CLASSIFICATION & MOLECULAR MARKERS OF LUNG ADENOCARCINOMA

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WHO CLASSIFICATIONS

- 1967 – Histologic Typing of Lung Tumours
- 1981 – Histologic Typing of Lung Tumours
- 1999 – Histologic Typing of Tumours of the Lung and Pleura
- 2004 – Pathology and Genetics: Tumours of the Lung, Pleura, Thymus and Heart
- 2015 - Pathology and Genetics: Tumours of the Lung, Pleura, Thymus and Heart
PERSONALIZED MEDICINE:
INCREASING RELEVANCE

INCREASING COMPLEXITY

- 1967 WHO
- 1981 WHO
- 1999 WHO
- 2004 WHO
- 2015 WHO

- H&E
- H&E & Mucin
- H&E, EM & IHC
- H&E, EM, IHC & Genetics
- H&E, Cytology, IHC, Genetics, Mucin, Radiology
Lung Carcinoma

- Small cell carcinoma
- Non-small cell lung carcinoma (NSCLC)
  - Adenocarcinoma (including bronchialalveolar carcinoma BAC)
  - Adenosquamous carcinoma
  - Squamous cell carcinoma
  - Large cell carcinoma
  - Large cell neuroendocrine carcinoma
EPITHELIAL TUMORS
Invasive Malignant - 2004

Adenocarcinoma
  Mixed pattern
  Acinar
  Papillary
Bronchioloalveolar carcinoma
Solid adenocarcinoma with mucin formation

Variants

WHO/IASLC CLASSIFICATION OF LUNG AND PLEURAL TUMORS
WHO Classification of Lung Cancer

Classification is based on resected specimen. On small biopsy, the differentiation of various subtypes of NSCLC is not reliable in many cases.

NSCLC - NOS

WHO Classification of Lung Cancer, 2004
Revised classification that emphasizes:

- Integrated multidisciplinary approach for classification is needed
- Classification in small biopsies and cytology specimen (was not addressed in 2004 WHO Classification)
- Tissue management by pathologists
International Association for the Study of Lung Cancer/American Thoracic Society/European Respiratory Society International Multidisciplinary Classification of Lung Adenocarcinoma

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Major Changes of Proposed Classification

- Stop usage of “bronchioalveolar carcinoma”
- Addition of minimally invasive carcinoma
- Classification of invasive carcinoma according to predominant subtype

Journal of Thoracic Oncology, Vol. 6, Number2, February 2011
MAJOR POINTS

- Small bx/cytology section-molecular revolution
  EGFR mutation & ALK rearrangement
- Bronchioloalveolar carcinoma replaced by 5 different Ad subtypes
- Adenocarcinoma in situ and Minimally invasive adenocarcinoma are new lesions with a 100% disease free survival if completely resected
- Invasive Ad classified by predominant subtype
- Invasive mucinous Ad replaces mucinous BAC
- Multidisciplinary correlation (esp. radiologic - pathologic correlation) is essential (size, TNM)
IASLC/ATS/ERS ADENOCARCINOMA CLASSIFICATION

- PREINVASIVE LESIONS
  - ATYPICAL ADENOMATOUS HYPERPLASIA
  - ADENOCARCINOMA IN SITU (≤3 cm, formerly BAC pattern) †
    - non-mucinous
    - mucinous
- MINIMALLY INVASIVE ADENOCARCINOMA (≤3 cm, a lepidic predominant tumor with ≤5mm invasion)

- INVASIVE ADENOCARCINOMA
  † Size should be specified. AIS and MIA should be completely sampled histologically
Adenocarcinoma in situ (AIS) which can be non-mucinous and rarely mucinous

2. Minimally invasive adenocarcinoma

3. Invasive adenocarcinoma with predominant nonmucinous lepidic pattern

4. Invasive adenocarcinoma with less than predominant nonmucinous lepidic pattern (probably most formerly clinically advanced adenocarcinomas with BAC pattern)

5. Invasive mucinous adenocarcinoma
ADENOCARCINOMA IN SITU NONMUCINOUS
MINIMALLY INVASIVE ADENOCARCINOMA NONMUCINOUS
IASLC/ATS/ERS ADENOCARCINOMA CLASSIFICATION

INVASIVE ADENOCARCINOMA

- **Lepidic pattern predominant** (formerly non-mucinous BAC pattern)
- Acinar pattern predominant
- Papillary pattern predominant
- Micropapillary pattern, predominant
- **Solid pattern predominant**

*(Comprehensive histologic subtyping: semiquantitative assessment of patterns in 5-10% increments)*
LEPIDIC PREDOMINANT
INVASIVE MUCINOUS ADENOCARCINOMA
ADENOCARCINOMA VARIANTS
1999 & 2004 WHO

