/poor prognosis
Largely confined to heavy smokers
Exquisitely chemo/radiotherapy sensitive
Rapidly relapses

Adenocarcinoma
“Better” prognosis
Most common subtype in nonsmokers
Surgery for early stage disease
Unique chemosensitivity profile
~60% have a defined oncogenic driver
“Targetable”

Squamous cell carcinoma
“Better” prognosis
Smokers
Surgery for early stage disease
Use of antiangiogenic agents associated with massive pulmonary hemorrhage
Minority with defined oncogenic driver
Limited targetability

Historically lumped together as “non small cell carcinoma”

Standardized pathologic criteria required to drive selection of patients for targeted therapies.
Challenges in classification

• ~70% of lung cancers are diagnosed and staged using small biopsies/cytology
  • Limited tumor cellularity often precludes confident assessment of morphology
  • Using morphology alone, up to 30% of small biopsy/cytology specimens cannot be classified beyond NSCLC, not otherwise specified

• Integrated use of IHC is critical to accurate diagnosis of small specimens
  • Indeterminate classification rate drops to <5%
  • BUT... judicious use of IHC is of paramount importance to conserve tissue for molecular studies

IASLC algorithm for Small Biopsies:

- Morphology
- Immunohistochemistry
- Molecular
P63 vs TTF1 in normal lung

• P63 expressed in airway basal cells, metaplastic epithelium; absent in pneumocyte population
• TTF-1 expressed in terminal airway respiratory epithelial cells and pneumocytes
Extensive (>50%) p63 expression is highly sensitive and specific for lung SCC. <50% expression is not specific for SCC.

<table>
<thead>
<tr>
<th>Tumor</th>
<th>Sox2</th>
<th>P63</th>
<th>TTF-1</th>
<th>CK5/6</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>≤1+</td>
<td>2+</td>
<td>3+</td>
<td>4+</td>
</tr>
<tr>
<td>ACA</td>
<td>34</td>
<td>23</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>SCC</td>
<td>32</td>
<td>2</td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>

ACA indicates adenocarcinoma; SCC, squamous cell carcinoma; TTF-1, thyroid transcription factor-1; ≤1+, fewer than 5% of tumor cells staining; 2+, 5% to 25% of tumor cells staining; 3+, 26% to 50% of tumor cells staining; 4+, >50% of tumor cells staining.

P63 and CK5/6 as markers for Squamous Carcinoma vs. Lung Adenocarcinoma and Neuroendocrine Carcinomas:

<table>
<thead>
<tr>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SCC vs.</td>
</tr>
<tr>
<td>Extensive positivity</td>
<td>ACA</td>
</tr>
<tr>
<td>p63</td>
<td>97</td>
</tr>
<tr>
<td>CK5/6</td>
<td>93</td>
</tr>
<tr>
<td>Any positivity</td>
<td>p63</td>
</tr>
<tr>
<td></td>
<td>97</td>
</tr>
</tbody>
</table>

## Subclassification of morphologic NSCLC-NOS

<table>
<thead>
<tr>
<th>Scenario</th>
<th>TTF1</th>
<th>P63</th>
<th>Diagnosis</th>
<th>Frequency</th>
<th>Next steps?</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>+ to +++</td>
<td>-</td>
<td>NSCLC, favor ACA</td>
<td>~90% of ACA 0% of SQC</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>+++</td>
<td>+ to +++ (in same cells)</td>
<td>NSCLC, favor ACA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>-</td>
<td>+++</td>
<td>NSCLC, favor SQC*</td>
<td>&gt;95% of SQC 0% of ACA</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>+++</td>
<td>+++ (in different cells)</td>
<td>NSCLC, NOS, possible adenosquamous carcinoma</td>
<td>Rare (~1% of NSCLC)</td>
<td>Formal classification deferred to resection</td>
</tr>
<tr>
<td>5</td>
<td>-</td>
<td>-</td>
<td>NSCLC, NOS*</td>
<td>~10% of ACA 0% of SQC</td>
<td>Mucin stain, napsin</td>
</tr>
<tr>
<td>6</td>
<td>-</td>
<td>+</td>
<td>NSCLC, NOS*</td>
<td>1% ACA 1% SQC</td>
<td>Mucin stain, CK5/6</td>
</tr>
</tbody>
</table>

