Best Practices in Oncologic Pathology

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

‘Know your enemy’
Sun Tzu, The Art of War.
Patient Selection – Why?

• Determinant of Management
  – There is nothing to be done
  – You are going to do something
  – Assumes you have something to do

• Best outcome from chosen management
  – Do no harm
  – The patient will benefit (by how much?)
  – Best outcomes from available therapy
  – Avoid predictable toxicities
  – Affordable practice?

• Pathological features as determinants of therapy decisions
Almost all recently introduced therapies require, or potentially require, a detailed pathological diagnosis.

New biomarkers? Personalised strategies = Potential to improve outcomes

Biomarkers in routine clinical use for adenocarcinomas

Overall Survival in NSCLC and Biomarkers

Median OS, months

1999 Docetaxel

2004 Pemetrexed: NSQ

2004 Erlotinib

1994 Vinorelbine

1996–99 Platinum-based doublet

2001 Docetaxel + chemotherapy

2006 Bevacizumab + chemotherapy: NSQ

2008 Pemetrexed + chemotherapy: NSQ

2012 Nab-paclitaxel + chemotherapy

2014 Ramucirumab + docetaxel

2014 Nintedanib + docetaxel: NSQ

2015 Nivolumab: NSQ

2015 Pembrolizumab: PD-L1 +ve

2015 Nivolumab: SQ

Medians OS >20 months with targeted therapies for patients with EGFR mutations

New biomarkers? Personalised strategies = Potential to improve outcomes

Biomarkers in routine clinical use for adenocarcinomas

Almost all recently introduced therapies require, or potentially require, a detailed pathological diagnosis.
Clinical Requirements of Diagnosis: Advanced Disease

Advanced disease, small samples

- WHO 2004 *et prev*
  - inapplicable
  - inaccurate
- NSCLC-NOS problematic
- NSCLC subtype matters

Now a critical determinant of Therapy Choice

Therapy evolved over time
During This Period of Early Evolution…

• When oncology thought lung cancer was one or maybe two diseases…

• Pathology had been subdividing lung cancer according to the WHO classification
  - 1967
  - 1981
  - 1999
  - 2004
  - 2015
During This Period of Early Evolution…

- When oncology thought lung cancer was one or maybe two diseases…

- Pathology had been subdividing lung cancer according to the WHO classification
  - 1967
  - 1981
  - 1999
  - 2004
  - 2015

But no one really cared outside of pathology due to a lack of therapeutic relevance…until recently
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

- Accuracy of specific diagnosis improved
Small Biopsy/Cytology: Thresholds of “Certainty”

Sure

Hmmm...

NSCLC-NOS

Cannot say
NSCLC – probably adeno-carcinoma

Tumour cells express Nuclear p63 Or p40 or CK5/6

NSCLC – probably squamous cell

TTF1 positive in tumour cell nuclei

NSCLC-NOS
Subtyping NSCLC Greatly Improved by IHC

- Predictive IHC has “levelled the playing field”
- Better diagnosis possible on poorer specimens
- Transformed small sample lung cancer diagnosis

Lung Cancer Classification and Sample type

WHO 1967-2015: intended for, and only applicable to, resected cases

- Small cell Carcinoma
- Squamous cell Carcinoma
- Adenocarcinoma
- Large cell carcinomas
- Salivary-type carcinomas
- Adenosquamous carcinomas
- Carcinoid tumours
- Salivary-type carcinomas

WHO 2015: a simplified classification intended for small sample diagnosis only

- Small cell carcinoma
- Squamous cell carcinoma
  - Probable squamous cell ca
- Adenocarcinoma
  - Probable adenocarcinoma
- NSCLC-NOS
  - NSCLC-NOS (null IHC)
- Carcinoid tumour
- Salivary-type (occasionally)

Only TWO IHC are required in most cases

TTF1 &
P40 or p63 (or CK5/6)
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.

Tissue Handling

- Standard fixation
- Cytology preparation to facilitate biomarker testing
- Integrated reporting
- Sample enrichment by micro- or macrodissection

Recommendation 1: Guidance on tissue handling

- Specimen processing
  - Standard fixation using 10% neutral buffered formalin (4% formaldehyde) is recommended [V, A]
  - Fixation time should be no less than 6 h, and no greater than 48 h [IV, A]
  - Sections for biomarker testing should ideally be cut immediately before analysis [IV, A]
- Cytology samples (cellblocks, stained direct smears or liquid-based preparations) can be used reliably to detect EGFR mutations and ALK rearrangements [III, A]. At this time, a cell block is the most widely applicable cell source
- The same pathologist should, if possible, review all available tumour material from the same patient including biopsies and cytology specimens to select the most suitable for biomarker analyses [IV, A]
- A pathologist should be involved in sample preparation for DNA extraction [V, A]
- Enrichment of samples by micro- or macrodissection to maximise tumour cell content before DNA extraction is recommended [III, A]