- Fetal adenocarcinoma (WDFA/99)
- Mucinous ("colloid")
- Mucinous cystadenocarcinoma
- Signet ring
- Clear cell
SIGNET RING FEATURES

- SOLID
- PAPILLARY
- ACINAR
- MICROPAPILLARY
NON-SMALL CELL LUNG CANCER: 70% PRESENT IN ADVANCED STAGE
SPECIAL STAINS ARE INTRODUCED TO CLASSIFY NSCLC-NOS FURTHER
### IASLC/ATS/ERS TERMINOLOGY FOR SMALL BIOPSIES AND CYTOLOGY

<table>
<thead>
<tr>
<th>2004 WHO CLASSIFICATION</th>
<th>2011 IASLC/ATS/ERS CLASSIFICATION</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>ADENOCARCINOMA</strong></td>
<td></td>
</tr>
<tr>
<td>Mixed subtype</td>
<td>Morphologic adenocarcinoma patterns clearly present: Adenocarcinoma, describe identifiable patterns present (including micropapillary pattern not included in 2004 WHO classification)</td>
</tr>
<tr>
<td>Acinar</td>
<td></td>
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<tr>
<td>Papillary</td>
<td></td>
</tr>
<tr>
<td>Solid</td>
<td></td>
</tr>
<tr>
<td>Bronchioloalveolar</td>
<td></td>
</tr>
<tr>
<td>No 2004 WHO counterpart – most will be solid adenocarcinomas</td>
<td>Morphologic adenocarcinoma patterns not present (supported by special stains): Non-small cell carcinoma, favor adenocarcinoma</td>
</tr>
</tbody>
</table>

| **SQUAMOUS CELL CARCINOMA** |                                   |
| Papillary                  | Morphologic squamous cell patterns clearly present: Squamous cell carcinoma |
| Clear cell                 |                                   |
| Small cell                 |                                   |
| Basaloid                   |                                   |
| No 2004 WHO counterpart    | Morphologic squamous cell patterns not present (supported by stains): Non-small cell carcinoma, favor squamous cell carcinoma |

| **LARGE CELL CARCINOMA**   |                                   |
|                           | Non-small cell carcinoma, not otherwise specified (NOS) |
IMMUNOHISTOCHEMICAL MARKERS

- **ADENOCARCINOMA** *(ONE MARKER)*
  - TTF-1 *(best)*, Napsin, PE-10

- **SQUAMOUS CARCINOMA** *(ONE MARKER)*
  - p40 *(best)*, p63, CK5/6, 34βE12
  - Desmocolin-3 *(need more testing)*

- **Cocktails** – nuclear/cytoplasmic antibodies
  - Adenoca – TTF-1/Napsin
  - Squamous – p63/CK5/6
Differentiate primary pulmonary adenocarcinoma from metastatic carcinoma

Differentiate adenocarcinoma from squamous cell carcinoma

Distinguish adenocarcinoma from mesothelioma

Determine the neuroendocrine status of the tumor

*Immunohistochemical stains*

NCCN Guidelines Version 2.20, 2013
Identification of driver mutations in tumor specimens from 1,000 patients with lung adenocarcinoma: The NCI’s Lung Cancer Mutations Consortium (LCMC)

- **KRAS** 107 (25%)
- **EGFR** 98 (23%)
- **ALK** rearrangements 14 (6%)
- **BRAF** 12 (3%)
- **PIK3CA** 11 (3%)
- **MET** amplifications 4 (2%)
- **HER2** 3, (1%)
- **MEK1** 2(0.4%)
- **NRAS** 1 (0.2%)
- **AKT1** 0(0%)

In 60% tumor driver mutation detected

*J Clin Oncol 29: 2011 (suppl; abstr CRA7506)*
Lung Adenocarcinoma
Activating Oncogenes

- Deletion and point Mutations
  - KRAS (30%)
  - EGFR (15%)

- Gene Amplification
  - EGFR (6-9%)

- Chromosomal rearrangement
  - EML4-ALK (5%)
  - ROS1 (2%)

EGFR, EML 4-ALK and KRAS are mutually exclusive
Molecular Testing Guideline for EGFR and ALK Tyrosine Kinase Inhibitors: Guideline from the College of American Pathologists, International Association for the Study of Lung Cancer, and Association for Molecular Pathology.

