Histopathology of NSCLC, IHC markers and WHO classification
WHO Classification

1967 HE
1981 HE & Mucin
1999 HE, Mucin & IHC
2004 HE, Mucin, IHC & Genetics
   for resections

2015 HE, Mucin, IHC, Genetics
   & Radiology
   includes small specimens
Small Samples: NSCLC Subtyping

- Established morphological criteria present:
  - Glandular differentiation and/or mucin → ADC
  - Intercellular bridges and/or keratinization → SCC

- Established morphological criteria absent – do IHC
Adenocarcinoma
Squamous cell carcinoma
Small Samples: NSCLC Subtyping

- Established morphological criteria present:
  - Glandular differentiation and/or mucin → ADC
  - Intercellular bridges and/or keratinization → SCC

- Established morphological criteria absent – do IHC:
  - TTF1+ (NapsinA) → NSCC, favor ADC
  - p40+ (p63, CK5/6) → NSCC, favor SCC
  - Inconclusive → NSCC, NOS
Immunohistochemistry in NSCLC

**Diagnostic**

- Primary vs. Metastasis
  - Breast: ER/GATA7, Colon: CDX2, Bladder: GATa3/CK20, Melanoma: S100/MelanA etc.
- Subtyping of NSCLC

**Predictive**

- ALK / ROS1
- PD-L1
- BRAF (V600E mutation specific Ab)
- EGFR (mutation specific AB)
Adenocarcinoma  Squamous cell carcinoma

TTF1  p40
Lung Cancer: Immunocytochemical Subtyping

Diagnosis: NSCLC, favor SqCC  NSCLC, favor AC
Adenocarcinoma / AC phenotype

Mutations analysis (NGS)
- EGFR
- KRAS
- HER2
- BRAF

Immunohistochemistry
- ALK
- ROS1

Swiss Lung Pathology Group Recommendations 2014

EGFR  KRAS  HER2  BRAF

ALK         ROS1

ALK FISH

ROS FISH

RET FISH
ALK Immunohistochemistry

- Screening for subsequent FISH analysis
- May replace FISH
<table>
<thead>
<tr>
<th></th>
<th>BenchMark XT</th>
<th>BOND-MAX</th>
</tr>
</thead>
<tbody>
<tr>
<td>D5F3</td>
<td>5A4</td>
<td>5A4</td>
</tr>
</tbody>
</table>

![Image of tissue sections](image_url)
ROS1 Immunzytochemie
(D4D6 Antikörper, Cell Signaling, BondMax Immunostainer)

HCC78
(positive control)
PD-L1 immunohistochemistry
- The candidate predictive marker for anti PD1/PD-L1 treatment -

Kerr et al, J Thorac Oncol 2015; 10:985
Resected NSCLC

• New classification of differentiated NSCLC
  – Adenocarcinoma
  – Squamous cell carcinoma

• New classification of undifferentiated NSCLC
  – Large cell carcinoma
Adenocarcinoma

2004 WHO

- Mixed subtype \(>90\%\)
- Acinar
- Papillary
- BAC
- Solid
- Variants

No established grading criteria
## Adenocarcinoma

<table>
<thead>
<tr>
<th>2004 WHO</th>
<th>2015 WHO</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Mixed subtype</td>
<td>• Preinvasive lesions</td>
</tr>
<tr>
<td>• Acinar</td>
<td>– Atypical adenomatous hyperplasia</td>
</tr>
<tr>
<td>• Papillary</td>
<td>– AIS (≤ 3cm) (≈ 0.2%)</td>
</tr>
<tr>
<td>• BAC</td>
<td>• Minimally invasive ADC (MIA)</td>
</tr>
<tr>
<td>• Solid</td>
<td>– ≤ 3cm with ≤ 5mm invasion (≈ 3%)</td>
</tr>
<tr>
<td>• Variants</td>
<td>• Invasive ADC</td>
</tr>
<tr>
<td></td>
<td>– G1: Lepidic (≈ 10%)</td>
</tr>
<tr>
<td></td>
<td>– G2: Acinar, papillary</td>
</tr>
<tr>
<td></td>
<td>– G3: Micropapillary, solid</td>
</tr>
<tr>
<td></td>
<td>• Variants</td>
</tr>
</tbody>
</table>
Atypical adenomatous hyperplasia (AAH) ≤ 5mm
Minimally invasive Adenocarcinoma

≤ 3cm
lepidic predominant

≤ 0.5cm invasion:
– invasive subtypes
– tumor cells infiltr. stroma

~ 100% disease-free survival
Minimally invasive Adenocarcinoma

≤ 3cm
lepidic predominant

≤ 0.5cm invasion:
– invasive subtypes
– tumor cells infiltr. stroma

no invasion of lymphatics, blood vessels or pleura, no tumor necrosis
Pleural invasion = Infiltration beyond elastic layer
→ Lepidic predominant invasive ADC
Invasive Adenocarcinoma

