Histopathology of NSCLC, IHC markers and pTNM classification

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The management of patients with lung cancer is becoming ever more dependant on a knowledge of the pathology of each patient’s disease.

‘Know your enemy’

Sun Tzu, The Art of War.
The management of patients with lung cancer is becoming ever more dependant on a knowledge of the pathology of each patient’s disease.

Lung cancer is:

• NOT a single disease
• NOR is it just two diseases: Small Cell Carcinoma and Non-small Cell Carcinoma
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Lung cancer is
• NOT a single disease
• NOR is it just two diseases: Small Cell Carcinoma and Non-small Cell Carcinoma

Non-Small Cell Carcinoma is NOT a specific biological entity
• more a classification of convenience driven by a lack of therapeutic choice
2015 WHO Classification of Lung Tumours (part 1!!)

• **1-2: Adenocarcinoma**
  - 1-2A Invasive adenocarcinoma
  - 1-2B Variants of invasive adenocarcinoma
  - 1-2C Minimally invasive adenocarcinoma
  - 1-2D Preinvasive lesions
    - 1-2D-i: Atypical adenomatous hyperplasia
    - 1-2D-ii: Adenocarcinoma in situ

• **1-3: Squamous cell carcinoma**
  - 1-3A: Keratinizing and nonkeratinizing squamous cell carcinoma
  - 1-3B: Basaloid carcinoma
  - 1-3C: Preinvasive lesion: Squamous ca in situ

• **1-4: Neuroendocrine Tumours**
  - 1-4A: Small cell carcinoma
  - 1-4B: Large cell neuroendocrine carcinoma
  - 1-4C: Carcinoid tumors
  - 1-4D: Preinvasive lesion: DIPNECH

• **1-5: Large cell carcinoma**

• **1-6: Adenosquamous carcinoma**

• **1-7: Sarcomatoid carcinoma**
  - 1-7A: Pleomorphic, spindle cell and giant cell carcinoma
  - 1-7B: Carcinosarcoma
  - 1-7C: Pulmonary blastoma

• **1-8: Other carcinomas**
  - 1-8A: Lymphoepithelioma-like carcinoma
  - 1-8B: NUT-carcinoma
Small Cell Carcinoma of the Lung

- Nuclear features key to diagnosis
- Neuroendocrine markers and TTF1 IHC positive but not required for diagnosis
- Accurately diagnosed on cytology
- Aggressive disease, usually Stage 4 at presentation

- Therapeutic relevance
  - chemotherapy choice
  - radiotherapy strategy
  - prognosis
So all those other, biologically diverse malignant diseases are NOT small cell carcinomas – so we call them non-small cell carcinoma (NSCLC)

- Adenocarcinoma
- Squamous cell carcinoma
- Neuroendocrine tumours apart from SCLC
- Large Cell Carcinoma
- Adenosquamous Carcinoma
- Sarcomatoid Carcinomas
- Others
There are at least two pathways of Lung Carcinogenesis

- Bronchial Squamous Dysplasia
- Atypical Adenomatous Hyperplasia (AAH)
- Invasive Squamous Cell carcinoma
- Invasive Adenocarcinoma
- Adenocarcinoma-in-situ
- Squamous carcinoma-in-situ
Central Bronchial Carcinogenesis?

The progenitor cells express p63, p40, Cytokeratins 5&6

Peripheral airway Carcinogenesis?

