Purpose of FNA in Patients with Lung Cancer

- Make a diagnosis
  - What kind of malignancy?
    - Adenocarcinoma
    - Squamous cell carcinoma
    - Adenosquamous carcinoma
    - Small cell carcinoma
    - Lymphoma
    - Sarcoma
    - Other
  - What mutations/markers are present that will....

- Direct patient care
  - Surgery
  - Chemotherapy and/or radiation therapy
  - Targeted therapy /immunotherapy

Historical Note

- Small cell vs. Non-small cell carcinoma
  - Small cell carcinoma
    - Usually advanced stage at presentation
    - Chemotherapy and/or radiation therapy
  - Non-small cell carcinoma
    - Treatment the same, based on disease stage and performance status
      - Surgery
      - Chemotherapy and/or radiation therapy
Personalized Medicine in Lung Cancer

KRAS

- Most common mutation, 25-35%
  - Caucasian
  - Smokers
  - Non-squamous NSCLC, especially mucinous adenocarcinoma
- Activating mutation
  - Downstream signaling and proliferation
  - 95% codons G12-G13
  - Mutually exclusive with EGFR/ALK mutations
- KRAS mut tumors are resistant to conventional and EGFR-TKI therapy
- Assay: mutational analysis
EGFR

- 10-20% of non-squamous NSCLC
  - Women
  - Non-smokers
  - Asian descent, 40%
  - Mostly adenocarcinoma
- Cellular transmembrane tyrosine kinase
  - Triggers signaling cascades regulating growth, cell survival, proliferation, angiogenesis, cell migration and differentiation
- Mutation in the tyrosine kinase domain
  - Increased cell survival and proliferation pathways
- 85-90% EGFR mutations involve deletion of exon 19 or mutations in exon 21
  - erlotinib (Tarceva) and afatinib (Gilotrif) for the first-line treatment

ALK

- 3-5% of non-squamous NSCLC
  - Younger patients
  - Mostly adenocarcinoma
- ALK is transmembrane TK receptor
- Cancer with an ALK rearrangement is highly sensitive to crizotinib, a TKI that binds to the ALK protein

Assay: mutational analysis (PCR, Sanger, NGS); MANDATORY in non-SCC or nonsmokers with NSCLC

Assay: IHC, FISH; MANDATORY in non-SCC or nonsmokers with NSCLC


Naidoo and Drailon; gotoper.com
**ALK FISH**

**ROS1**

- 1-2% of non-squamous and NOS, NSCLC  
  - Young and never-smoker
- TKR of the insulin receptor family
- ROS1 and ALK rearrangements are mutually exclusive
- Currently, no formal screening guideline  
  - Patients respond to crizotinib
**MET**

- 4–6% of NSCLC
- Any histological type
- Uncommon in previously untreated NSCLC
- Test: FISH; MET:MET control ratio > 2 or copy #>5 (e.g. MET amplified)
  - MET exon 14 skipping
- Confers poor prognosis
- Plays a role in acquired resistance to EGFR inhibitors in patients with EGFR-mutated tumors

**Met Skipping**

- METex14 activates oncogenic signaling and is a potential MET-targeting therapy cancer genomic predictive biomarker.
- METex14 genomic variants can occur via diverse genomic aberrations involving the splice sites, resulting in in-frame skipping of the juxtamembrane domain encoding exon 14.
- METex14 not only can represent an important oncogenic variant, but also can serve as genomic predictive biomarker for MET-targeting therapy.

**BRAF**

- 1–4% of all non-squamous NSCLC
  - Mostly Adenoca.
- More likely to be found in former/current smokers
  - V600E (50%), G469A (39%), and D594G (11%)
- In the vast majority of cases, BRAF mutations are non-overlapping with other oncogenic mutations found in NSCLC

---


BRAF

https://www.mycancergenome.org/content/disease/lung-cancer/braf
Morphology and Mutations