* Exclude metastatic disease.
p40: A p63 isoform with improved specificity for lung squamous cell carcinoma

Table 2: Sensitivity and specificity of p63 vs p40 for squamous cell carcinoma

<table>
<thead>
<tr>
<th></th>
<th>Unquantified reactivity</th>
<th>&gt;5% Reactivity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sensitivity (%)</td>
<td>Specificity (%)</td>
</tr>
<tr>
<td>p63</td>
<td>100</td>
<td>60</td>
</tr>
<tr>
<td>p40</td>
<td>100</td>
<td>98</td>
</tr>
</tbody>
</table>

p63 shows nonspecific staining of DLBCL

Recommendations for subclassification of morphologic NSCLC-NOS

- p40 (ΔNp63) is a more specific isoform of p63 and is preferred but should be present in ≥50% of tumor cells to make a diagnosis of SCC
- Immunoreactivity for fewer than 10% of cells (even with p40) should not be considered diagnostic of SCC
- 10-50% p40+ cells should be interpreted based on intensity and diagnostic context

Best Practices Recommendations for Diagnostic Immunohistochemistry in Lung Cancer
Yatabe Y and IASLC Pathology Committee
What about adenosquamous carcinoma?

TTF-1 and p40 should highlight DISTINCT populations of tumor cells. TTF-1 + p40 (or p63) coexpression ≠ adenosquamous carcinoma.
A few words about lung adenocarcinoma markers
TTF-1: clone matters

• 8G7G3/1: 77% sensitive, 100% specific for lung adenocarcinoma
• SPT24: 81% sensitive for lung adenocarcinoma BUT also stains 6% of lung squamous carcinomas
• SP141: newer clone with less data, but appears similar to SPT24
### Beware!

**TTF-1 expressed by non-lung adenocarcinomas**

<table>
<thead>
<tr>
<th>Cancer Type</th>
<th>8G7G3/1</th>
<th>SPT24</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n=</td>
<td>positive (%)</td>
</tr>
<tr>
<td><strong>Ovarian carcinoma</strong></td>
<td>615</td>
<td>22 (3.6%)</td>
</tr>
<tr>
<td><strong>Endometrial adenocarcinoma</strong></td>
<td>215</td>
<td>17 (7.9%)</td>
</tr>
<tr>
<td><strong>Uterine cervical adenocarcinoma</strong></td>
<td>92</td>
<td>3 (3.3%)</td>
</tr>
<tr>
<td><strong>Uterine cervical squamous carcinoma</strong></td>
<td>7</td>
<td>0 (0%)</td>
</tr>
<tr>
<td><strong>Breast adenocarcinoma</strong></td>
<td>297</td>
<td>4 (1.5%)</td>
</tr>
<tr>
<td><strong>Colon adenocarcinoma</strong></td>
<td>594</td>
<td>11 (1.8%)</td>
</tr>
<tr>
<td><strong>Gastric adenocarcinoma</strong></td>
<td>170</td>
<td>3 (1.8%)</td>
</tr>
</tbody>
</table>

Aberrant TTF-1 expression in 5% of colon carcinomas when using clones SPT24 and SP141.

Napsin A: a protease whose expression is regulated by TTF-1

- Monoclonal Napsin A is preferred to polyclonal
- Polyclonal Napsin A has only 70% specificity with significant expression in 30% of non-lung primary tumors (especially GI)
- Primary lung tumors may rarely be TTF-1 negative/Napsin A positive
- Napsin A expressed in pulmonary macrophages

Napsin A/TTF-1 double stain

Napsin A is not recommended as a first line stain– similar performance characteristics as TTF-1 but may be more difficult to interpret.

