MORPHOLOGY AND MOLECULAR TESTING IN NON-SMALL CELL CARCINOMA OF LUNG NEW FRONTIER IN CYTOPATHOLOGY PRACTICE

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NON-SMALL CELL LUNG CANCER: 70% PRESENT IN ADVANCED STAGE

http://whobuebooks.iarc.fr/

2015 Classification: Impact on Management of Advanced Lung Cancer Patients

- Criteria/terminology for small bx/cytology
- More accurate histologic subtyping
- Strategic management of small tissues
- Streamlining workflow for molecular testing
- Need for local multidisciplinary team

THERAPEUTIC ADVANCES IMPACTED NEED FOR MORE ACCURATE HISTOLOGIC DIAGNOSIS AND MOLECULAR TESTING

- Predictive of response
  - EGFR mutation (adenoca) – TKI’s
  - Adenoca or NSCC-NOS – pemetrexed
  - ALK fusion (adenoca)- crizotinib
- Predictive of toxicity
  - Bevacizumab – contraindicated in life-threatening hemorrhage in squamous carcinoma
**CLASSIFICATION OF LUNG CANCER NOW REQUIRES GENETIC TESTING**

- EGFR
- ALK fusions
- BRAF V600E
- ROS1 fusions
- RET fusions
- MET splice site Exon 14 mutations

**TARGETABLE GENETIC CHANGES IN SQUAMOUS CELL CARCINOMA**

- PTEN
- FGFR1 AMP
- PIK3CA
- AKT1
- Unknown (34%)

**Evolution of molecular testing of lung adenocarcinomas at MSKCC**

- EGFR only
- Sequenom MassARRAY™
- PCR-based for Exon 19 Δ, Exon 21 mutations
- Sanger Sequencing

- Next-generation sequencing
- MSK-IMPACT™

- Integrated Mutation Profiling of Actionable Cancer Targets
- Sequenom MassARRAY™
- Exon 19 Δ, ALK FISH
- EGFR Exon 19 Δ
- ALK FISH
- RET FISH
- ROS1 FISH

**Initial Therapy of Advanced Adenoca or NSCLC-NOS**

- Adenocarcinoma
- Large cell ca
- NSCLC-NOS

- EGFR Mutations
- Pos ALK fusion
- Neg ALK fusion
- Unknown EGFR Mutation & ALK Status

- Erlotinib/Gefitinib
- Cisplatin
- Bevacizumab
- Crizotinib
- Pemetrexed
- Cabozantinib
- Claplatin
**Initial Therapy of Advanced Adenocarcina or NSCLC-NOS**

- Adenocarcina
  - EGFR Mutation
    - Exon 19 del
    - Exon 21 L858R, L861X
    - Exon 18 G719A/S
  - Neg EGFR mut
    - Pos ALK fusion
  - Neg EGFR mut
    - Neg ALK fusion
  - Unknown EGFR Mutation & ALK Status
  - Erlotinib/Gefitinib
  - Crizotinib
  - Pemetrexed
  - Bevacizumb
  - Cisplatin

**LUNG ADENOCARCINOMA**

CLASSIFICATION IN SMALL BIOPSY AND CYTOLOGY SPECIMENS

Because this was never addressed by WHO, by necessity other histologies needed to be addressed

**SMALL BIOPSY/CYTOLOGY LUNG CANCER DIAGNOSIS: IN USA OVER 132,000 CASES IN 2017**

- 2017: ACS estimates for USA:
  - 222,500 Lung Cancers
  - 85% NSCLC = 189,125 (15% SCLC)
- 70% Advanced Stage = 132,388
  - Unresectable: Diagnosed by small biopsies/cytology

**PHASE III STUDY COMPARING CISPLATIN PLUS GEMCITABINE WITH CISPLATIN & PEMETREXED IN ADVANCED NSCLC**


**PSEUDOSQUAMOUS SOLID ADENOCARCINOMA**

- TTF-1
- Mucicarmine

- IN THIS STUDY APPROXIMATELY 20% OF CASES REPRESENT NSCLC-NOS

- EGFR Exon 19 Deletion

Modified from Mark Kris, Thoracic Oncology, MSKCC
**Pseudokeratinizing Adenocarcinoma**

