Cool Stains for Hot Diagnoses in Thoracic Pathology

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Disclosure

• No disclosures

Learning Objectives

• Know about recently introduced immunostains that are useful in the differential diagnosis of thoracic malignancies.

• Be aware of the importance of PD-L1 immunostain in non-small cell lung carcinoma to identify patients that might benefit from anti-PD-1/anti-PD-L1 immunotherapy.
Learning Objectives

- Learn indications for the use of histochemical stains in non-neoplastic lung diseases.

Outline

- Introduction to thoracic pathology
- Case presentations featuring immunohistochemical and histochemical stains of particular interest
- Pearls for each stain/section
- Take home points

Thoracic Pathology

Anterior Mediastinum

SVC

Thymus

Tracheal

BCV

Rt Lung

Lt Lung

Pleura

Heart
Thoracic Pathology

Mediastinal Compartments

- Prevascular (anterior)
  - Thymus
  - Fat
  - Lymph nodes
  - Lt brachiocephalic vein

- Visceral (middle)
  - Trachea
  - Esophagus
  - Lymph nodes
  - Heart, Aorta, SVC
  - Intrapericardial PAs
  - Thoracic duct

- Paravertebral (posterior)
  - Paravertebral soft tissues

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Thoracic Pathology

- Lung cancer
- Metastases
- Other lung tumors
- Pleural neoplasms
- Mediastinal neoplasms
- Non-neoplastic lung disease

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Lung Pathology

- Neoplastic
  - Metastasis
  - Carcinoma
  - Small Cell Carcinoma
- Non-Neoplastic
  - Sarcoma
  - Carcinoid
  - Lymphoma
  - Non-Small Cell Carcinoma
  - Lymphoma
Lung Cancer

- Lung cancer = most common cancer worldwide
  - 1.8 mio new cases (2012)
- Lung cancer = most common cause of death from cancer worldwide
  - 1.6 mio deaths (2012)
- Mortality to incidence ratio overall 0.87

IARC, WHO, Cancer Today. Fact sheets 2012

Lung Cancer Incidence (per 100,000)

IARC, WHO, Cancer Today. Fact sheets 2012

US Lung Cancer Statistics

Year of Diagnosis / Death
http://seer.cancer.gov
US Lung Cancer Mortality

<table>
<thead>
<tr>
<th>Year of Death</th>
<th>Death per 100,000</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Men</td>
</tr>
</tbody>
</table>

Primary Lung Carcinoma (SEER)

- Non-small cell carcinoma
- Small cell carcinoma (13%)
- Adenocarcinoma (44%)
- Squamous cell carcinoma (23%)
- Other (18%)
- Large cell carcinoma (2%)

Case

- 58 yo female
- Smoker
- Slight cough
  - Imaging studies - spiculated 1.8 cm nodule right middle lobe lung
  - Right middle lobectomy

Adenocarcinoma
Gland formation and/or mucin production

Case Follow Up
- 3 years later adenocarcinoma right lower lobe lung
- 2 years later metastases to breast and bone

Adenocarcinoma
- Gland formation
- Mucin production
- Lung: Pneumocyte marker expression (70% of lung adenocarcinoma)
Primary Lung Adenocarcinoma

Napsin

TTF 1

TTF-1 Clones in Lung AdenoCa

<table>
<thead>
<tr>
<th>Clones</th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>8G7G3/1</td>
<td>79.3*</td>
<td>98.0*</td>
</tr>
<tr>
<td>SPT24</td>
<td>84.1*</td>
<td>86.8*</td>
</tr>
</tbody>
</table>

SP141 appears similar to SPT24

Smits, AJJ et al. 2015. Appl Immunohistochem Mol Morphol. 23:416-23


PD Adenocarcinoma
Pearls – TTF-1

• Expressed in most lung adenocarcinoma
• Multiple clones with different sensitivities and specificities
• Not specific to lung (thyroid; rarely also other sites)
Case

- 70 yo male, smoker, persistent cough
- 2.3 cm cavitary nodule right lower lobe lung
- Additional pulmonary nodules
- Wedge resection
Case Follow Up

- Multiple lung nodules – squamous cell carcinomas and lung adenocarcinomas
- Some resected, some radiated
- Doing well 3 years after diagnosis

