Lung Cytology and Histology: Getting What You Need from Small Samples

Dr. Jennifer Brainard
Section Head Cytopathology
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

Advances in Lung Cancer Diagnosis

• Advances in bronchoscopic techniques
  – Endobronchial ultrasound-guided (EBUS) FNA and electromagnetic navigation bronchoscopy (ENB) FNA
  – Rapid on site evaluation (ROSE)
• Advances in cytologic diagnosis and therapy
  – Use of immunostains to distinguish types of lung carcinoma
  – Molecular diagnostics in non-small cell lung carcinoma
Lung Carcinoma

- A leading cause of cancer mortality
- 87% of lung cancer deaths related to tobacco use
- Non-small cell lung cancer (NSCLC) represents 85% of all primary pulmonary malignancies
- Majority of patients (>75%) present with regional or distant metastases
- Nodal stage is most important determinant of resectability and prognosis

Estimated Cancer Deaths in the US in 2014

<table>
<thead>
<tr>
<th>Cancer Site</th>
<th>Men</th>
<th>Women</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lung &amp; bronchus</td>
<td>26%</td>
<td>15%</td>
</tr>
<tr>
<td>Prostate</td>
<td>13%</td>
<td></td>
</tr>
<tr>
<td>Colon-rectum</td>
<td>9%</td>
<td></td>
</tr>
<tr>
<td>Pancreas</td>
<td>7%</td>
<td></td>
</tr>
<tr>
<td>Liver &amp; intrahepatic</td>
<td>5%</td>
<td></td>
</tr>
<tr>
<td>Larynx</td>
<td>4%</td>
<td></td>
</tr>
<tr>
<td>Esophagus</td>
<td>4%</td>
<td></td>
</tr>
<tr>
<td>Urinary bladder</td>
<td>3%</td>
<td></td>
</tr>
<tr>
<td>Non-Hodgkin</td>
<td>3%</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 (%)

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

- Accurate diagnosis and staging is critical
- Surgery is most appropriate for patients with disease confined to lung and hilar LN
- Tissue confirmation of suspected mediastinal LN metastasis is recommended
- Mediastinoscopy is current gold standard
- EBUS-FNA is an accurate and less invasive staging method
Lung Carcinoma Staging

• Clinical staging often differs from pathologic staging
• Both “over” and “under” staging are possible
• Staging modalities include:
  – CT
  – PET
  – Mediastinal sampling

Advanced Bronchoscopy at Cleveland Clinic

• Performed by interventional pulmonologists
• Centralized procedures
• Patients under general anesthesia
• ROSE (rapid on-site evaluation) in all cases
• 6-7 total passes if positive for non-small cell carcinoma
• Multiple passes (usually 3) with lymphoid tissue in benign lymph nodes
Diagnostic Bronchoscopy 2017

<table>
<thead>
<tr>
<th>Sampling Method</th>
<th># Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>EBUS-TBNA</td>
<td>1108</td>
</tr>
<tr>
<td>EMN-NA</td>
<td>180</td>
</tr>
<tr>
<td>Conventional TBNA</td>
<td>426</td>
</tr>
<tr>
<td>Brushings</td>
<td>156</td>
</tr>
<tr>
<td>TBBx</td>
<td>1383</td>
</tr>
<tr>
<td>EBBx</td>
<td>292</td>
</tr>
<tr>
<td>Total</td>
<td>1675</td>
</tr>
</tbody>
</table>

Primary lung cancer diagnosis/staging: 616
Adequacy rate: 96%

EBUS Staging

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Average number of nodes</td>
<td>6.85</td>
</tr>
<tr>
<td>evaluated per case</td>
<td></td>
</tr>
<tr>
<td>Average number of nodes sampled</td>
<td>3.45</td>
</tr>
<tr>
<td>per case</td>
<td></td>
</tr>
<tr>
<td>Average size of nodes sampled</td>
<td>8.45 mm</td>
</tr>
<tr>
<td>per case</td>
<td></td>
</tr>
</tbody>
</table>
On-Site Adequacy Assessment (ROSE)