A good quality double stain may optimize your sensitivity for lung adenocarcinoma.

Napsin A expression in LCNEC may indicate biology more similar to NSCLC (as opposed to SCLC).

Other “Second-line” markers

- **CK5/6**: diffuse positivity favors squamous cell carcinoma, but specificity is poor
- **CK7**: More common in adenocarcinoma but lung squamous cell carcinoma is often positive too
- **Mucicarmine**: If you can discern mucinous differentiation then it is an adeno- may be most helpful in solid subtype tumors
  - on resection specimen, mucin staining is used to discriminate between adenocarcinoma and large cell carcinoma.
Non small cell carcinoma, NOS, TTF-1-/P40-

Consistent radiology findings
Smoking history
Past lung cancer history

NSCLC, NOS

If advanced, reflex to PD-L1, ALK, ROS1 staining

Review clinical history

Ambiguous radiographic findings
Never smoker
Known non-lung cancer history

Exclude metastases

Broad spectrum keratin or CK7/CK20

Positive

GATA3

Breast (SOX10)
Urothelial
Lung SCC>ACA (weak)

CDX2

GI
Pancreas (SMAD4)
Enteric lung ACA

PAX8

RCC
Thyroid
Thymoma
Mullerian tract
Lung NET

Negative

SOX10, S100
rule out melanoma

LCA
rule out lymphoma

Neuroendocrine issues

• NSCLC with neuroendocrine differentiation is not really a “thing”
  • NSCLC morphology with some incidental chromogranin, synaptophysin, or CD56 expression does not define a unique clinical entity
  • Neuroendocrine IHC is discouraged unless morphologic features of NE differentiation are seen

• NSCLC with NE morphology and IHC confirmation = large cell neuroendocrine carcinoma
  • Requires positivity with at least one of chromo., synapto. or CD56
Small cell lung carcinoma

- Is principally a morphologic diagnosis.
- Clinical correlation is helpful—if patient is never smoker, think twice.
- Consider ddx:
  - basaloid squamous cell carcinoma
  - lymphoma
  - metastatic melanoma
  - rhabdomyosarcoma, Ewing sarcoma
  - Carcinoid (esp small crushed bx)
SCLC: Beware aberrant transcription factor expression!

Diagnosis: Diffuse large B cell lymphoma?
Pulmonary DLBCL? Nope.
SCLC: some annoying realities

#1: Neuroendocrine markers typically weak, focal, in contrast to carcinoids
SCLC: some annoying realities

#2: 10-15% of SCLC are TTF1 negative (and non-pulmonary small cells may be TTF1+)

Iida et al. Hum Pathol 79:127-134. 2018
#3: The Small Cell- Large Cell Neuroendocrine carcinoma distinction is hard. Don’t let presence of cytoplasm lead you astray when features are otherwise c/w SCLC.
IASLC classification of small biopsies, take home points:

- Distinguish ACA and SQC whenever possible
- The molecular profile of an ACA will dictate targeted therapy
- Rational and prudent use of IHC is critical to conserve tissue for molecular profiling
  - Two first-line markers:
    - TTF1 and p63 or p40
    - Less established/less specific markers (napsin, mucin, CK7, CK5/6) should be considered second line
Predictive IHC for lung cancers
## Essential Biomarkers for Metastatic NSCLC Patients