Squamous Cell Carcinoma

- Usually central location → lobar or entire lung collapse
- Arise from bronchial epithelium
- > 90% occur in cigarette smokers
- Arsenic - strongly associated with SQCC
- Most frequent tumor type to cavitate
Squamous cell carcinoma

Keratinization and/or Intercellular bridges

Squamous Cell Carcinoma - IHC

<table>
<thead>
<tr>
<th>Marker</th>
<th>Sensitivity %</th>
<th>Specificity %</th>
<th>Other Tumors</th>
</tr>
</thead>
<tbody>
<tr>
<td>CK5/6</td>
<td>47-100</td>
<td>44-92</td>
<td>Mesothelioma, Thymoma</td>
</tr>
<tr>
<td>p63</td>
<td>78-100</td>
<td>35-100</td>
<td>AdenoCa, Small cell Ca, Small cell Ca</td>
</tr>
<tr>
<td>TTF-1</td>
<td>-</td>
<td>-</td>
<td>Lung adenoCa, Thyroid Ca</td>
</tr>
<tr>
<td>DSG-3</td>
<td>98 (lung)</td>
<td>99 (lung)</td>
<td>Pancreas / Colon, AdenoCa</td>
</tr>
<tr>
<td>p40</td>
<td>100</td>
<td>98-100</td>
<td>AdenoCa (3%)</td>
</tr>
</tbody>
</table>

**Pearls – Squamous Cell Carcinoma**

- Keratin pearls
- p40, DSG3, CK5/6 indicate squamous differentiation
- No marker is entirely specific
- No squamous marker helps to distinguish between lung origin or metastasis

**SQCC vs Other NSCLC**

- SQCC **not eligible for molecular marker** testing and respective treatment
- Bevacizumab (anti-VEGF antibody) + platin-based chemotherapy (chemo) in advanced NSCLC
  - → ↑ Overall survival
  - → ↑ Time to progression
- Pulmonary hemorrhage (few SQCC pat.)
Small Biopsies in Lung Cancer

- 70% of lung cancers - advanced at diagnosis
- Treatment other than surgery
- Small biopsy might be the only specimen available for diagnosis

Case

- 89 yo male, longtime smoker
- 4.6 cm centrally located mass left upper lobe lung
- Needle core biopsy
- No surgical candidate
Squamous Cell Carcinoma

Case

- 74 yo woman, lifetime non-smoker
- 3.6 cm peripheral mass right lower lobe lung
- Found during follow up of breast carcinoma
- No surgical candidate
Lung Adenocarcinoma

Role of Personalized Medicine in Lung Cancer

- 70% of lung cancers - advanced at diagnosis
  → Treatment other than surgery needed
- 1st line therapy (chemo, radiation) might fail
- Advances in molecular studies
- Advances in tumor biology
  → Targeted therapies

Targeted Therapy in Advanced Lung Cancer

Develop agents that:
• Target specific molecular pathways in malignant cells ("driver mutations")
• Preferentially kill malignant cells
• Relatively innocuous to benign cells

Molecular Targets & Pathways in NSCLC

<table>
<thead>
<tr>
<th>EGFR</th>
<th>ALK</th>
<th>ROS1</th>
<th>Her2</th>
<th>MET</th>
<th>RET</th>
<th>FGFR</th>
</tr>
</thead>
</table>

RAS → PI3K → RAF → Akt → PTEN → mTOR

Proliferation → Resistance to apoptosis → Invasion/Metastasis → Angiogenesis

Lung Adenocarcinoma
Molecular Abnormalities in Lung Adenocarcinoma

- KRAS 30%
- EGFR 15-20%
- Other/Unknown >30%
- ROS 1-2%
- HER 2 2%
- BRAF 1-5%
- MET 1-7%
- ALK 5%
- RET 1-2%

ALK Rearrangement

- EML4-ALK fusion oncogene → upregulation
  → ALK protein expression (IHC)
- 2.6-6.7% of NSCLC
- Adenocarcinomas, solid, signet ring cell
- Light or never smokers
- Younger patients

Case

- 53-yo Asian female
- 2.7cm nodule RUL
- Wedge resection
Patient Follow Up

- Visceral pleural invasion
- Positive lymph nodes
  → Stage IIIA → Lobectomy not indicated
- Chemotherapy and radiation
  → Radiation-induced pneumonitis
- 3-years later – bone and possibly brain mets
- Crizotinib → interval response of bone met

Patient Follow Up

- 5 months later – progression of bone disease, new pulmonary mets
- Radiation and continuation of crizotinib
### ALK Testing – Multiple Clones

- IHC compared to gold standard (FISH, RT-PCR)

<table>
<thead>
<tr>
<th>Clone</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1A4</td>
<td>100</td>
<td>70.3 - 99.1</td>
</tr>
<tr>
<td>D5F3</td>
<td>91.4 - 95</td>
<td>99.5 - 100</td>
</tr>
</tbody>
</table>

Gruber K et al. 2015. JTO 10:713-6.  