- Rapid on-site evaluation (ROSE) increases the value of the sampling procedure for patients (diagnostic yield 90.5% vs 81.2% adequacy)
- Allows additional passes/needle redirection
- Allows specimen triage to optimize testing including gross evaluation of the needle rinse
- Provides an opportunity to stop sampling
- Preliminary diagnosis to guide urgent therapy when needed


On-Site Adequacy Assessment

- Involved personnel: cytotechnologists, cytology fellows, cytotechnology students and cytology prep techs (2018)
- Attending cytopathologist in every case
- 2 smears from each pass maximum
- Critical attention to smear technique to avoid diagnostic pitfalls related to artifacts
- Emphasis on triage: flow cytometry, microbiologic cultures, need for IHC and molecular
- Cell blocks routinely obtained from all sites

Adequacy Assessment for FNAB

- 2 smears per pass maximum with remainder in fixative (Cytolyt)
- Attention to smear technique is critical to avoid diagnostic pitfalls related to artifacts
- Most triage decisions need to be made at ROSE: flow cytometry, microbiologic cultures, need for IHC and molecular
04/26/2018

Remaining sample placed into CytoLyt® solution.

Needle withdrawn from bronchoscope, small drop placed onto a slide for smearing; remainder of sample rinsed into CytoLyt® solution.

Two smears are made from each pass, one is air-dried and the other placed in jar of alcohol.

Air-dried slide is stained with Diff Quik® for rapid on-site adequacy assessment.

If positive for carcinoma, additional passes obtained and rinsed directly into CytoLyt® and formalin.

Air-dried slide and Alcohol fixed slide.

Sample in formalin processed in surgical pathology.

Cell Block from FNAB (Methanol-fixed)

Carcinoid Neoplasm
Flow Cytometry

- 1 million lymphocytes need for full analysis
- 1 dedicated pass generally sufficient

CMS Memo 031618

- The Centers of Medicare & Medicaid Services (CMS) is providing clarification related to FNA and ROSE under CLIA 1988
- A slide assessment that provides only a determination of specimen adequacy is not considered to be a slide examination for purposes of determining workload limits in accordance with 42 CFR 493.1274(d)
On-Site Adequacy Assessment (ROSE)

- Adequacy for a “positive” diagnosis is defined as sufficient material for morphologic diagnosis and ancillary testing for cell typing, treatment and prognosis
- Multidisciplinary approach with close communication is required
- Preliminary diagnosis akin to a frozen section

Adequacy Criteria

Endobronchial Ultrasound-Guided Transbronchial Fine-Needle Aspiration
The University of Minnesota Experience, With Emphasis on Usefulness, Adequacy Assessment, and Diagnostic Difficulties

- Lymphoid sample:
  - ≥ 40 lymphocytes/ high magnification field; OR
  - Clusters pigment-laden macrophages
  - Diagnostic smears (granulomas/tumor) require fewer
- <40 lymphocytes/ high magnification field: not a lymphoid sample

Adequacy Criteria

<table>
<thead>
<tr>
<th>Author</th>
<th>Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Choi et al, 2016</td>
<td>Tissue core size (≥ 2cm), anthracotic pigment, increased lymphocyte density (≥ 40 lymphocytes/hpf, mean 10 fields)</td>
</tr>
<tr>
<td>Nayak et al, 2010</td>
<td>Over 5 low-power fields with at least 100 lymphocytes and less than 2 groups of bronchial cells per low-power field OR germinal center fragments</td>
</tr>
<tr>
<td>Feller-Kopman et al, 2009</td>
<td>Moderate to abundant lymphocytes and/or pigmented macrophages</td>
</tr>
<tr>
<td>Alsharif et al, 2008</td>
<td>&gt;40 lymphocytes per high magnification field or clusters of pigment laden macrophages</td>
</tr>
<tr>
<td>Patelli et al, 2002</td>
<td>Lymphocytes compose at least 30% of cellularity</td>
</tr>
</tbody>
</table>
Adequate lymphoid sample