Cancer Diagnosis for Triage for Molecular Testing

- Follow WHO classification rules
- Restrict use of IHC
- Minimise NSCLC NOS
- Maximise tissue left for molecular analysis

**Recommendation 2:** What is an acceptable rate for NSCLC not otherwise specified (NOS) diagnosis in the small biopsy/cytology diagnostic setting and how can this be achieved?

- A diagnosis of NSCLC-NOS should be given in <10% of cases [IV, A]
- This figure is achieved with the judicious use of immunohistochemistry in morphologically indeterminate cases. A recommended approach should include TTF-1 to predict adenocarcinoma. For predicting squamous cell carcinoma, p63 or p40 and CK5/6 testing are useful [IV, A]

Is There Enough Material for All Diagnosis?

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

Two biopsy fragments <1 mm

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

LET’S WORK
ONCOLOGY FROM BOEHRINGER INGELHEIM

Boehringer Ingelheim
Adenocarcinomas, Targets and Therapy

Only three of these targets have agents through approval by FDA.
Very few suitable targets – very few additive oncogenes

The commonest alterations are inactivating mutations in tumour suppressor genes

Panel of 54 Genes With Potentially Druggable Alterations

<table>
<thead>
<tr>
<th>Therapy class</th>
<th>Gene target</th>
<th>Smokers</th>
<th>Never Smokers</th>
<th>Targeted therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>S/T kinase inhibitors</td>
<td>AURKC</td>
<td></td>
<td></td>
<td>AMG900, CYC116,</td>
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<tr>
<td>RTK ligands</td>
<td>BRAF</td>
<td></td>
<td></td>
<td>GSK1218436, Vemurafenib.</td>
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<tr>
<td>EGFR tyrosine kinase inhibitors</td>
<td>VEGFA</td>
<td></td>
<td></td>
<td>Fictiluzumab, Ritubumab.</td>
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<tr>
<td>Other receptor tyrosine kinase inhibitors</td>
<td>EGFR, ERBB3, ERBB4</td>
<td></td>
<td></td>
<td>Afiblercept, Bevacizumab, Thalidomide.</td>
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<tr>
<td>Cytoplasmic tyrosine kinase inhibitors</td>
<td>FLT3, IGFR1, MET</td>
<td></td>
<td></td>
<td>AEE 788, AV-412, AZD931, etc.</td>
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<tr>
<td>HDAC inhibitors</td>
<td>HDAC1, HDAC2, HDAC6</td>
<td></td>
<td></td>
<td>AZD9391, MM121, U-1287/AMG886.</td>
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<tr>
<td>Integrins</td>
<td>HIF1A, BIRC5</td>
<td></td>
<td></td>
<td>BMS-599626, Pertubin.</td>
</tr>
<tr>
<td>Multiple classes</td>
<td>ITGB1, ITGB3, PTP2</td>
<td></td>
<td></td>
<td>AZD4547, BGL398, Dovitinib, etc.</td>
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<tr>
<td>Notch pathway</td>
<td>DLL4, CTSK</td>
<td></td>
<td></td>
<td>Axitinib, Cediranib, Lenvatinib, etc.</td>
</tr>
<tr>
<td>Others</td>
<td>IL6, PRKC8, THBS1, PIK3CG</td>
<td></td>
<td></td>
<td>AMG 479, AVE1642, Bilbob22, etc.</td>
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<tr>
<td>PI3K inhibitors</td>
<td>RICTOR, PTEN</td>
<td></td>
<td></td>
<td>AMG 208, ARQ197, Amsuvitinib, etc.</td>
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<tr>
<td>Proteasome inhibitors</td>
<td>GSK3B, TERT, TERT</td>
<td></td>
<td></td>
<td>AT9283, Amuvatinib, Cabacainib, etc.</td>
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<tr>
<td>Telomerase inhibitors</td>
<td>TNFRSF10B, KRAS</td>
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<td>PF-00562271.</td>
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<tr>
<td>Vaccines</td>
<td>MFGEB, MMP14</td>
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<td></td>
<td>Dasatinib.</td>
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<tr>
<td>VDAs</td>
<td>TP53, ANPEP</td>
<td></td>
<td></td>
<td>XL047, [1]</td>
</tr>
</tbody>
</table>