<table>
<thead>
<tr>
<th>TARGET</th>
<th>EGFR</th>
<th>ALK</th>
<th>ROS1</th>
<th>BRAF V600E</th>
<th>PD-L1</th>
</tr>
</thead>
<tbody>
<tr>
<td>TREATMENT</td>
<td>EGFR TKIs</td>
<td>Crizotinib, alectinib, other ALK inhibitors</td>
<td>Crizotinib</td>
<td>Dabrafenib &amp; trametinib</td>
<td>Immunotherapy</td>
</tr>
<tr>
<td>IHC?</td>
<td>EGFR mutation-specific IHC</td>
<td>ALK IHC</td>
<td>ROS1 IHC</td>
<td>BRAF V600E (VE1) IHC</td>
<td>PD-L1 IHC</td>
</tr>
</tbody>
</table>
EGFR mutation-specific IHC

- Applies only to the two major mutational hotspots
  - L858R has excellent sensitivity and specificity
  - Ex19del has excellent specificity, low sensitivity due to variability of mutation

- Some institutions use these stains reflexively for specimens too small for molecular testing

- Nice ancillary/confirmatory tool for confirming unexpected results

We et al. Mod Pathol. 2013 Sep;26(9):1197-203
Immunohistochemistry (IHC) is an equivalent alternative to FISH for ALK testing.

- D5F3 and 5A4 antibodies only
- Pooled estimates of IHC performance relative to FISH:
  - 97% sensitivity
  - 99% specificity

ALK (D5F3) Companion diagnostic assay approved for crizotinib and alectinib ALK therapy

ALK IHC pitfall!

Combined adenocarcinoma-small cell carcinoma

ALK IHC- false positive?

Glandular component
ALK IHC in SCLC

• This case was negative for an ALK rearrangement by both FISH and NGS testing
• Case reports and series report ALK expression in SCLC that cannot be confirmed by FISH or molecular methods
• Frequency unclear- up to 5% of SCLC with aberrant ALK expression
• Mechanism of expression is unclear, but has been reported in other contexts as well, including Ewing Sarcoma
ROS1 IHC should be used as a screening test, with molecular/FISH confirmation of positive results.
ROS1 IHC: Performance compared to cytogenetic/molecular assays

Pooled estimates: 96% sensitivity, 93.5% specificity.

ROS1 IHC pitfalls

• Low level endogenous ROS1 expression seen in some lung tumors lacking ROS1 rearrangements

• Reactive pneumocytes can show moderate to strong expression

• False positive ROS1 results are usually weak and patchy (1+) in most studies.
BRAF V600E IHC testing in lung cancer

<table>
<thead>
<tr>
<th>Study</th>
<th>Antibody</th>
<th>n</th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Routhier et al. <em>Hum Path</em> 2013</td>
<td>VE-1, anti-B-Raf</td>
<td>25</td>
<td>100</td>
<td>85-90</td>
</tr>
<tr>
<td>Ilie et al. <em>Ann Oncol</em> 2013</td>
<td>VE-1</td>
<td>250</td>
<td>90</td>
<td>100</td>
</tr>
<tr>
<td>Sasaki et al. <em>Lung Cancer</em> 2013</td>
<td>VE-1</td>
<td>26</td>
<td>100</td>
<td>95</td>
</tr>
</tbody>
</table>

Too little data to support a recommendation for use of BRAF V600E mutation-specific IHC in lung cancer.
And, finally, PD-L1
# IHC assays applied in clinical trials

<table>
<thead>
<tr>
<th>Drug</th>
<th>mAb/Platform</th>
<th>Scoring criteria</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pembrolizumab (Keytruda)</td>
<td>22C3 (pharmDx, Agilent)/Link 48 Autostainer</td>
<td>≥50% tumor cells for 1st line, ≥1% tumor cells for ≥2nd line†</td>
<td>Companion diagnostic SP263 assay can be used in some countries</td>
</tr>
<tr>
<td>Nivolumab (Opdivo)</td>
<td>28-8 (pharmDx, Agilent)/Link 48 Autostainer</td>
<td>≥1% tumor cells†</td>
<td>Complementary diagnostic only in non-squamous NSCLC 22C3 assay can be used in Japan</td>
</tr>
<tr>
<td>Atezolizumab (Tecentriq)</td>
<td>SP142/BenchMark ULTRA Autostainer</td>
<td>Tumor cells and/or tumor infiltrating immune cells‡</td>
<td>Complementary diagnostic</td>
</tr>
<tr>
<td>Durvalumab (Imfinzi)</td>
<td>SP263/BenchMark ULTRA Autostainer</td>
<td>≥ 25% tumor cells††</td>
<td></td>
</tr>
<tr>
<td>Avelumab (Bavencio)</td>
<td>73-10 (Agilent)</td>
<td>≥80% tumor cells†</td>
<td></td>
</tr>
</tbody>
</table>