Lymph Node Adequacy

- 174 patients underwent staging EBUS FNA prior to tumor resection from 9/6/2011 - 8/15/16
- 330 of 578 lymph nodes sampled by EBUS-FNA were resected
- Cytology-histology concordance in 311/330 (94%)
- Discrepancies related to sampling in all cases
- Majority of discrepant cases had adequate lymphoid sample on review (83%) but a significant minority did not (17%)
- Nodal metastases less than 2mm in 10 discrepant cases


Accurate Classification of Lung Carcinoma

- Dramatic evolution in understanding of non-small cell lung cancer, which comprises 80% of lung cancers
- Shift from single common disease to collection of relatively uncommon diseases with different genetic drivers
- Recognition of distinct molecular subtypes
- New targeted drugs for EGFR gene mutations and EML4/Alk gene rearrangements
Therapies Targeted to Mutations in Lung Cancer

Histology-based subtyping

Adenocarcinoma 55%
Squamous cell carcinoma 34%
Others 11%


Updated Molecular Testing Guideline for the Selection of Lung Cancer Patients for Treatment With Targeted Tyrosine Kinase Inhibitors

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

Neal J. Lindeman, MD; Philip T. Cagle, MD; Dara K. Ajani, MD, PhD; Maria E. Arda, MD; Mary Beth Babby, MD; Eric H. Barenboim, MD; Carol Cilussi, MD, MSCI, FACP; Satpriya Dwarakanath, MD; MD, PhD; Neil A. Myers, MD, PhD; Keith Kern, MD, CNS; David J. Aislabie, MD, PhD; Marc Leenstra, MD; Ian E. Novak, MD, PhD; Lezlie Ried, MD; John Templeton, MD; Benjamin Schirmer, MD, PhD; Jeffrey Y. Seiden, MD, PhD; Aria Shuqair, MD, PhD; Andrew T. Sun, MD; Christie E. Verna, PhD, MD-DO; Mary M. Waves, MD, PhD; Traci S. Migli, MD, PhD;

Strong Recommendation

Physicians must use **EGFR, ALK** and **ROS1** molecular testing to select lung adenocarcinoma patients for **EGFR, ALK** or **ROS1** targeted therapies, irrespective of clinical characteristics or when adenocarcinoma cannot be excluded.

(ALK immunohistochemistry is equivalent to ALK FISH)

**Strong Recommendation**

Physicians must use *EGFR*, *ALK* and *ROS1* 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 (Stage IIIIB and IV).


---

**Strong Recommendation**

In patients with lung adenocarcinoma who harbor sensitizing *EGFR* mutations and have progressed after treatment with an *EGFR*-targeted TKI, physicians must use *EGFR* T790M mutational testing when selecting patients for third-generation *EGFR*-target therapy.


---

**Recommendation**

Pathologists may use either cell blocks or other cytologic preparations as suitable specimens for lung cancer biomarker molecular testing.
**Recommendation**

In some clinical settings in which tissue is limited and/or insufficient for molecular testing, physicians may use a cell-free plasma DNA assay to identify *EGFR* mutations. 

*(Liquid biopsy)*

---

**The 2015 WHO Classification of Tumors of the Lung**

- 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?
- How can we do all of this on small specimens?
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


Questions to Answer

- Can small biopsies be used to type tumors accurately?
- What antibodies are best to use?
One adenocarcinoma marker, one squamous cell carcinoma marker and a mucin stain

Antibodies for Lung Non-small Cell Carcinomas

- Adenocarcinoma
  - TTF-1
  - Napsin A
  - Carcinoembryonic antigen
  - CK7

- Squamous cell carcinoma
  - p63
  - p40
  - CK5/6
Adenocarcinoma Antibodies

- **TTF-1**
  - Sensitive
  - Specific
    - Ventana 8G7G3/1
  - 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
  - Is not positive in high grade neuroendocrine carcinomas such as large cell neuroendocrine carcinomas


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
    - BIOCARE/ACI3066A (Mouse monoclonal)
  - Very sensitive
  - Very specific
    - No p40 = Not squamous cell
  - Nuclear antibody