Archives of Pathology & Laboratory Medicine
June 2013, Vol. 137, No. 6, pp. 828-860
Lung Carcinoma

*Distinction is critical between:*

- Adenocarcinoma
- Pure squamous cell carcinoma
- Pure small cell carcinoma
- Pure neuroendocrine carcinoma

For EGFR and Alk testing
Lung Carcinoma

Lung carcinoma with mixed histology (adenosquamous, adeno/small cell) can have EGFR mutation or Alk rearrangement. Testing is required if possibility of adenocarcinoma component cannot be excluded.
It is important to retain sufficient tissue for molecular testing after establishing diagnosis of adenocarcinoma.
EGFR mutations are seen more common (50%) in:

- Women
- Never smoker
- Asian

Selection of patients for EGFR mutation testing is dependent on subtype of lung cancer not on clinical information.
Common Mutations Identified in **EGFR** Gene

**EGFR transcript**
- Exons 1–16
- Exon 17
- Exons 18–24
- Exons 25–28

**EGFR TK domain (exons 18-21)**
- 18
- 19
- 20
- 21

- G719
- Deletions
- D770_N771 insNPG
- T790M
- L858R
- L861

EGFR TK Mutations

Common

- Exon 19 in-frame deletion
- Exon 21 L858R mutation (Lysine to Arginine)

Both mutations result in activation of TK domain and associated with sensitivity to TKI.
EGFR Mutations

- Exon 18 Gly719 (sensitive)
- Exon 19 deletion (sensitive)
- Exon 20 insertion (resistance)
- Exon 20 Thr790Met (acquired resistance)
- Exon 21 Leu858Arg (sensitive)
Frequency of EGFR Mutations in Lung Adenocarcinoma

- 32% in East Asia
- 7-15% in Caucasians
- 2% in African America
- About 30% in Saudi population (unpublished data)

Resistance to EGFR-TKIs

- **Primary resistance**
  - KRAS mutations and Alk gene rearrangement
  - EGFR mutations not sensitive to EGFR TKIs (rare, ~2%) – ex 20 insertion
  - BRAF mutations (rare, ~3%)

- **Acquired resistance**
  - Second EGFR mutation: T790M (50% of cases)
  - MET amplification (some)
  - Pi3k mutations
  - Transformation to small cell lung ca
Tissue Sampling Methods in NSCLC

Three main methods of obtaining tumour samples

- Excised during surgery
- Bronchoscopic biopsy (for central lesions)
- Guided needle biopsy (for peripheral lesions)

Preservation of DNA is essential (e.g. formalin-fixed, paraffin-embedded tumour sample)

Preferably use primary tumour tissue
  - when this is not available, may consider metastatic tissue, pleural effusion or blood
Testing for Mutation

Tumor Sample Collection

Sectioning (at least 50% tumors)

DNA Extraction

Amplification

Sequencing
Limitations of Mutation Detection by Direct Sequencing

- Sequencing will not detect proportions of tumor cells below the sensitivity level (25%).
- Microdissection routinely used to increase tumor content (eliminate non-neoplastic areas).
- Blocks or unstained sections for DNA extraction should be from the most cellular areas with >50% tumor cells.
- Select sections without excessive inflammatory response.
Adequacy of EGFR Testing

- Adequacy is determined by malignant cells content and DNA quality and not sample type.
- Specimen should be fixed in 10% NBF for 6-48 hours.
- Cell blocks are preferred over smears for cytology samples.
ALK-rearranged Adenocarcinoma

- 2-7% of adenocarcinomas
- Younger patients
- Never smoking
- Higher stage
- Solid tumor growth, frequent signal cells with abundant intracellular mucin

Similar to EGFR mutation positive patient except they are younger and male
### SIGNAL CLASSIFICATION

<table>
<thead>
<tr>
<th>Patterns observed in native ALK</th>
<th>Patterns observed in split 3'-5' ALK</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image1" alt="Pattern 1" /></td>
<td><img src="image2" alt="Pattern 2" /></td>
</tr>
<tr>
<td><img src="image3" alt="Pattern 3" /></td>
<td><img src="image4" alt="Pattern 4" /></td>
</tr>
</tbody>
</table>

- **Red and green separated by <2 signal diameters**
  - Classified as negative
- **Red and green separated by ≥2 signal diameters**
  - Classified as positive
- **Red and green separated by ≥2 signal diameters**
  - Classified as positive
- **Red and green separated by <2 signal diameters**
  - Classified as negative

### SPECIMEN CLASSIFICATION

- **Nonrearranged tumors:**
  - Rearrangement-positive cell rate <15% of cells
- **Rearranged tumors:**
  - Rearrangement-positive cell rate ≥15% of cells
1st Reader-50 tumor cells

- >50% Positive
- 10%-50% Positive
- <10% Positive

Equivocal

2nd Reader-50 tumor cells

- 1st + 2nd Readers-100 tumor cells

- ≥15% Positive: Specimen is positive for ALK rearrangement
- <15% Positive: Specimen is negative for ALK rearrangement