† membranous staining, †† membranous and/or cytoplasmic staining
‡ IHC3 (tumor cell [TC]3 or immune cell [IC]3): PD-L1 expression in >50% of tumor cells or >10% of immune cells, IHC 2/3 (TC2/3 or IC2/3): PD-L1 expression in >5% of tumor cells or immune cells, IHC1/2/3 (TC1/2/3 or IC1/2/3): PD-L1 expression in ≥1% of tumor cells or immune cells, IHC0 (TC0 and IC0), PD-L1 expression in <1% of tumor cells and <1% of immune cells
PD-L1 Immunohistochemistry: Real World Practice

- IASLC Pathology Committee international survey of PD-L1 staining practices (September, 2018)
- SP263 is predominant commercial assay used in clinical practice
- >25% use E1L3N lab-developed test in clinical practice

PD-L1 IHC assays used in 25 academic institutions

<table>
<thead>
<tr>
<th>Clone</th>
<th>Commercial assay</th>
<th>LDT</th>
</tr>
</thead>
<tbody>
<tr>
<td>22C3</td>
<td>8 (32%)</td>
<td>10 (40%)</td>
</tr>
<tr>
<td>28-8</td>
<td>5 (20%)</td>
<td>3 (12%)</td>
</tr>
<tr>
<td>SP142</td>
<td>7 (28%)</td>
<td>5 (20%)</td>
</tr>
<tr>
<td>SP263</td>
<td>14 (56%)</td>
<td>3 (12%)</td>
</tr>
<tr>
<td>73-10</td>
<td>0</td>
<td>1 (4%)</td>
</tr>
<tr>
<td>E1L3N</td>
<td>0</td>
<td>7 (28%)</td>
</tr>
</tbody>
</table>

Mari Mino-Kenudson, Massachusetts General Hospital
PD-L1 expression heterogeneity

Same patient, two contemporaneous samples:

Endobronchial FNA: 90% TPS
Supraclavicular LN excision: 40% TPS

Only 62-78% concordance between paired primary/metastatic site biopsies?

Technical and Interpretative Issues with PD-L1 Immunohistochemistry

Although there is good global correlation between several approved PD-L1 IHC platforms and sample types (Blueprint studies)....

“10% to 20% of cases will be classified differently as above or below clinical cutoffs, depending on the assay used” - Hendry et al. JTO Sept 2018

• There is significant interobserver deviation around a “gold standard” score
• Individual pathologists change their assessment 8-10% of the time around the 1 and 50% cutpoints
• And training doesn’t do much to help (concordance at 50% cutpoint 75.3 → 78.7% following training) – Cooper et al. Clin Cancer Res 2017

The tumor-immune interaction is complex and patient selection cannot be reduced to a single binary biomarker.
Wrap up

• For NSCLC, if morphologic features are diagnostic of ACA or SCC, no further workup required
• Only TWO first line diagnostic IHC markers when evaluating a case of suspected NSCLC:
  • TTF-1 - clone 8G7G3/1
  • P40 preferred over p63
• Always check the clinical history- especially when working up NSCLC, NOS
• Interpret SCLC biopsies within the clinical context and be aware of potential mimics
• Predictive IHC for ALK, ROS1, PD-L1 is widely accepted/in use
• Stay tuned for more on BRAF V600E IHC