TTF-1 versus p40 IHC in NSCLC

<table>
<thead>
<tr>
<th></th>
<th>TTF-1</th>
<th>p40</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative Predictive Value (NPV)</td>
<td>62-81%</td>
<td>100%</td>
</tr>
<tr>
<td>Positive Predictive Value (PPV)</td>
<td>89-100%</td>
<td>90-94%</td>
</tr>
</tbody>
</table>

**Rules to Follow**

- **p40** has a very strong NPV
  - If it is negative, it is NOT a squamous cell carcinoma
  - BIOCARE/ACI3066A (BC28 Mouse monoclonal)

- **TTF-1** has a strong PPV
  - If it is positive, it is an adenocarcinoma
  - VENTANA 8G7G3/1 (Mouse monoclonal)

- Diffuse and strong p40 expression is squamous cell carcinoma
  - If TTF-1 +: think adenosquamous cell carcinoma
  - If just rare + cells for p40, ignore it

- If both are negative, it favors adenocarcinoma based on molecular testing and signed out as:
  - **Biopsy:** NSCLC, not otherwise specified (NOS)
  - **Resection:** Large cell carcinoma

**Cases with Targetable Mutations 2004-2010**

- n=816 Total NSCLC were tested for molecular mutations
- n=336 NSCLC with mutations
- 70% kRAS mutations
- 30% EGFR mutations
- 12 Squamous cell carcinomas by p63+
- 5 Squamous cell carcinomas by p40+
  - 2 EGFR mutations
    - 1 targetable mutation
  - 3 kRAS mutations
  - Using current diagnostic criteria, 1 targetable mutation was missed

*Source: Vincenten JP, et al. Lung Cancer 2015;89:19-26*

**When to Molecular Test Lung Tumors with Squamous Cell Carcinoma**

(Expert Consensus Opinion)

- 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
Questions to Answer

• Can small biopsies be used to type tumors accurately?
• What antibodies are best to use?
• How can we do all of this on small specimens?

Molecular Testing of Lung Cancer Patients for EGFR Mutations, ALK and ROS1 Rearrangements
Cleveland Clinic

• All primary diagnoses of not squamous NSCLC, regardless of stage
• All recurrences of not squamous NSCLC
• All adenosquamous cell carcinomas
• Squamous cell carcinomas in unusual circumstances
  – Non-smoker
  – At request by clinician
• All NSCLC for PD-L1
  – DAKO Antibody 22C3: In-house LDT (Ventana platform)

Tissue for Tumor Type

Immunohistochemistry

• Bronchoscopic biopsy
• Needle Core Biopsy
• Cell Block
  – Cell block (methanol or formalin-fixed)
Determining Tumor Type in Small Biopsy Specimens

Non-small cell carcinoma

NSCLC, NOS
TTF-1, p40

TTF-1, p40 +

Morphologic features present

Adenocarcinoma

Squamous cell carcinoma

Molecular Studies Including PD-L1

PD-L1

NSCLC, not otherwise specified (biopsy)
Large cell carcinoma (resection)

NSCLC, Adenocarcinoma

TTF-1, - p40 + CYTOKERATIN

Tissue for Ancillary Studies

- Immunohistochemistry
  - p40
  - TTF-1
  - Pancytokeratin
  - Alk D5F3:
  - Screen for EML4-Alk
  - PD-L1

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

Fine Needle Aspiration Biopsy
CytoLyt® Cell Pellet
Sample collected in CytoLyt, placed into PreservCyt, and centrifuged to generate cell pellet. The cell pellet is divided and used to make a ThinPrep slide and a cell block. The residual cell pellet is resuspended and saved. IHC is performed on cell block sections for subtyping of non-small cell carcinomas if needed. NGS is performed on non-squamous NSCLCs on material from residual cell pellet and for ALK rearrangements on ThinPrep slide; PD-L1 on FFPE sample.

Formalin-fixed samples include TBBX, EBBX or FNA/core from lymph node.