- TTF-1
- p40

**2015 WHO Terminology for Small Biopsies and Cytology**

<table>
<thead>
<tr>
<th>2015 WHO Resections</th>
<th>Small Biops/Cytology</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adenocarcinoma</td>
<td>Morphologic adenocarcinoma patterns clearly present: Adenocarcinoma, describe identifiable patterns present</td>
</tr>
<tr>
<td>Acinar</td>
<td></td>
</tr>
<tr>
<td>Micropapillary</td>
<td></td>
</tr>
<tr>
<td>Solid</td>
<td></td>
</tr>
<tr>
<td>No 2004 WHO counterpart – Most will be solid adenocarcinomas</td>
<td></td>
</tr>
<tr>
<td>Squamous cell carcinoma</td>
<td>Morphologic squamous cell patterns not present (supported by special stains; i.e. TTF-1 +; p40 -): Non-small cell carcinoma, favor adenocarcinoma</td>
</tr>
<tr>
<td>Keratinizing</td>
<td>Squamous cell carcinoma</td>
</tr>
<tr>
<td>Nonkeratinizing</td>
<td></td>
</tr>
<tr>
<td>Basaloid</td>
<td></td>
</tr>
<tr>
<td>No 2004 WHO counterpart</td>
<td>Morphologic squamous cell patterns not present (supported by special stains; i.e. p40 +; TTF-1 -): Non-small cell carcinoma, favor squamous cell carcinoma</td>
</tr>
<tr>
<td>Large cell carcinoma</td>
<td>Non-small cell carcinoma, not otherwise specified (NOS)</td>
</tr>
</tbody>
</table>

**2015 WHO Classification**

- Non-small cell carcinoma, favor squamous cell carcinoma
- Non-small cell carcinoma, favor adenocarcinoma

**Immunohistochemical Markers**

- **Adenocarcinoma (One Marker)**
  - TTF-1 (best), Napsin, PE-10
- **Squamous Carcinoma (One Marker)**
  - p40 (best), p63, CK5/6, 34βE12
  - Desmocollin-3 (need more testing)
- Cocktails – nuclear/cytoplasmic antibodies
  - Adenoc – TTF-1/Napsin
  - Squamous – p63/CK5/6

**NSCLC Diagnosed by Light Microscopy in Small Biopsies/Cytology**

- Squamous cell carcinoma: 20-30%
- NSCLC-NOS: 20-40%
- Adenocarcinoma: 40-50%

- Historically NSCLC-NOS has been encouraged because there was no reason to classify these tumors further.
- As a result 20-40% of NSCLC in small biopsies/cytology are currently being diagnosed as NSCLC-NOS.
**IMMUNOHISTOCHEMISTRY FOR MUTATION/FUSION SPECIFIC ANTIBODIES**

- ALK
- EGFR
  - EGFR L858R
  - EGFR E746
- ROS1

**ALK Rearranged Adenocarcinoma**

- ALK IHC (D5F3)
- ALK FISH

**EGFR EXON 21 L858R MUTATION SPECIFIC AB**


**EGFR EXON 19 DELETION MUTATION SPECIFIC AB**

**EGFR MUTATION SPECIFIC ANTIBODIES**

- Exon 19 deletion
  - All 20 cases with 15-bp deletion were MS Ab positive (sensitivity 100%, specificity 99%)  
  - 35 other than the common 15bp deletion – 49% stained positively (sensitivity 74%)
- EGFR L858R mutation
  - 17/18 cases were positive with MS Ab (sensitivity 95%, specificity 99%); better if use 2+/3+ for positive


**NSCLC – FAVOR ADENOCARCINOMA TOUCH PREP CYTOLOGY**

**CYTOLOGY IS A POWERFUL TOOL FOR CLASSIFYING NSCLC**

Suitability of Thoracic Cytology for New Therapeutic Paradigms in Non-Small Cell Lung Carcinoma

High Accuracy of Tumor Subtyping and Feasibility of EGFR and KRAS Molecular Testing