The progenitor cells Express TTF1

The Terminal Respiratory Unit TRU

Lung stem cells
Darrell N. Kotton - Alan Fine
Adenocarcinoma

• Commonest subtype of lung cancer
• Associated with tobacco carcinogenesis
• Commonest subtype **by far** in never smokers
• Addictive oncogenic drivers are frequent in adenocarcinomas NOT associated with tobacco carcinogenesis

• Relatively inaccurately diagnosed by morphology alone
• ONLY 75-80% express TTF1

• Therapeutic relevance
  • Chemotherapy choice, Surgery choice
  • Anti-angiogenic agents for safety and efficacy
  • Testing for addictive oncogenic targets
  • Testing strategy for immuno-oncology therapy?
Five histological patterns of adenocarcinoma: Most cases are mixtures, Pure forms are rare
Post operative survival vs predominant pattern in pulmonary adenocarcinoma

AIS, MIA

Lepidic

Acinar

Papillary

Solid, Micropapillary

‘High Grade’ Adenocarcinoma Histology and benefit from Adjuvant chemotherapy

Yoshizawa A et al. Mod Pathol 2011; 24, 653-664
Russell PA et al. J Thorac Oncol 2011; 6,1496-1504
Warth A et al. J Clin Oncol 2012; Mar 5 epub
Squamous Cell Carcinoma

- Still common in populations who smoke
- Archetypal cancer of central, bronchial tobacco-driven carcinogenesis
- Rare in never smokers; rarely driven by addictive oncogene
- Relatively accurately diagnosed by morphology
- Most strongly express p63, p40, CK5/6

Therapeutic relevance
- Chemotherapy choice
- Toxicity and efficacy of anti-angiogeneics
- Choice of molecular testing
- Immunotherapy decisions
Neuroendocrine tumours other than SCLC

Large Cell Neuroendocrine Carcinoma (LCNEC)
• High grade neuroendocrine carcinoma
• Strongly associated with tobacco carcinogenesis
• Molecularly similar to SCLC
• Generally a diagnosis for surgically resected tumours only, however..........
• Requires immunohistochemistry

• Therapeutic relevance
  • Chemotherapy choice?
  • Uncertainty due to diagnostic problems in advanced disease
Neuroendocrine tumours other than SCLC

**Typical Carcinoid**
- Usually central bronchial tumour, Obstructive pneumonia
- Paradoxical lesion
  - Low grade, wrong location
- Metastatic disease rare
  - 10% regional nodes
  - Distant metastases very rare

**Atypical Carcinoid**
- Very rare, Relatively aggressive
- Mitoses & necrosis – area dependent: $2\text{mm}^2$

**Diagnosis on small samples vs surgical material**

**Therapeutic relevance**
- Context important
  - bronchial polyp or peripheral nodule
- Confusion with SCLC in biopsy or cytology
Large Cell Carcinoma

- ONLY diagnosed on surgical resection
- NEVER a diagnosis on small biopsy or cytology
- Most cases (66%) re-assigned as squamous or adenocarcinoma by IHC (WHO 2015)

- Therapeutic relevance
  - Relatively aggressive tumour
  - KRAS mutation dominant
Adenosquamous Carcinoma

- Relatively rare tumour
- Relatively aggressive tumour
- Peripheral or central?
- Requires minority component to comprise at least 10% of the lesion
- A surgical resection diagnosis
  - Small biopsy or cytology suspicion only
  - Morphology vs IHC

- Therapeutic relevance
  - Manage like adenocarcinoma
Sarcomatoid Carcinoma

• Very rare lesions

• **Pleomorphic carcinoma** if >10% of lesion shows pleomorphic, spindle or giant cells

• Usually combined with squamous cell or adenocarcinoma

• Surgical resection for definitive diagnosis

• Therapeutic relevance
  • Chemoresistant
  • KRAS mutations relatively frequent
  • Found in TKI-recurrent disease
  • MET exon14 skipping mutations
The subtyping accuracy of NSCLCs in small biopsy and cytology was inaccurate by morphology alone

• Previous WHO classifications not designed for small samples
• This drove the adoption of the NSCLC-NOS diagnosis
• Which became a problem when therapy diversified by histology

• Most NSCLC-NOS diagnosis came from differentiated tumours
  • 67% were adenocarcinoma when resected Edwards S et al 2000

• Immunohistochemistry has transformed this diagnostic landscape
  • NSCLC-NOS rates should be <10% cases
Bronchial Basal cells express p63, p40 and CK5/6

Invasive Squamous Cell Carcinoma (~100% express these markers)

Normal

Dysplasia

Squamous CIS

TRU epithelium expresses TTF1

Normal

AAH

AIS

Invasive Adenocarcinoma (75-80% express TTF1)
NSCLC – probably adenocarcinoma

Tumour cells express Nuclear p63

TTF1 positive in tumour cell nuclei

NSCLC – probably squamous cell
Subtyping NSCLC: How good?