Fine Needle Aspiration Biopsies

- For NGS, DNA extraction may be performed on the cell pellet, the cell block or the supernatant from CytoLyt specimen, which contains cell free cDNA
- Cell block involves cutting of sections, melting and cleansing of paraffin
Next Generation Sequencing

- 50 gene hotspot lung panel
- Report results for 5 genes
  - EGFR
  - KRAS
  - BRAF
  - ERBB2
  - MET

EML4/ALK Rearrangement

ALK Testing: IHC

D5F3 Clone
FISH for EML4-ALK

Minca EC et al. Jnl Thorac Oncol 2014 April 464-468

---

**PD-L1 Antibodies**

<table>
<thead>
<tr>
<th>Antibody</th>
<th>DAK</th>
<th>ARO</th>
<th>RKL1</th>
</tr>
</thead>
<tbody>
<tr>
<td>DAK</td>
<td>DAK</td>
<td>DAK</td>
<td>DAK</td>
</tr>
<tr>
<td>ARO</td>
<td>ARO</td>
<td>ARO</td>
<td>ARO</td>
</tr>
<tr>
<td>RKL1</td>
<td>RKL1</td>
<td>RKL1</td>
<td>RKL1</td>
</tr>
</tbody>
</table>

- DAK: Dako
- ARO: A реализаций
- RKL1: RSL измерения

<table>
<thead>
<tr>
<th>% Tumor cells</th>
<th>% Tumor cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;1%</td>
<td>&lt;1%</td>
</tr>
<tr>
<td>&gt;50%</td>
<td>&gt;50%</td>
</tr>
</tbody>
</table>

Rimm D. et al. IASLC, Chicago September 2016

---

**PD-L1 Lung Cancer Grading**

- <1%
- ≥1% <50%
- >50%

Rimm D. et al. IASLC, Chicago September 2016
Formalin-fixed lymph node sample

PD-L1 stain; clone 22C3

Cleveland Clinic Positive and Adequacy Rates 2016

- Next Generation Sequencing for EGFR
  - 542 NGS performed: ~15-20% EGFR Mutations
  - 4 Quantity not sufficient
  - 0.74% Failure rate on NGS
  - 99% Adequacy rate

- Alk
  - 425 Specimens Tested: 5% Positive
  - FFPE: 11+/90 2 unsatisfactory
  - ThinPrep: 9+/335 2 unsatisfactory
  - 0.94% Unsatisfactory Rate on ALK
  - 99% Adequacy rate
Cleveland Clinic Adequacy Rates 2017

- Next Generation Sequencing for EGFR
  - 720 NGS performed
  - 16 Quantity not sufficient total (2.0%) includes outside cases
  - 2 Cytology samples called “adequate” at ROSE not sufficient (0.55%)
  - 98% Adequacy rate

- Alk
  - 708 Specimens Tested:
  - ThinPrep: 371 0 unsatisfactory
  - 100% Adequacy rate

ROSE Procedures

<table>
<thead>
<tr>
<th></th>
<th>2016</th>
<th>2017</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Cases with ROSE</td>
<td>2188</td>
<td>2228</td>
</tr>
<tr>
<td>Total ROSE fee codes</td>
<td>15510</td>
<td>15242</td>
</tr>
</tbody>
</table>

Practical Advice

- ROSE with attention to triage/gross of needle rinse
- Unified process for ordering molecular tests including cellularity assessment
- Molecular tech rotation in cytology and vice versa
- Regular molecular/pulmonary pathology/cytopathology meetings
- AP molecular coordinator positions
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 to optimized using biopsy and cytology specimens.

Special Thanks

• Carol Farver MD
• Jordan P Reynolds MD
• Erika Doxtader MD
• Yu-Wei Cheng PhD
• Kimberly McDonald
• Thomas Gildea MD
• Francisco Almeida MD

ROSE Procedures

<table>
<thead>
<tr>
<th></th>
<th>2016</th>
<th>2017</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Cases with ROSE</td>
<td>2188</td>
<td>2228</td>
</tr>
<tr>
<td>Total ROSE fee codes</td>
<td>15510</td>
<td>15242</td>
</tr>
</tbody>
</table>
Challenging Cases in EBUS-Guided FNA: Things are Often Not as They Seem