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### PD-L1 - An Emerging Biomarker in NSCLC

**PD-L1 in Immune System**

**PD-L1 in NSCLC**

- **PD1 - PD-L1 interaction**
  - → inhibition of T cells → Tumor growth
- **Anti-PD1 or anti-PD-L1 drugs**
  - → block PD1-PD-L1 interaction
  - → boost host anti-tumor immune response
  - → Inhibit tumor growth
- **Unselected NSCLC – response rate ≈ 20%**
- **Second or higher line therapy**

References:
- Bang YJ et al. J Clin Oncol 33. 2015 (Suppl. Abstract 4001)
- Kerr KM et al. 2016 Arch Pathol Lab Med. Epub

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**Importance of PD-L1 Inhibition**

- **Responses in lung adenoCa and SQCC**
- **PD-1/PD-L1 blockade → durable responses in other met. tumors (melanoma, urinary bladder, kidney, prostate, breast, colon, head / neck, germinal tumors, lymphomas)**

References:
- Bang YJ et al. J Clin Oncol 33. 2015 (Suppl. Abstract 4001)
- Kerr KM et al. 2016 Arch Pathol Lab Med. Epub

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**Pembrolizumab versus Chemotherapy for PD-L1–Positive Non–Small-Cell Lung Cancer**

Martin Reck, M.D., Ph.D., Delays Rodriguez-Albreu, M.D., Andrew G. Robinson, M.D., Rina Hui, M.B., B.S., Ph.D., Tibor Csészti, M.D., Andrea Fàllop, M.D., Maya Gottfried, M.D., Niri Peled, M.D., Ph.D., Ali Tafreshi, M.D., Sinead Cuffe, M.D., Mary O’Brien, M.D., Suman Raik, M.D., Katsuaki Hotta, M.D., Ph.D., Melanie A. Leiby, Ph.D., Gregory M. Lubiniecki, M.D., Yue Shentu, Ph.D., Reshma Rangavala, M.D., Ph.D., and Julie R. Brahmer, M.D., for the KEYNOTE-010 Investigators

Reference:
Pembrolizumab – anti-PD-1
- 305 patients with untreated high stage IV NSCLC
- PD-L1 expression \( \geq 50\% \) tumor cells (clone 22C3)
- Randomized to pembrolizumab or chemotherapy

**Anti-PD-1 vs Chemotherapy**

Rech M et al. NEJM 2016.

<table>
<thead>
<tr>
<th></th>
<th>Anti-PD-1</th>
<th>Chemo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Progression-free Survival</td>
<td>10.3 mos</td>
<td>6.0 mos</td>
</tr>
<tr>
<td>Overall Survival</td>
<td>p=0.005</td>
<td></td>
</tr>
</tbody>
</table>

Rech M et al. NEJM 2016.
Challenges of PD-L1-Testing

- What predicts response to PD-1/PD-L1 inhibitors:
  - PD-L1 expression on tumor cells, TILs or both?
- Multiple anti-PD1, anti-PD-L1 drugs approved for clinical trials or treatment
- Drugs tested in conjunction with different anti-PD-L1 clones by IHC

**PD-1/PD-L1 Inhibitors - NSCLC**

<table>
<thead>
<tr>
<th>Drug</th>
<th>FDA Approval</th>
<th>Clone/Platform</th>
<th>% +Tumor cells</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pembrolizumab</td>
<td>High stage NSCLC</td>
<td>22C3 DAKO</td>
<td>≥50</td>
<td>Companion</td>
</tr>
<tr>
<td>Nivolumab</td>
<td>≥ 2nd line NSCLC</td>
<td>28-8 DAKO</td>
<td>≥1</td>
<td>Complementary</td>
</tr>
<tr>
<td>Atezolizumab</td>
<td>≥ 2nd line UCC</td>
<td>SP142 Ventana</td>
<td>≥ 5 IC</td>
<td>Complementary</td>
</tr>
<tr>
<td>Durvalumab</td>
<td>In development</td>
<td>SP263 Ventana</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**PD-L1 - Poor Concordance**