Nastasia Rothman, MD, PhD,* Suzanne M. Brandt, MD,* Carrie S. Sigel, MD,* Marie A. Freivalskaya, MPA, CT (ASCP), PT; Gregory J. Boyle, MD, PhD; J. William D. Travis, MD,* Maseena F. Zafar, MD,* and Andrei L. Moreira, MD, PhD*

J Thoracic Oncol 6:451-8, 2011

**INVASIVE MUCINOUS ADENOCARCINOMA CYTOLOGY DRUNKEN HONEYCOMBING**

**TISSUE MANAGEMENT**

- Each group of thoracic physicians (clinicians, radiologists, surgeons, pathologists, molecular biologists) must develop a strategy to manage tissues
- Obtaining biopsies or cytology samples
- Optimal processing by laboratories/pathologists for diagnosis AND molecular studies
- Pathologists should be the leader of this
MSKCC COPATH ORDERING SETS FOR MOLECULAR TESTING

- Molecular Lung Adenoca (NGS – IMPACT)
- Molecular – T790M
  - 15 Unstained slides for resections
  - 20 Unstained slides for small biopsies
- Specific orders for ALK and ROS1 FISH
- Other platforms (i.e. Sequenome)

Molecular Processing: 1 vs 2 Blocks

- Two Block Setting
  - Diagnostic IHC; if adeno: TTF-1, ALK (D5F3) and EGFR (L858R), PD-L1
  - Second block: Run Group Stains: Molecular Lung (4 choices) – USS directly to DMP
- One Block Setting
  - Diagnostic IHC: i.e. TTF-1, ER, CDX2
  - Unstained Recut (20 small bx, 15 resection)
  - Slides returned to fellow – send USS with H&E to DMP with paper form

KEY PRINCIPLES

- Minimize diagnostic stains to maximize tissue for molecular studies
- Molecular testing is reliable on FFPE tissues – even very small samples
- Unstained slides (n=15-20) provide adequate DNA if sufficient tumor
- Cytology fluids (i.e. pleural) – cytospin and make cell block (for IHC/molecular)

NEW DEVELOPMENTS IN ADVANCED LUNG CANCER DIAGNOSIS

- Immunotherapy – PD-L1 Immunohistochemistry
- Cell free DNA analysis (liquid biopsy)
PD-L1 IHC Assays for Lung Cancer

<table>
<thead>
<tr>
<th>Drug</th>
<th>Assay</th>
<th>PD-L1 scoring</th>
<th>Cut-offs assessed in clinical trials</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nivolumab</td>
<td>28-8</td>
<td>Tumor cells</td>
<td>1%, 5%, 10%</td>
</tr>
<tr>
<td>Pembrolizumab</td>
<td>22C3</td>
<td>Tumor cells</td>
<td>1%, 5%, 50%</td>
</tr>
<tr>
<td>Atezolizumab</td>
<td>SP142</td>
<td>Tumor cells</td>
<td>1%, 5%, 50%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Immune cells</td>
<td>1%, 5%, 10%</td>
</tr>
<tr>
<td>Durvalumab</td>
<td>SP263</td>
<td>Tumor cells</td>
<td>25%</td>
</tr>
<tr>
<td>Avolumab</td>
<td>73-10</td>
<td>Tumor cells</td>
<td>1%</td>
</tr>
</tbody>
</table>

Courtesy of Ming Tsao

PD-L1 IHC: CHALLENGES
- Five different IHC clones, staining platforms & scoring criteria
- Limited tissue – cannot perform all assays after genomic testing
- Heterogeneity of staining
- Need for standardization of testing and interpretation of results
- Lack of data on cytology specimens

CELL FREE DNA ASSAYS

- Detection of circulating tumor cells – new technology with some potential
- FDA approved CellSearch System for circulating tumor cell detection
- In patients with a genomically defined solid tumor – may be clinically useful
- However, not validated for lung cancer diagnosis and its lower sensitivity could delay diagnosis compared to tissue biopsy

SUMMARY
- 2015 WHO Classification provides diagnostic criteria and terminology to be used in small bx and cytology
- Need strategic approach to use of small specimens not only for diagnosis but for molecular testing
- Rapidly evolving field requires following new technology (IHC, molecular)
- Need multidisciplinary team
- Computer IT input is critical