Dr. Jennifer Brainard  
Section Head, Cytopathology  
Pathology and Laboratory Medicine  
Cleveland Clinic

Case 1

• 81 year-old female with history of chronic obstructive pulmonary disease and 50 pack-year smoker; quit 14 years ago  
• Winter, 2012: wheezing and productive cough, no hemoptysis; treated with antibiotics  
• 5/2012 CXR: 5.7 cm right infra hilar mass; CT showed mediastinal lymphadenopathy  
• 6/2012: EBUS FNA performed of subcarinal LN
Diagnosis

• Non-small cell carcinoma, favor adenocarcinoma

Case #2

• 66 year old man diagnosed with extensive stage small cell carcinoma in 8/08
• Treated with 6 cycles of chemotherapy (cisplatin and etoposide) and prophylactic cranial irradiation
• Partial response to chemotherapy with stable disease for 20 months
• Chest CT 4/10 showed increasing size of persisting lung nodules and hilar lymph nodes
ROSE: “Small cell carcinoma differential”, additional workup required
Diagnosis:

• Basaloid squamous carcinoma

Nomenclature confusing:
- Pure basaloid carcinoma is a subtype of large cell carcinoma; lacks squamous or glandular differentiation but may see focal keratin; similar immunophenotype

Basaloid Squamous Carcinoma

• Squamous carcinoma with basaloid morphology
• At least focal intercellular bridges and individual cell keratinization
• Nomenclature confusing:
  - Pure basaloid carcinoma is a subtype of large cell carcinoma; lacks squamous or glandular differentiation but may see focal keratin; similar immunophenotype
Basaloid Squamous Carcinoma

- Endobronchial or peripheral location
- Characteristic lobular growth with peripheral palisade of basal-type cells
- Central necrosis is often seen
- Stroma may be hyalinized or mucoid
- High mitotic rate
- Individual cells have hyperchromatic dense small nuclei lacking nucleoli

Squamous cell carcinoma, basaloid type
Squamous cell carcinoma, basaloid type

Basaloid Squamous Carcinoma: Cytology

- Large fragments of tumor
- Small cuboidal cells with scant cytoplasm and hyperchromatic finely granular chromatin
- Inconspicuous nucleoli
- Necrosis including single cell necrosis may be seen
- Focal keratinization generally present

Differential Diagnosis

- Small cell carcinoma
### Basaloid Squamous Ca vs. Small Cell Ca

<table>
<thead>
<tr>
<th>Cytologic Feature</th>
<th>Small Cell Carcinoma</th>
<th>Basaloid Squamous Ca</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell aggregates</td>
<td>Present, usually small and focal</td>
<td>Prominent; large aggregates</td>
</tr>
<tr>
<td>Necrosis with nuclear fragments</td>
<td>Present</td>
<td>Present</td>
</tr>
<tr>
<td>Molding</td>
<td>Present</td>
<td>Absent</td>
</tr>
<tr>
<td>Squamous differentiation</td>
<td>Absent</td>
<td>Present; focal</td>
</tr>
</tbody>
</table>

### Immunohistochemistry:

- **Basaloid squamous carcinoma:**
  - Cytokeratin 5/6: Positive
  - P63: Positive
  - TTF-1: Negative

- **Small cell undifferentiated carcinoma:**
  - Cytokeratin 5/6: Negative
  - P63: Negative
  - TTF-1: Positive

*Original biopsy*
Take Home Points:

- Know the patient’s history and pay attention to the current clinical setting—look for clinical clues
- Prepare for the unexpected
- Obtaining material for cell blocks at the time of ROSE can keep the cytologist out of trouble
- Immunohistochemistry is necessary to differentiate basaloid squamous carcinoma from small cell carcinoma
- Basaloid squamous carcinoma may be primary in the lung or secondary from other sites, particularly the head and neck

Case #3

- 65 year old man with persistent productive cough
- Never smoker
- Left hilar mass with hypermetabolic mediastinal adenopathy on PET
- Suspected stage III lung cancer clinically
- EBUS with multiple node sampling
Diagnosis

• Hodgkin lymphoma associated with a sarcoid-like reaction
Adenocarcinoma with sarcoid-like reaction