➢ Predictive IHC has ‘levelled the playing field’
➢ Better diagnosis possible on poorer specimens
# Lung Cancer Classification and sample type

<table>
<thead>
<tr>
<th>WHO 2004 (et prev): intended for, and only applicable to, resected cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Small Cell Carcinoma</td>
</tr>
<tr>
<td>• Squamous Cell Carcinoma</td>
</tr>
<tr>
<td>• Adenocarcinoma</td>
</tr>
<tr>
<td>• Large cell carcinomas</td>
</tr>
<tr>
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</tr>
<tr>
<td>• Adenosquamous carcinomas</td>
</tr>
<tr>
<td>• Carcinoid tumours</td>
</tr>
<tr>
<td>• Salivary-type carcinomas</td>
</tr>
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</table>

<table>
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<th>WHO 2015: a simplified classification intended for small sample diagnosis</th>
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</thead>
<tbody>
<tr>
<td>• Small Cell Carcinoma</td>
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<tr>
<td>• Squamous Cell Carcinoma</td>
</tr>
<tr>
<td>• Probable Squamous Cell Ca</td>
</tr>
<tr>
<td>• Adenocarinoma</td>
</tr>
<tr>
<td>• Probable Adenocarcinoma</td>
</tr>
<tr>
<td>• NSCLC-NOS</td>
</tr>
<tr>
<td>• NSCLC-NOS (null IHC)</td>
</tr>
<tr>
<td>• Carcinoid tumour</td>
</tr>
<tr>
<td>• Salivary-type (occasionally)</td>
</tr>
</tbody>
</table>
Testing algorithm

2 x 1mm tissue fragments

On average only 20% is tumour

Diagnose & subtype lung cancer
Squamous Adeno etc

Immuno-Histochemistry IHC If required

Biomarker testing dictated by histology and protocol

Sections for DNA extraction

EGFR, KRAS, BRAF mutation (NGS panels)

Sections for Biomarker IHC & FISH

ALK, ROS1 EGFR PD-L1

Morphology-based tests

‘Test tube’ tests

IHC should be used SPARINGLY for diagnosis
pTNM classification (7th edition: adopted by UICC and AJCC)
New proposals for TNM8 by IASLC – from Jan 2017

• Tumour Nodes Metastases
• pTNM based upon pathological examination
Carcinoma in situ: pTis

Squamous Carcinoma In situ

Adenocarcinoma In situ - AIS
Pulmonary nodule with small solid area surrounded by GGO
This focus must be LESS THAN 5mm dia

Most of this lesion is In situ disease

Minimally Invasive Adenocarcinoma
MIA – pT1a(mi)

Focus of Invasive Patterns of Adenocarcinoma

This focus must be LESS THAN 5mm dia

pT1a(mi)
The lesion MUST NOT Involve a main bronchus

**pT1a**

\[ \leq 2\text{cm} \]

**pT1b**

\[ >2\text{cm but } \leq 3\text{cm} \]

**TNM7**
The lesion involving the main bronchus is a superficial spreading lesion with invasion limited to the bronchial wall – pT1a
pT1a
≤ 1cm

pT1b
>1cm but ≤ 2cm

pT1c
>2cm but ≤ 3cm

TNM8
T1a
T1b
T1c
T2: tumours over 3cm but ≤ 7cm

pT2a  >3cm ≤ 5cm

pT2b  >5cm ≤ 7cm
TNM8 - T2: tumours over 3cm but ≤ 5cm

pT2a  >3cm ≤ 4cm

pT2b  >4cm ≤ 5cm
Pleural invasion upstages a tumour to pT2a
Regardless of size. This tumour is associated with atelectasis of a whole lobe. Obstructive pneumonitis extending to the hilar region but not involving the whole lung.
Regardless of size, this tumour is associated with atelectasis of a whole lobe, obstructive pneumonitis extending to the hilar region, or involving the whole lung.
Small tumour but it involves main bronchus

