Lung Cancer Diagnosis: Role of Pathology and Genetic analysis

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DISCLOSURE OF INTEREST

Consultancy
AbbVie, AstraZeneca, Bayer, Boehringer Ingelheim, Bristol-Myers Squibb,
Eli Lilly, Merck Serono, Merck Sharp & Dohme, Novartis,
Pfizer, Roche, Ventana

Honoraria (speaker)
AstraZeneca, Boehringer Ingelheim, Bristol-Myers Squibb,
Eli Lilly, Merck Serono, Merck Sharp & Dohme, Novartis,
Pfizer, Roche, Ventana
The management of patients with lung cancer is becoming ever more dependant on a knowledge of the pathology and genetics 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

‘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 and genetics 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

Non-Small Cell Carcinoma is NOT a specific biological entity
- more a classification of convenience that WAS driven by a lack of therapeutic choice

This is no longer the case..............
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
  - Emerging biomarker issues
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:

1. Atypical Adenomatous Hyperplasia (AAH) → Adenocarcinoma-in-situ → Invasive Adenocarcinoma
2. Bronchial Squamous Dysplasia → Squamous carcinoma-in-situ → Invasive Squamous Cell carcinoma
Two different stem cell populations in the lung

Bronchial Basal cells express p63, p40 and CK5/6

TRU epithelium expresses TTF1

Normal

Dysplasia

Squamous CIS

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

Invasive Adenocarcinoma (75-80% express TTF1)

TRU

Terminal Respiratory Unit
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**
- Surgery choice
- Chemotherapy choice (Pt & Pemetrexed)
- 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

Lepidic

Acinar

Papillary

Solid

Micropapillary
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 Stage 1 only
Russell PA et al. J Thorac Oncol 2011; 6,1496-1504 Stages 1-3
Warth A et al. J Clin Oncol 2012; Mar 5 epub Stages 1-4
Tsao MS et al JCO 2015
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 (Pt & gemcitabine)
  - Toxicity and efficacy of anti-angiogenesics
  - 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 in some cases
- Generally a diagnosis for surgically resected tumours only, however..........
- Requires immunohistochemistry

- Therapeutic relevance
  - Chemotherapy choice?
  - Uncertainty due to diagnostic problems in advanced disease
### Other tumour types

<table>
<thead>
<tr>
<th>Type</th>
<th>Therapeutic relevance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carcinoid tumours</td>
<td>- Diagnostic confusion with HGNEC on biopsy</td>
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<tr>
<td></td>
<td>- Central vs Peripheral disease</td>
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<tr>
<td>Large cell carcinoma</td>
<td>- Aggressive tumours</td>
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<tr>
<td>Adenosquamous carcinoma</td>
<td>- Manage like adenocarcinoma</td>
</tr>
<tr>
<td>Sarcomatoid carcinomas</td>
<td>- Chemoresistant</td>
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<tr>
<td></td>
<td>- KRAS mutations relatively frequent</td>
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<td></td>
<td>- Found in TKI-recurrent disease</td>
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<td></td>
<td>- MET exon14 skipping mutations</td>
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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 became 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
NSCLC – probably adenocarcinoma

TTF1 positive in tumour cell nuclei

Tumour cells express Nuclear p63

NSCLC – probably squamous cell

NSCLC – probably adenocarcinoma
Subtyping NSCLC: How good?

- Biopsy or Cytology plus IHC: 6%
- Cytology Morphology: 40%
- Biopsy Morphology: 25%

- Predictive IHC has ‘levelled the playing field’
- Better diagnosis possible on poorer specimens

p40 & TTF1

TWO MARKERS ONLY!!!
Histology-IHC diagnostic practice UK

About 20% centres have NOS rates over 10%

Wide range of IHC used

Lung Pathology accounts for anything from 1 – 13++ hours per week per pathologist surveyed.

Cane P et al. Histopathology 2015

IHC over-used
Lung Cancer Classification and sample type

**Resection diagnosis**

**WHO 2004 (et prev): intended for, and only applicable to, resected cases**
- Small Cell Carcinoma
- Squamous Cell Carcinoma
- Adenocarcinoma
- Large cell carcinomas
- Sarcomatoid carcinomas
- Adenosquamous carcinomas
- Carcinoid tumours
- Salivary-type carcinomas

**WHO 2015: a simplified classification intended for small sample diagnosis**
- Small Cell Carcinoma
- Squamous Cell Carcinoma
  - Probable Squamous Cell Ca
- Adenocarcinoma
  - Probable Adenocarcinoma
- NSCLC-NOS
  - NSCLC-NOS (null IHC)
- Carcinoid tumour
- Salivary-type (occasionally)
Diagnosis does not stop with tumour subtype

Primary Lung cancer

- Small cell lung cancer

- NSCLC

  - Squamous

  - Not squamous de facto Adenocarcinoma

Biomarker testing not routine, outside of clinical trials

PD-L1 IHC testing

Testing for Oncogenic drivers
- EGFR
- ALK
- ROS1
- BRAF

Therapy determined by Histology and/or Biomarker findings

No actionable target found

Reflex versus Bespoke testing
Who orders the testing?
Pathologist or Oncologist?
Diagnosis ➤ Testing algorithm – dual track