<table>
<thead>
<tr>
<th>Molecular Alterations</th>
<th>Subtype of tumor morphology</th>
<th>Targeted therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>EML4</td>
<td>Non-mucinous</td>
<td>Erlotinib</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Gemcitabine</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Afatinib</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Decitabine</td>
</tr>
<tr>
<td>ROS1</td>
<td>Mucinous</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Solid (with mucin production)</td>
<td></td>
</tr>
<tr>
<td>ALK</td>
<td>Solid (with signet ring cells)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mucinous (with atypical invasion)</td>
<td></td>
</tr>
<tr>
<td>ROR1</td>
<td>Bronchial adenocarcinoma</td>
<td>Cisplatin</td>
</tr>
<tr>
<td></td>
<td>Ringer ring cells</td>
<td></td>
</tr>
<tr>
<td>BAP1</td>
<td>Mucopapillary</td>
<td>Vemurafenib (only for V600E)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Dabrafenib</td>
</tr>
<tr>
<td>MET</td>
<td>Papillary</td>
<td>Trametinib</td>
</tr>
<tr>
<td></td>
<td>Solid</td>
<td>Selumetinib</td>
</tr>
<tr>
<td></td>
<td>Any</td>
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</table>

**Immune Checkpoints: PD-1/PD-L1**

- PD-1 is a negative co-stimulatory receptor expressed mainly on T cells
- Binding of PD-1 to PD-L1 and PD-L2 blocks TCR signaling and therefore T cell activation
- Expression of PD-L1 on tumor cells can suppress immune surveillance and thus permit tumor growth
Drug mAb/Platform Scoring criteria Comments

**Pembrolizumab (Keytruda)**
22C3 (DAKO pharmDx)/ Link 48 Autostainer ≥50% tumor cells for 1st line, ≥1% tumor cells for ≥2nd line†

**Nivolumab (Opdivo)**
28-8 (DAKO pharmDx)/ Link 48 Autostainer ≥1% tumor cells†
Predictive of response only in non-squamous NSCLC

**Atezolizumab (MPDL3280)**
SP142/BenchMark ULTRA Autostainer Tumor cells and/or tumor infiltrating immune cells‡
Complementary diagnostic

**Durvalumab (MEDI4736)**
SP243/BenchMark ULTRA Autostainer ≥25% tumor cells††

**Avelumab (MSB0010718C)**
73-10 (DAKO pharmDx) ≥1% tumor cells†

† 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, IHC 1/2/3 (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

Courtesy of Dr. Mari Mino-Kenudson
Determinants of Response: Tumor Infiltrating Lymphocytes?


PDL1/CD8 Multiplex IHC

- Template for reporting
- PD-L1/CD8 IMMUNOHISTOCHEMICAL STAINING
  - A multiplex stain for PD-L1/CD8 was performed.
  - 100 or more tumor cells are available in this specimen.
  - PD-L1 shows no membranous staining of tumor cells.
  - PD-L1 shows intense/imperious staining of tumor cells.
  - CD8+ tumor infiltrating lymphocytes are absent or rare (score of 0).
  - CD8+ tumor infiltrating lymphocytes are few and scattered, involving <5% of tumor cells (score of 1).
  - CD8+ tumor infiltrating lymphocytes are present, associated with 5-25% of tumor cells (score of 2).
  - CD8+ tumor infiltrating lymphocytes are numerous, associated with >25% of tumor cells (score of 3).

Additionally, CD8+ T-cells comprise X% of stromal nucleated cells.

Metastatic Adenocarcinoma to GEJ

Before Anti-PD1 therapy

Courtesy of Dr. Justin Gainor
### New Paradigm for 1st Line Management

- **Non-Small Cell Lung Cancer**
  - **Nonsquamous**
  - **Squamous**

### Novel Biologic Therapies for Advanced NSCLC Approved by the US Food and Drug Administration

<table>
<thead>
<tr>
<th>Agent</th>
<th>Mechanism</th>
<th>Indication</th>
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<td>Small molecule EGFR inhibitor</td>
<td>NSCLC with EGFR mutations; NSCLC with ALK rearrangements; NSCLC with ROS1 rearrangements; progressed or resistant of crizotinib; NSCLC with ROS1 and ALK inhibitors; NSCLC with ROS1 rearrangements; progressed or resistant of ceritinib</td>
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<td>Bevacizumab</td>
<td>Anti-VEGF monoclonal antibody</td>
<td>NSCLC with EGFR mutations; Non-SqCC (contraindicated in SqCC because of risk of pulmonary hemorrhage); Non-SqCC (risk of effeciency level of TQ)</td>
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<td>Pembrolizumab</td>
<td>Anti-PD-1 antibody</td>
<td>Previously treated advanced NSCLC</td>
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### Diagnostic Work-Up of Lung Mass