CD30
Sarcoid-Like Reaction

- Occurs in approximately 4% of patients with carcinoma and 13.8% of patients with Hodgkin lymphoma
- Non-necrotizing granulomas in lymph nodes
- PET positive and difficult to distinguish from malignancy on imaging
- May precede the diagnosis of malignancy, occur concurrently or occur later
Take Home Points:

• Know the patient's history and pay attention to the current clinical setting—look for clinical clues
• Prepare for the unexpected
• Obtaining material for cell blocks at the time of ROSE can keep the cytologist out of trouble
• When you find granulomas, keep looking
• Sarcoidosis is a clinical/pathologic diagnosis. Communication with the pulmonologist is imperative.
• If the cytologic diagnosis does not fit the clinical presentation of the patient, additional sampling may be needed

Case #4

• 88 year old man; former smoker
• History of stage 1B adenocarcinoma of RUL status post lobectomy 2/10
• Doing well since with no evidence of recurrence
• Follow up CT 2/13 shows nodule in RLL and diffuse adenopathy
• EBUS FNA of 4L and station 7 lymph nodes
Diagnosis

• Small cell carcinoma with a possible component of non-small cell carcinoma (mixed small cell carcinoma)

Another example……

• 77 year old woman never smoker
• 2007: Stage 1 lung adenocarcinoma
• 2011: Adenocarcinoma in pleural fluid; EGFR mutation positive
• 2012: Near complete response to Erlotinib
• 2013: New left lung mass and left hilar adenopathy
Adenocarcinoma pleural fluid

Adenocarcinoma pleural fluid

Left lung mass
Diagnosis

• Keratinizing squamous cell carcinoma
• EGFR mutation positive (exon 19 deletion)

Lung Tumor Heterogeneity

• Varying morphology may be seen in primary tumor, between primary and metastasis, and between metachronous tumors
• May be related to chemotherapy (resistance mechanism)
• Consider the possibility of second primary tumor, especially if presents after 2 years
• Role for a lung “stem cell” currently being investigated

Take Home Points:

• Know the patient’s history and pay attention to the current clinical setting—look for clinical clues
• Prepare for the unexpected
• Obtaining material for cell blocks at the time of ROSE can keep the cytologist out of trouble
• Lung cancers are morphologically heterogeneous, so it is not unusual to see varying morphology
Case 5

- 55 year old man
- Non-smoker with recurrent bouts of pneumonia requiring home oxygen therapy
- Right lower lobe consolidation with an endobronchial lesion on CT scan
- Clinical impression was lung cancer
Diagnosis

- Purulent acute inflammation compatible with abscess
- Food particles/vegetable material consistent with aspiration
- Filamentous bacteria consistent with Actinomyces
Infection

• Don’t forget to think about infection, as it may mimic malignancy
• Knowledge of clinical history and imaging necessary
• ROSE allows triage for microbiologic culture

Take Home Points

• Discussions between clinicians and pathologists are essential throughout the entire patient encounter
• The patient’s history is important
• ROSE helps to ensure an adequate specimen for diagnosis and all necessary ancillary testing

Case #6

• 41 year old man
• Non-smoker with a chronic cough
• Lung nodule and adenopathy
• EBUS FNA of a station 7 lymph node
Diagnosis

- Metastatic medullary thyroid carcinoma

Metastatic Carcinoma

- Don’t forget to think about metastasis in any lung FNA
- Knowledge of clinical history and imaging necessary
- Isolated or endobronchial metastases do occur
- Immunohistochemistry may be needed for confirmation
Take Home Points

• Discussions between clinicians and pathologists are essential throughout the entire patient encounter for optimal use of pathology specimens for diagnosis and molecular markers.
• ROSE helps to ensure an adequate specimen for diagnosis and all necessary ancillary testing.

Take Home Points

• Specifically classifying non-small cell carcinoma based on morphology alone is quite challenging.
• Immunohistochemistry is very useful and can be used in a tissue sparing manner.
• Molecular testing can be performed successfully in cytology samples using an approach that allows for archival material for future testing.