- On average only 20% is tumour
- Diagnose & subtype lung cancer
  - Squamous
  - Adeno etc
- Immuno-Histochemistry IHC
  - Only if required

Biomarker testing dictated by histology and protocol
- Sections for DNA extraction
- EGFR, BRAF mutation (NGS panels)

Test tube tests
- ALK
- ROS1
- PD-L1

Morphology-based tests
- Sections for Biomarker IHC & FISH

IHC should be used SPARingly for diagnosis

2 x 1mm tissue Fragments

IHC should be used SPARingly for diagnosis
Why Do We Need Biomarkers for therapy?

Precision medicine is a reality for many tumour types

Avoidance of harm?
- There are toxicities from these drugs
- Is there a subgroup of patients who fair worse on targeted treatment?
- Alternative treatment would be better

Not all patients respond to and benefit from all treatments
- Enrich the treatment population for benefit
- *How many to treat, to get one response?*
- Benefit is relative to standard of care

Financial burden of expensive therapy
The Hallmarks of Cancer.....

.....have become therapeutic targets

Virtually every new therapy for NSCLC in last 10 years is biomarker-dependant

Hanahan D, Weinberg RA. Cell 144; 2011
Mutational heterogeneity in cancer

- Vast majority of mutations are not drivers; not therapeutic targets
- Most lung cancers are ‘driven’ by multiple genomic changes; ‘oncogene expedience’
- However, high tumour mutation burden has become ‘item of interest’…………..

Enormous variation in mutations:
tobacco carcinogenesis

Lung carcinomas in smokers
are heavily mutated

“Driver” mutations as targets?

- **Oncogene expedience**
  - Commonest scenario
  - Smoking-induced
  - Limited therapeutic response

- **Oncogene addiction**
  - NOT tobacco-related (mostly)
  - Mutations or fusions
  - GOOD drug targets
  - Dramatic effects
  - Resistance

Genetically complex
Genetically simple
Adenocarcinomas, Targets and Therapy

Only four of these targets have agents through approval by FDA & EMA

- **EGFR mutation**
- **ALK rearrangement**
- **ROS1 rearrangement**
- **BRAF mutation**

Only a minority are ‘genetically simple’ with an addictive oncogenic driver, most are ‘genetically complex’

Squamous Cell Carcinomas, Targets and Therapy

Very few suitable targets – very few addictive oncogenes
The commonest alterations are inactivating mutations in tumour suppressor genes

Delta like 3 (DLL3) in SCLC

- In SCLC and some LCNEC ASCL1 induces overexpression of DLL3 & inhibits Notch 1
- DLL3 Antibody drug conjugate
  - (Pyrrolobenzodiazepine Dimer Toxin) (Rovalpituzumab tesirine- RovaT)
    - In mouse PDX SCLC durable response
    - Phase 1 in recurrent SCLC: 18% response,
    - Related to DLL3 IHC expression (>50% TPS)

Rudin C et al, Lancet Oncol 2017,18(42-51)
Updated Molecular Testing Guideline for the Selection of Lung Cancer Patients for Treatment With Targeted Tyrosine Kinase Inhibitors

Guideline From the College of American Pathologists, the International Association for the Study of Lung Cancer, and the Association for Molecular Pathology

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Metastatic non-small cell lung cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up†

D. Planchard, S. Popat, K. Kerr, S. Novello, E. F. Smit, C. Faivre-Finn, T. S. Mok, M. Reck, P. E. van Schil, M. D. Hellmann & S. Peters, on behalf of the ESMO Guidelines Committee

Arch Pathol Lab Med. doi: 10.5858/arpa.2017-0388-CP
J Mol Diagn. doi.org/10.1016/j.jmoldx.2017.11.004
Ann Oncol 29: 2018
Resistance mechanisms in TKI Recurrence

Emergent clones resistant to therapy
Induction of resistance mutations
Adoption of bypass tracks

Phenotypic alteration
Small cell, squamous cell or sarcomatoid carcinoma

EGFR TKI Resistance

ALK TKI Resistance
The Immune Response is a Very Complex Multifactorial ‘Reaction’

Somewhere in here there are predictive factors which will enrich for immunotherapy response!

The Tumour and the Host

Immunotherapy Biomarkers

Different ways of looking at the same problem?

- Tumour Mutational Burden or Surrogates
- Targeted panels
- Specific mutations
- MSI-High

PD-L1 IHC: a therapy specific biomarker

Gene-expression signature

Tumour micro-environment Tumour Inflammation

Chalmers ZR et al. Genomic Med 2017
Biomarkers - biological features associated with disease behaviour

Clinical Biomarkers are far from perfect

- Naïve approach
  - Single versus combination
- Binary factor
- Biological continuum
- Some therapy-predictive biomarkers are also prognostic….or are we misinterpreting data?