- E1L3N
- SP263
- SP142
PD-L1 IHC Blueprint Study
- 39 NSCLC
- % PD-L1 + tumor cells
- Comparable for clones 22C3, 28-8, SP263
- Lower for clone SP142
- 37% of patients would have been treated differently dependent on clone used
- More variability for immune cell staining

H&E
22C3 28-8
SP142 SP263

Heterogeneity of PD-L1 Expression
- Within a single tumor (maybe focal or patchy)
  - Sampling bias in biopsies?
- Between independent primary NSCLC
  (agreement, 52.2%)
- High level of agreement between intrapulmonary metastases (88.9%)

PD-L1 – Heterogeneous Expression

• Does a lab need multiple platforms and clones to fulfill all clinical requirements?
• Which PD-L1 expression threshold is useful?
• Report PD-L1 expression on immune cells?
  → Currently no standard

**PD-L1 IHC**

**Our Current Approach**

- FDA-approved clone SP263
- Membranous staining counts
- Report of % positive tumor cells (vs negative)
- Report whether immune cells are positive or negative
- Require 100 tumor cells present

PD-L1 IHC

> 90% tumor cells express PD-L1
Be aware: Macrophages

Immune cells positive for PD-L1
**Pearls – PD-L1 Testing**

- Multiple clones available
- Staining not concordant between clones
- Staining might be heterogeneous → Sampling
- Clones require different staining platforms
- Reporting not standardized
Case History

- 36 yo man, non-smoker → ED
- Dry, harsh, non-productive cough since 10 days
- Cold symptoms
- Intermittent headaches
- Fever (101-103 °F) for 3 days
  → Antibiotics
Case History

- No improvement
- Developed chest tightness, wheezing

6 cm
2 weeks later

10 cm

Mediastinal Mass FNA-Biopsy
Differential Diagnosis
- Undifferentiated Carcinoma
- Lymphoma
- Germ cell tumor
- Ewing sarcoma
- Malignant mesothelioma
- Metastasis

Negative Immunostains
CD20, CD30, CD31, CD34, CD43, CD99, TTF-1, S-100, OSCAR keratin, desmin, OCT4, HMB45
Diagnosis

**NUT Carcinoma**

= Carcinoma with t(15;19)

Case Follow-up

- Chemoradiation therapy
- Restaging revealed progression
- Enrolled in phase I clinical trial with bromodomain inhibitor
- Worsening respiratory symptoms, pleural effusions
- Died 4 months after diagnosis
Case Follow-up

Autopsy:
- Necrotic bulky tumor extending to mediastinum, pleura, hilum, bilateral lungs, trachea, thoracic aorta, diaphragm
- Compression and invasion of airways and vasculature

NUT Carcinoma

- Described in early 1990s
- Rare
- Location:
  - Midline predominance (90%)
  - Most common: Thorax (57%), head & neck


NUT Ca – Clinical Presentation

- Rapid tumor growth
- Pleuritic chest pain, non-productive cough, SOB, weight loss (thoracic tumor)
- No sex predominance
- Age, median 16-50yrs (range, 0.1 – 78)

17 yo woman
9.2 cm mass
obliterating rt
mainstem
bronchus
Diffuse mets

Undifferentiated
morphology

Abrupt squamous
differentiation
NUT Ca – Cytogenetics/Molecular
Rearrangement and translocation of \textit{BRD4-NUT} - t(15;19)(q14;p13.1) (70%)

- FISH
- RT-PCR
- Karyotype

Because of \texttt{PPV} of IHC – confirmatory FISH, PCR, cytogenetics are not necessary

1. Dey A et al. PNAS. 2003; 100:8758-63.

Pearls - NUT Carcinoma

- Aggressive
- Midline
- Undifferentiated carcinoma with abrupt squamous differentiation
- NUT immunostain: speckled nuclear stain
- t(15;19)
- FISH or RT-PCR
Case

- 52-year old male presented with chest pain and fever
- Imaging: patchy bilateral GGO
Non-necrotizing Granuloma in Wall of Small Vessels