Acinar predominant

Papillary predominant

Micropapillary predominant

Solid predominant with mucin

Semiquantitative assessment in 5-10% increments
>50% („BAC“)
lepidic

Anami et al
JTO 2009

Warth A, J Clin Oncol 2012

Histologic pattern of AC & prognosis
Spread Through Alveolar Spaces (STAS)

40-50% of AC

Travis WD, ECC 2014
Spread Through Alveolar Spaces (STAS)

Recurrence

Lobectomy group

Limited resection group

Kadota K et al, J Throac Oncol 2015
Adenocarcinomas: major points

- Small biopsies & cytologies: molecular revolution
- BAC replaced by 5 different AC subtypes
- AIS/MIA new lesions with a 100% DSF if resected
- Invasive AC classified by predominant subtype
- Invasive mucinous AC replaces mucinous BAC
- Multidisciplinary correlation is essential
Large Cell Carcinoma

2004 WHO

- LCC
- LCNEC
- Basaloid carcinoma
- Lymphoepithel.-like CA
- Clear cell CA
- Rhabdoid phenotype
# Large Cell Carcinoma
Redefined by Immunohistochemistry and Genomics

<table>
<thead>
<tr>
<th>Reference</th>
<th># of cases</th>
<th>Immunohistochemistry panel</th>
<th>Other studies</th>
<th># reclassified as ADC</th>
<th># reclassified as SqCC</th>
<th># unclassified</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monica et al. [12]</td>
<td>54</td>
<td>DSC3, TTF-1</td>
<td>N/A</td>
<td>24 (44%)</td>
<td>26 (48%)</td>
<td>4 (8%)</td>
</tr>
<tr>
<td>Barbareschi et al. [51]</td>
<td>56</td>
<td>TTF-1, p63, CK5, CK7, Napsin A, p40, DSC3</td>
<td>miR205 and miR21 profiling</td>
<td>19 (34%) with IHC alone</td>
<td>14 (25%) with IHC alone</td>
<td>23 (41%) with IHC alone</td>
</tr>
<tr>
<td>Rekhtman et al. [11**]</td>
<td>102</td>
<td>TTF-1, p40</td>
<td>N/A</td>
<td>62 (61%)</td>
<td>20 (20%)</td>
<td>20 (20%)</td>
</tr>
<tr>
<td>Rossi et al. [52*]</td>
<td>74</td>
<td>TTF-1, p63, CK5/6, CK7, Napsin A, p40, DSC3, chromogranin, synaptophysin, CD56</td>
<td>N/A</td>
<td>40 (80%)</td>
<td>6 (12%)</td>
<td>4 (8%)</td>
</tr>
</tbody>
</table>

ADC: 55%
SqCC: 26%
Null: 19%

## Large Cell Carcinoma

### 2004 WHO

- LCC
- LCNEC
- Basaloid carcinoma
- Lymphoepithel.-like CA
- Clear cell CA
- Rhabdoid phenotype

### 2015 WHO

- LCC
  - Null-IHC features
  - With no stains available
- TTF1+ $\rightarrow$ solid ADC
- P40 + $\rightarrow$ non-keratinizing SCC
Large Cell Carcinoma

<table>
<thead>
<tr>
<th>2004 WHO</th>
<th>2015 WHO</th>
</tr>
</thead>
<tbody>
<tr>
<td>• LCC</td>
<td>• LCC (mucin -, null-IHC)</td>
</tr>
<tr>
<td>• LCNEC</td>
<td>• NE Tumors</td>
</tr>
<tr>
<td>• Basaloid carcinoma</td>
<td>• SqCC</td>
</tr>
<tr>
<td>• Lymphoepithel.-like CA</td>
<td>• Other carcinomas</td>
</tr>
<tr>
<td>• Clear cell CA</td>
<td>• Cytol. pattern, not subtype</td>
</tr>
<tr>
<td>• Rhabdoid phenotype</td>
<td>• Cytol. pattern, not subtype</td>
</tr>
</tbody>
</table>
## Squamous Cell Carcinoma

<table>
<thead>
<tr>
<th>2004 WHO</th>
<th>2015 WHO</th>
</tr>
</thead>
<tbody>
<tr>
<td>• SqCC</td>
<td>• Keratinizing</td>
</tr>
<tr>
<td></td>
<td>• Non-keratinizing (p40+/TTF1-)</td>
</tr>
<tr>
<td></td>
<td>• Basaloid CA (p40+/TTF1-)</td>
</tr>
</tbody>
</table>
Summary

- Small samples
  - Save tissue for predictive markers. Also use cytology
- ADC
  - Defined by morphology or TTF-1 expression
- SqCC
  - Defined by morphology or p40 expression
- LCC
  - Only on resections
  - No squamous, adeno- or neuroendocr. diff. by morphology and IHC
Thank you