T2a

Tumour > 2cm from carina
Small tumour but it involves main bronchus

Regardless of distance from the Carina as long as it is not involved

T2a
TNM7

pT3

> 7cm

85mm
TNM8

T3 category SIZES changes to

- 5 cm but
- no greater than 7 cm
Chest wall

Invasion of Mediastinal pleura
Chest wall
Superior sulcus
Phrenic nerve
Diaphragm
Parietal pericardium

Main bronchus within 2cm of carina

TNM7

pT3
pT3

Atelectasis or Obstructive Pneumonitis of the entire lung

‘downgraded’ to T2
In TNM8
pT3

‘Same lobe’
Intrapulmonary metastases
Satellite nodules may be histological findings but there is no definition of a ‘nodule’
pT4 Invasion of
Mediastinal structures
Heart
Great vessels
Trachea
Recurrent laryngeal nerve
Oesophagus
Vertebral body
Carina
pT4
‘Different ipsilateral lobe’
Intrapulmonary metastases

Issues with Pulmonary Metastases versus synchronous Primary tumours
TNM8

>7cm is now T4

pT4

85mm
Which part or element of adenocarcinoma should be measured to determine T status?

- Invasive tumour
- Lepidic growth
- Adenocarcinoma in situ
pN disease identified by pathological examination
Histology can define node positive disease
M1a

Contralateral lung metastases
Pleural nodules
Malignant pleural or pericardial effusion

M1b

Distant Metastases
• Liver, Adrenals, Bone, Brain, Skin, etc etc
• Cervical nodes above scalene are also M1

M1a or M1b defines Stage 4 disease
<table>
<thead>
<tr>
<th>M1a</th>
<th>M1b</th>
<th>M1c</th>
</tr>
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<td>Contralateral lung metastases</td>
<td>Single extrathoracic metastasis is a single organ (incl lymph node)</td>
<td>Distant Metastases</td>
</tr>
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<td></td>
<td>• Liver, Adrenals, Bone, Brain, Skin, etc etc</td>
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<tr>
<td>Malignant pleural or pericardial effusion</td>
<td></td>
<td>• Cervical nodes above scalene are also M1</td>
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M1a or M1b defines Stage IVa
M1c defines Stage IVb
TNM defines Stage – Stage defines prognosis

**A**

**TNM7**

<table>
<thead>
<tr>
<th>7th Ed.</th>
<th>Events / N</th>
<th>MST</th>
<th>24 Month</th>
<th>60 Month</th>
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<tbody>
<tr>
<td>IA</td>
<td>1837 / 11423</td>
<td>NR</td>
<td>94%</td>
<td>83%</td>
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<tr>
<td>IB</td>
<td>2168 / 7711</td>
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<td>71%</td>
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<tr>
<td>IIA</td>
<td>1514 / 3702</td>
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<td>57%</td>
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**B**

**TNM8**

<table>
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<th>Proposed</th>
<th>Events / N</th>
<th>MST</th>
<th>24 Month</th>
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<tbody>
<tr>
<td>IA1</td>
<td>139 / 1389</td>
<td>NR</td>
<td>97%</td>
<td>90%</td>
</tr>
<tr>
<td>IA2</td>
<td>823 / 5633</td>
<td>NR</td>
<td>94%</td>
<td>85%</td>
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<tr>
<td>IA3</td>
<td>875 / 4401</td>
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<td>92%</td>
<td>80%</td>
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<tr>
<td>IB</td>
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<tr>
<td>IIA</td>
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<td>IIB</td>
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<td>IIIC</td>
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<td>30%</td>
<td>12%</td>
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