- **Pathology**
  - Cytopathology of lung, nodal and/or mediastinal site
  - Determine Cancer or noncancerous process
    - Infection
    - Sarcoid
  - Define type of cancer: small cell versus NSCLC
    - Morphology
    - IHC if needed
- **Ancillary testing for personalized targeted therapy**
  - Diagnostic Genetic Mutational Analysis
  - Immune check-point PD1 IHC
Biopsy Methods

Recent advances in the pathology and molecular genetics of lung cancer is pivotal review for cytopathologists

Table 1: Summary of minimally invasive sampling techniques.

<table>
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<tr>
<th>Technique</th>
<th>Advantages</th>
<th>Disadvantages</th>
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<td>Can be performed during therapeutic bronchoscopy</td>
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<td>Easy access to mediastinal lymph nodes (staging) and central lung lesions</td>
<td>Significantly lower risk of pneumothorax and other complications than other bx methods</td>
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ROSE

- Specimen adequacy
  - Shortens procedure time
  - Reduces ND samples
  - Cost effective
- Triage of tissue
  - Culture, flow cytometry, FFPE tissue for ancillary testing
  - Diagnosis and staging for malignant tumors
- Directs patient care
  - Confirmation of clinical diagnosis
  - Unexpected findings
- Disadvantages: time and poor reimbursement
Specimen Adequacy

**Insufficient**
- Not representative, e.g.
  - Not diagnostic of a mass or not enough lymphocytes to represent a node
- Not defined specifically
  - 5 low-power fields with >100 lymphocytes
  - Most cellular 40x field with >40 lymphs
  - Germinal centers or granulomas


**Sufficient**
- No defined amount
- Individual comfort level
- Representative of the lesion sampled - mass or node
- Don’t make a malignant diagnosis unless you can sign it out on the spot!

Tissue Processing SOP

**FNA**
- Direct smears
  - ROSE
- Needle rinse into LBC
  - Cell block
  - Can use for IHC, NGS and FISH as well

**Core biopsy**
- Into formalin
  - Surface HE
  - 2 blanks
  - TTF-1; Napsin A/p63 dual stain if needed
  - PD/L1/CD8 multiplex
- Save tissue for NGS, FISH
A superior method for cell block preparation for fine-needle aspiration biopsies: Colloidion Bag technique

2015 Classification Changes

- Adenocarcinoma
  - No longer use "bronchioloalveolar" carcinoma (now lepidic)
  - Addition of pre-malignant lesions [in-situ and ADH] and minimal invasive
- Squamous cell carcinoma
  - Keratinizing, non-keratinizing, basaloid, in-situ
- Emphasizes utility of IHC and molecular subtyping

Small Cell Carcinoma

- Hyperchromatic, crowded groups
  - Single cells
- Small cells
  - Scant to no cytoplasm
  - Round, oval nuclei
    - Molding
    - Stippled chromatin
    - Small nucleoli/chromocenters
- Mitoses, necrosis
Small Cell Carcinoma

core biopsy

Necrosis

Crush artifact

Synaptophysin

Chromogranin

Table 1: WHO classification of neuroendocrine tumours

<table>
<thead>
<tr>
<th>Grade</th>
<th>WHO</th>
<th>WHO classification</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
<td>Neuroendocrine tumour, Grade 1</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td>Neuroendocrine tumour, Grade 2</td>
</tr>
<tr>
<td>3</td>
<td></td>
<td>Neuroendocrine tumour, Grade 3</td>
</tr>
</tbody>
</table>

Table 2: Comparison of neuroendocrine tumours

<table>
<thead>
<tr>
<th>WHO classification</th>
<th>NCIC-CCDC</th>
<th>WHO classification</th>
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<tr>
<td>Neuroendocrine tumour, Grade 1</td>
<td>Neuroendocrine tumour, Grade 1</td>
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<td>Neuroendocrine tumour, Grade 2</td>
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<tr>
<td>Neuroendocrine tumour, Grade 3</td>
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</table>