It Is Worthwhile Finding an Actionable Genetic Alteration in Lung Cancer

Using Multiplexed Assays of Oncogenic Drivers in Lung Cancers to Select Targeted Drugs

Driver detected – Targeted Rx

Testing Algorithm for NSCLC

Morphology +/- IHC

Non-small cell lung cancer

Squamous cell carcinoma

EGFR & ALK testing ONLY if never or long time ex-smoker

Chemotherapy if negative

EGFR or ALK TKI if positive

Non-squamous cell carcinoma (de facto Adenocarcinoma)

EGFR mutation

ALK fusion gene testing

Chemotherapy if negative

Immunohistochemistry ONLY when needed
Guidelines for EGFR Testing

- Test adenocarcinomas
- Caveats in never smokers
- Range of mutations to be tested
- Independent of methodology

Recommendation 3: Routine EGFR somatic mutation testing has been recommended
- All non-squamous tumours in patients with advanced/recurrent disease should be tested for EGFR mutation [I, A]
- Selected squamous tumours (from patients with minimal or remote smoking history) should strongly be considered for testing [IV, B]
- A wide coverage of mutations in exons 18–21 is strongly encouraged, including those associated with resistance to some therapies. At a minimum, when resources or material are limited, the most common activating mutations (Exon19del, L858R) should be determined [I, A]
- Any methodology employed should be validated by an external quality assurance programme [V, A]
Targeted Therapy in ABSENCE of Target May Cause Harm

Patients with advanced, previously untreated pulmonary adeno

Guidelines for ALK Testing

- Test adenocarcinomas
- Caveats in never smokers
- Screening by IHC
- Confirm by FISH
- IHC as primary test?

**Recommendation 4: Is there sufficient evidence to support routine testing for ALK rearrangement?**

- All non-squamous tumours in patients with advanced/recurrent disease should be tested for ALK rearrangement [II, A]
- Selected squamous tumours (from patients with minimal or remote smoking history) should strongly be considered for testing [III, B]
- Definitive assessment of ALK rearrangement is determined by FISH [I, A]
- IHC methods may be employed for screening and may become validated for therapy [IV, B]
- Methodologies employed should be validated by an external quality assurance programme [V, A]
What Is the Biggest Challenge to Achieving Diagnosis and Molecular Testing in NSCLC Patients?

- Resources - reimbursement
- Access to testing
- Knowledge and awareness

- TISSUE!
  - Don’t waste it
  - Handle with care
Most Lung Cancer Samples Are Small Biopsies or Cytology-Type Samples

![Image](image1.png)

60 mm diameter Adenocarcinoma in left upper lobe
Is There Enough Material to Test for Everything Required?

- Total failure all tests 1-2%
- Partial failure 6-7%
- EGFR tests in EBUS samples 2-25% fail
- ALK FISH 10-20% cases not enough cells
- Multiple sequential tests 30% fail
- NGS 20-40% failure

Only 10-25% of this tissue will actually be tumour
Biomarkers

• Biological features which are associated with disease behaviour

• The ideal biomarker: always correct
  – Easy and practical to measure
  – Present or absent
  – Stable and functionally unique
  – 100% predictive

• Usually biologically related to the system being examined
  – The drug target
  – A co-factor of the drug target
  – A factor negating drug effect

A biological rationale makes us more confident?
Prognostic Versus Predictive Biomarkers

- Prognostic biomarker informs about the natural history of the disease in the absence of treatment; risk stratification

- Predictive biomarker informs about the likely progress of disease when treated in a particular way; predicts response to treatment

- Some therapy-predictive biomarkers are also prognostic

"Prediction is very difficult, especially if it's about the future."
--Nils Bohr, Nobel laureate in Physics
Predictive Biomarkers and Therapy Selection

• Nature of the target
  – Addictive oncogenes
  – Expedited oncogenes
  – Other factors

• Nature of the test
  – Present or absent
  – Biological continuum
  – DNA, RNA, protein
  – Single factor or multiple components?
Methods of Biomarker Analysis

- Change in DNA sequence
- Change in Gene copy #
- Transcription
- mRNA transcript
Methods of Biomarker Analysis

- DNA mutational analysis
- FISH/CISH
- Microarray
- mRNA transcript
- RT-PCR

Change in DNA sequence
Change in Gene copy #

Transcription
Single Mutation Tests

**Approaches**
- Sanger sequencing
- Allele-specific tests
  - Commercial platforms
  - Digital PCR

**Issues**
- Cost
- Sensitivity
- Specificity
- Practicality
- Flexibility
- Turnaround time
In Situ Hybridisation:
Rearrangements/Translocations, Copy Number