- How can we improve our biomarker analysis?
‘Biopsy’ techniques in lung cancer diagnosis

Handling the tissue in pathology

- Sputum cytology
- Bronchial brushings and washings
- Fluids
- FNA cytology / EBUS-EUS – primary or mets
- Transbronchial biopsy
- Bronchial biopsy
- Pleural biopsy
- Core biopsy – primary or mets
- Mediastinoscopy
- Lymph node excision
- VATS biopsy / resection
- Thoracotomy tumor excision

Increase in cell number and tissue architecture

Advances in imaging, endoscopy, and other Interventional techniques

General tendency towards less invasive techniques to secure a diagnosis

EBUS-EUS, endobronchial ultrasound-endoscopic ultrasound; FNA, fine-needle aspiration; VATS, video-assisted thoracoscopic surgery
Which level to test? How will you do it?

Change in DNA sequence?

- Mutation
- Rearrangement
- Gene copy number

mRNA transcript

- Fusion gene transcripts
- Gene expression levels
- Gene expression profiles

Protein expression

Biological activity
Oncogenesis
Drug target

‘Test tube’ or Morphology

Transcription

Translation

PROTEIN
Qualitative versus quantitative biomarker data

- PD-L1 protein by IHC
- Gene copy number
- >15% for ALK FISH
- Tumour mutational burden
- Gene expression signature thresholds
- Mutant allele frequency

➢ Combination of biomarkers may improve predictive power
Next-generation sequencing (NGS) and other ‘multiplex’ technology?

- **Multiplex testing is certainly the future**
- NGS: Multiple mutations *and* fusion genes *and* gene copy number
- Experience so far is mixed
  - Good solution to the ‘genomic biomarker shopping list’
  - Relatively costly (depends where you practice)
  - Requires more tissue?... and more time?
  - Generates lots of (incomprehensible) data

- **And what about proteins?**
  - Not covered by NGS
  - Multiplex IHC is a reality
  - Need for orthogonal testing?
Does the test represent the patient’s disease burden?

- The challenge of heterogeneity and sampling error
  - Lack of test sensitivity?
  - Real biology
  - Trial samples
- Can we solve the heterogeneity issue?
  - Maybe not
  - Biomarkers in blood?

- Test accuracy and consistency
  - Standardization
  - Laboratory Accreditation
  - External Quality Assurance

As predictive biomarkers proliferate, Standardization and EQA will become more important.
Is there enough material for all diagnosis?

- Morphologic diagnosis
- Immunohistochemistry
- Molecular testing
- Conserve tissue
- Don’t waste

On average only 10-25% of this tissue is tumour!

Two biopsy fragments <1mm
The plastic cassettes used for processing tissue are also used to support the paraffin wax embedded block.

Abundant tumour tissue in a block taken from a resected tumour.

Lung biopsy fragments 1mm or less.

Although each section shows 5 fragments, only two remain in the block (left), after sections are cut for IHC and molecular testing.

Cell pellet formed from EBUS procedure.

Sections cut from the block, mounted on glass slide and stained with Haematoxylin and eosin (H&E).
Currently, these morphology-based tests are immunohistochemistry and FISH assays.
Sections are marked to indicate the zone(s) with highest % tumour content.

A single section may give enough DNA for Tumour Genotyping.

The marked zone(s) are scraped from the slide and DNA extracted.

Three serial sections of small lung biopsy

There is tumour present

The lab knows how much!

Percentage of tumour nuclei in the sample

DNA or RNA
Testing platforms in use
Single gene tests or Multiplex testing (NGS)

Data generation
Data integration
Report generation

DNA and/or RNA extraction and related chemistry necessary for the particular tests or platforms in use
Is tissue testing finished?  

The ‘Liquid Biopsy’

Cell free DNA, not circulating tumour cells

- Easy access
- Monitoring disease progress
- Single mutations – Large screening panels
  - Known vs unknown
- Fewer processing issues?
- Huge dilutional effects
  - Need for uber-sensitive technology?
- Representative of the patients disease?
- Is the biomarker unique to the disease in question?
- Who is responsible – Pathologist or other?
Now and in the future...

Predictive Biomarker testing – NSCLC

- **EGFR** mutation
- **ALK** rearrangement
- **PD-L1 IHC**
- **ROS1** rearrangement
- **BRAF** mutation

- **HER2** mutation
- **KRAS** mutation
- **MET** amplification
- **MET** exon14 skip mutations
- **RET** rearrangement
- **NTRK1** rearrangement
- **NRG1** rearrangement
- **TMB** or surrogate
- Tumor “inflammation”
- And there are others!

Practice is driven by:

- Evidence
- Drug availability
  - Routine vs Trials
  - Relapse on TKI
- Test reimbursement issues
  - Who pays
- Access to technology
  - Next-generation sequencing
  - Immunohistochemistry


TMB, tumor mutation burden
Conclusions

- Pathology does have a role!
- Diagnosis
- Staging
- Subtyping
- Biomarker analysis
- Facilitating mutation testing
- Assimilation of data in context
- Participate in the MDT