Large Cell Neuroendocrine Tumor

Well-differentiated NET (carcinoid)
- Monotonous small cells
- Round to oval nuclei with stippled chromatin
- No significant nuclear molding
- No necrosis or mitotic activity

Well-differentiated NET (carcinoid)
Well-differentiated NET (carcinoid)

- Monotonous small cells
- Round to oval nuclei with stippled chromatin
- No significant nuclear molding
- No necrosis or mitotic activity (confirmed with ki-67)

Squamous Cell Carcinoma

- Hyperchromatic crowded groups
- Large polygonal cells
  - Dense cytoplasm
  - Keratinization
  - Dark, angulated nuclei
Squamous Cell Carcinoma

- Nests of tumor cells
- Dense cytoplasm
  - No mucin
- Hyperchromatic nuclei, +/- nucleoli
- +/- Keratinization
- Necrosis

No need for IHC!

Adenocarcinoma

- Hyperchromatic crowded groups
  - Honeycombed sheets
  - Papillary groups
  - Luminal borders
  - Single cells
- Polygonal cells
  - Loose, clear cytoplasm
  - +/- mucin
  - Round nuclei
    - Less dense chromatin than SCC
    - Nucleoli

Adenocarcinoma

- Mucinous
- Micropapillary
- Lepidic (bronchioloalveolar)
- Acinar
Adenocarcinoma

- Lepidic pattern
- Acinar pattern
- Mucinous pattern

No need for IHC!

Adenocarcinoma

- NSCLC on smears
- Adenocarcinoma on core
- No need for IHC

Adenosquamous Carcinoma

- Glandular and squamous morphology
Adenosquamous Carcinoma

Non-small Cell Lung Cancer
(adenocarcinoma by IHC)

Non-small Cell Lung Cancer
(squamous by IHC)
Immunohistochemistry Typing of Cytokeratin-Positive, Morphologically Undifferentiated NSCLC in Biopsy/Cytology Specimens

<table>
<thead>
<tr>
<th>Adenocarcinoma Marker: TTF-1</th>
</tr>
</thead>
<tbody>
<tr>
<td>+ focal or diffuse</td>
</tr>
<tr>
<td>NSCC, favorable adenocarcinoma</td>
</tr>
<tr>
<td>NSCC, favorable adenocarcinoma</td>
</tr>
<tr>
<td>NSCC, favorable adenocarcinoma</td>
</tr>
<tr>
<td>NSCC, favor squamous cell carcinoma</td>
</tr>
<tr>
<td>NSCC, NOS</td>
</tr>
<tr>
<td>NSCC, NOS</td>
</tr>
</tbody>
</table>

Adenocarcinoma diffuse: >10% of cells positive; focal: 0% to 10% of cells positive; NOS: not otherwise specified; NSCC: non-small cell carcinoma; NSCLC: non-small cell lung cancer; TTF-1, thyroid transcription factor 1.

Pitfalls with IHC Staining

Metastatic Adenocarcinoma (metastatic breast cancer)

Gata3 ER
Metastatic Adenocarcinoma
(metastatic bladder cancer)

Gata3

Rapid molecular testing of NSCLC

Principle steps: Post-procedure

66 y.o. woman, former <5 pack-year smoker

- presented with a cough and rapidly progressive dyspnea with exertion for the last 2 weeks, now requiring 6L O2 at rest and severe left lower back pain making her bedridden

- Imaging studies showed a 3.1 cm LLL mass, multiple bilateral lung nodules, mediastinal lymphadenopathy as well as features of lymphangitic spread, and a L5 vertebral body lesion extending the left L5-S1 neuroforamen
NSCLC: adenocarcinoma

ROS1

Immunohistochemical images of NSCLC with ROS1 positive staining.
Summary

Carcinoma

Small Cell Carcinoma

Synapto+ Chromo+

Adenocarcinoma

Squamous Cell carcinoma

TTF-1 + Napsin A + P40-

Adenocarcinoma

Adenosquamous carcinoma

Squamous Cell carcinoma

NSCLC, NOS

Acknowledgements

• Dr. Mari Mino-Kenudson
• Dr. Justin Gainor
• Google