Polarizable

Not Polarizable
Microcrystalline cellulose

Talc

Movat Pentachrome

Diagnosis
Non-necrotizing granulomas with foreign body material c/w i.v. drug abuse
<table>
<thead>
<tr>
<th>Color</th>
<th>Tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td>Black</td>
<td>Nuclei; elastic fibers</td>
</tr>
<tr>
<td>Yellow</td>
<td>Collagen fibers, reticular fibers</td>
</tr>
<tr>
<td>Blue</td>
<td>Ground substance; mucin</td>
</tr>
<tr>
<td>Bright red</td>
<td>Fibrin</td>
</tr>
<tr>
<td>Red</td>
<td>Muscle</td>
</tr>
</tbody>
</table>

![Bronchiole](image1)

![Pulmonary artery](image2)

![Bronchiole](image3)

![Pulmonary artery](image4)
Pearls – Movat Pentachrome

- Highlights elastic tissue, collagen and reticulin fibers, mucin, fibrin and muscle – all in once
- Useful for CV, interstitial lung disease
- Spectacular in iv drug abuse
Case

- 77-yo male with progressive dyspnea on exertion and slight left anterior chest wall pain
- Asbestos exposure while Cutting siding with a saw at age 17
  Worked in ship industry later
- Former smoker
Case

- 1 year later – worsening dyspnea, left pleural effusion
- Patient died
- Autopsy was performed
Diagnosis

Malignant Mesothelioma, Sarcomatoid Type

Malignant Mesothelioma

- Rare - 17 / 1 Mio / year in the US
- Tumor of mesothelial surfaces (pleura, peritoneum, pericardium)
- 70% attributed to asbestos exposure
- Only few people with asbestos exposure develop disease
- Average latency between exposure and disease – 30-40 years
Malignant Mesothelioma

- Most patients > 60 yo
- Male to female 4:1
- Histologic subtypes:
  - Epithelioid
  - Biphasic
  - Sarcomatoid
Sarcomatoid

Malignant Mesothelioma
- Poor prognosis
- Average median survival 9-12 months after diagnosis
- Median survival
  - 19 months – epithlioid subtype
  - 13 months – biphasic
  - 8 months – sarcomatoid

Malignant Mesothelioma
- Treatment:
  - Extrapleural pneumonectomy
  - Pleural decortication
  - Chemotherapy, radiation
- Distinction from metastatic carcinoma
### Frequently Used Stains

<table>
<thead>
<tr>
<th>Mesothelial</th>
<th>Lung</th>
<th>Colon</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calretinin</td>
<td>TTF1</td>
<td>CK20</td>
</tr>
<tr>
<td>WT1</td>
<td>CK7</td>
<td>CDX2</td>
</tr>
<tr>
<td>D2-40</td>
<td>pCEA</td>
<td></td>
</tr>
<tr>
<td>CK5/6</td>
<td>MOC31</td>
<td></td>
</tr>
<tr>
<td>CK7</td>
<td>No marker is perfect!</td>
<td></td>
</tr>
</tbody>
</table>

### H&E

- MOC31
- pCEA
- WT1
- Calretinin
- CK5/6

### Malignant Mesothelioma

**Distinction from reactive mesothelial proliferation**

- **Morphology:**
  - Invasion into adipose tissue, skeletal muscle and/or lung
  - Tumefactive growth
- Loss of BAP1 expression (IHC)
- Homozygous deletion of CDKN2A
Invasion into fat

Case

- 75 yo male
- History of colon carcinoma 2 years prior – resected
- Presents now another colon carcinoma → right hemicolectomy.
- Small nodules on the surface of the small bowel were sampled

No invasion
Keratin

WT-1+, Calretinin+, CK5/6+
pCEA-, MOC31-

Diagnosis

• Atypical mesothelial proliferation
Loss of BAP1 expression in tumor cells

Case

- Malignant mesothelioma, epithelioid type
- Died 4 years later of widespread malignant mesothelioma

Pearls – Malignant Mesothelioma

- Rare but aggressive disease
- Usually asbestos-related
- Distinction from metastases (IHC)
- Distinction from reactive process (BAP1, FISH for CDKN2A homozygous deletion)
Take Home Message

No Stain is Perfect

But

Stains Work Great as a Team