**Fluorescence ISH (FISH)**
- Standard
- Experience
- Costly
- Possibly slow
- Morphologically challenging

**Chromogenic ISH (CISH)**
- New, little experience
- Cheaper? Faster?
- Morphology better
- Copy numbers OK
- Break-aparts much harder
RNA-Based Tests

- Highly sensitive and specific
- RNA is labile – most diagnostic material is not a reliable source
- Harder to implement
- Expression arrays still to find their place
Methods of Biomarker Analysis

Change in DNA sequence
- DNA mutational analysis
- Microarray
- RT-PCR

Change in Gene copy #
- FISH/CISH

Transcription
- mRNA transcript

Next-generation sequencing
Next-Generation Sequencing

- Huge capacity for multiplex testing
- How many genes? – How many samples?
  - Whole genome or exome
  - Multiplex panels
- Biostatistics crucial to output
- Input material – Quality and Quantity
- Mutation > Fusions > Copy numbers
- Allele frequency (mutation dose)
Methods of Biomarker Analysis

- Change in DNA sequence
- Change in Gene copy #
- DNA mutational analysis
- FISH/CISH
- Microarray
- Next-generation sequencing
- mRNA transcript
- RT-PCR
- Protein
- Immunohistochemistry

Transcription

Translation
Immunohistochemistry

**Upsides**
- Familiar technology
- Relatively cheap, relatively fast
- Morphology-based

**Downsides**
- Antibody specificity can be an issue
- Performance depends on
  - Antibody
  - Detection system
  - Tissue processing
  - Pathologist
- Subjective assessment
- Poor standardisation
- Poor record in lung cancer
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)

Morphology-based tests
- ALK, ROS1
- EGFR
- PD-L1

Sections for Biomarker IHC & FISH

2 x 1 mm tissue fragments

“Test tube” tests
A Testing Algorithm

Single tests?

Sequencing of tests?

Parallel testing approach?

Multiplex testing?

Reflex or Bespoke testing

• IHC
  – Part of morphologic diagnosis
  – Screening test
  – Quick tests
  – Confirmation of NGS?

• Panel testing on DNA/RNA
  – NGS
  – Panels for tumours?
  – Panels for drugs?
  – Panels for resistance?
Is the Technology Working?

- Standard procedures and protocols
- LDTs or commercial kits?
- Internal quality controls
- External quality assurance
- Laboratory monitoring
- Pathology review
  - Do results “fit”?
  - “Rubbish in / Rubbish out”
External EQA helps improve procedures over time
HER2 IHC UK laboratory Pass Rates: 2003-2012

94%
Laboratory ‘learning curve’

6%
Standardised tests

Understanding scoring methodology

Courtesy Dr. K Miller, Director, UKNEQAS.
Trials and Biomarker Tests

- Therapeutic trial
- Biomarker test selection
- Selection of patients based upon the test allows a higher probability of a better outcome
- Does real world testing have to recapitulate what was done in the trial?
- Does the test perform the same outside the trial reference laboratory?
- Conflicts driven by
  - Pharma
  - Regulators
  - Science
  - Laboratories
Biomarker Tests: Setting Expectations

• Test performance is influenced by many things
  – The drug
  – The biology of the target
  – Test accuracy (sensitivity, specificity)
  – Test reliability

• Expectations have to be set accordingly
Biomarker Test Reporting

- In the context of the pathological diagnosis
- Detailed enough to allow therapy selection
- Clear enough to avoid ambiguity
- Describe methodology such that “caveats” are understood
- Includes comment on therapeutic implications without being prescriptive
Biomarkers From Plasma DNA Testing?

- CTC may NOT be a representative population
- cfDNA reflects the overall picture in the patient? Evidence?
- cfDNA mutations: targeted testing vs. multi-gene screening
- Technical issues

EGFR Mutation Testing
Sensitivity in Meta-analysis – 62%

- 27 studies
- 3110 patients
Plasma DNA Testing Now…

• Known target
  – EGFR T790M mutation

• Screening test for T790M
  – Be prepared to rebiopsy if needed
  – Sensitivity still a challenge
  – Other factors not covered by a plasma test

• Molecular before Radiological before Clinical Relapse
  – Would you intervene any earlier?
In Conclusion

• Pathology now a key determinant of therapy choice in NSCLC

• Delivery of this pathology service requires
  – Knowledge of tumour biology and biomarkers
  – Access to appropriate testing
  – Testing needs to be “fit for purpose”
  – Adequate material to test
  – Good communication between ALL stakeholders