Role of the pathologist in the diagnosis and mutational analysis of lung cancer

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Disclosure

JRG is a paid advisor to and speaker for AstraZeneca, Boehringer-Ingelheim, Bristol-Myers Squibb, Dako, Lilly & Co, Merck, Merck Sharp and Dohme, Novartis, Pfizer, Roche and Ventana
Classification of carcinoma of the lung

- small cell lung cancer (SCC)
- non-small cell lung cancer (NSCLC)
  - Strictly, all malignant epithelial neoplasms other than SCC
  - In practice, squamous or adenocarcinoma
SPECIMEN
LEFT UPPER LOBE BRONCHIAL BIOPSY

MACROSCOPIC DESCRIPTION
Fragments of white tissue measuring up to 4mm

MICROSCOPY
This bronchial mucosa and submucosa are infiltrated by poorly differentiated carcinoma with a ‘squamoid’ growth pattern, although desmosomes and keratin are not evident. Some cells may contain vacuoles, possibly of mucin.

The features are those of a poorly differentiated non-small cell carcinoma, probably adenocarcinoma.
SPECIMEN
LEFT UPPER LOBE BRONCHIAL BIOPSY

MACROSCOPIC DESCRIPTION
Fragments of white tissue measuring up to 4mm

MICROSCOPY
This bronchial mucosa and submucosa are infiltrated by poorly differentiated non-small cell carcinoma. The growth pattern is 'squamoid', but desmosomes and keratin are not evident. Occasional cells contain vacuoles, possibly mucin.

Immunochemistry reveals expression of cytokeratins of class 7 as well as of TTF-1. There is no expression of p63. These features support a diagnosis of adenocarcinoma.

PREDICTIVE PROFILING

EGFR GENE MUTATIONS
Epidermal growth factor (EGFR) mutation analysis has been performed to determine suitability of this patient's non-small cell lung cancer (NSCLC) for treatment with tyrosine kinase inhibitors (TKIs).

Analysis was done using RT-PCR (Scorpions/ARMS methodology, Qiagen Therascreen EGFR kit). The Therascreen kit detects the following 29 somatic mutations:

- Exon 18: G719X
  - The kit detects, but does not distinguish between, any from the following 3 mutations; G719A (c.21567G>A p.G719A) G719S (c. 2155G>S p.G719S) G719C (c.2155G>C p.G719C)
- Exon 19: deletions
  - The kit detects, but does not distinguish between, any from a total of 19 deletions; Exon 20: T790M Exon 20: S768I Exon 20 : Insertions
  - The kit detects, but does not distinguish between, any from the following 3 mutations; c.2307_2308ins9 p.V769_D770insASV c.2319_2320insCAC p.H773_V774insH c.2319_2311insGGT p.D770_N771insG

Test sensitivity
The limit of sensitivity is stated to be 1% mutated EGFR alleles in a wild-type background

Results
NO MUTATIONS IN THE EGFR GENE HAVE BEEN DETECTED

Conclusion
This patient is unlikely to respond to TKIs active against NSCLCs with sensitising mutations in the EGFR gene.

Any remaining DNA from this patient's sample will be stored in the laboratory archives.

ALK GENE REARRANGEMENT
Immunochemistry using the Ventana system and Roche D5F3 antibody has been performed to detect the ALK fusion protein.

STRONG, GRANULAR EXPRESSION OF THE PROTEIN IS DETECTED. Such expression correlates with rearrangement of the ALK gene and indicates that this patient is likely to respond to TKIs active against NSCLCs with this genetic abnormality

PD-L1 EXPRESSION
Expression of PD-L1 has been sought by immunochemistry using the Dako 22C3 antibody as a guide to the likely sensitivity of the tumour to pembrolizumab.

Expression of PD-L1 on cell membranes is as follows:
Neoplastic cells: overall expression 80% (strong 30%; moderate 30%)
Immune cells: overall expression 10% (weak 10%)
In terms of guiding the use of pembrolizumab, this specimen should be considered POSITIVE for PD-L1 expression
Tailored therapy for NSCLC

- **Therapies dependent on morphology**
  - **Antifolate**
    - pemetrexed (Alimta)
  - **Anti-VEGFA monoclonal antibody**
    - bevacizumab (Avastin)

- **Therapies dependent on genetic aberrations**
  - **Small molecule tyrosine kinase inhibitors (TKIs)**
    - erlotinib (Tarceva), gefitinib (Iressa), afatinib (Giotrif), osimertinib (Tagrisso),
      crizotinib (Xalkori), ceritinib (Zykadia), alectinib (Alecensa)

- **Therapies dependent on protein expression**
  - **Anti-PD-1 and PD-L1 monoclonal antibodies**
    - nivolumab (Opdivo), pembrolizumab (Keytruda), atezolizumab (Tecentriq),
      durvalumab, avelumab
  - **Anti-EGFR monoclonal antibody**
    - necitumumab (Portrazza)
Predictive profiling of NSCLC

- EGFR gene mutations
  - ~12%
- ALK gene rearrangement
  - <5%
- PD-L1 protein expression
  - depends on ‘cut-off’
A challenge!
A high quality specimen
There’s no such thing as a biopsy that's too big.
Accurate diagnosis

squamous

‘NSCLC-NOS’

adenocarcinoma
Accurate diagnosis

- p40/p63 for squamous differentiation
- TTF-1 for glandular differentiation
Accurate profiling
EGFR gene mutation
Accurate profiling

ALK gene rearrangement

- FISH only
- Immunochemistry only
- Immunochemistry and FISH
- Immunochemistry for screening with FISH if immuno-positive
- CAP/IASLC/AMP; NCCN; JLCS; RCPath UK

Ventana D5F3

Abbott Vysis FISH
Immune checkpoints

- T-cell responses are regulated by a balance of activating and inhibitory ‘checkpoint’ signals.
- Neoplastic cells protect themselves from immune attack by dysregulating these checkpoints.
- Therapeutic targeting of these checkpoints restores the immune response and promote destruction of the neoplastic cells.

Activating receptors:
- CD28
- OX40
- CD137

Inhibitory receptors:
- CTLA-4
- PD-1
- TIM-3
- LAG-3
PD-L1 expression confers protection

Dako 22C3 anti-PD-L1
## Multiple tests for PD-L1 expression

<table>
<thead>
<tr>
<th></th>
<th>Pembrolizumab Merck, Sharp &amp; Dohme</th>
<th>Nivolumab Bristol-Myers Squibb</th>
<th>Durvalumab AstraZeneca</th>
<th>Atezolizumab Roche/Genentech</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>TARGET</strong></td>
<td>PD-1</td>
<td>PD-1</td>
<td>PD-L1</td>
<td>PD-L1</td>
</tr>
<tr>
<td><strong>DETECTION</strong></td>
<td>Dako 22C3</td>
<td>Dako 28-8</td>
<td>Roche SP263</td>
<td>Roche SP142</td>
</tr>
<tr>
<td><strong>RELEVANT EXPRESSION</strong></td>
<td>Surface of tumour cells</td>
<td>Surface of tumour cells</td>
<td>Surface of tumour cells</td>
<td>Surface of tumour cells and immune cells</td>
</tr>
<tr>
<td><strong>CRITERIA FOR ‘POSITIVITY’</strong></td>
<td>≥ 1% or ≥ 50% expression</td>
<td>≥1% expression</td>
<td>≥25% expression</td>
<td>TC expression 0-3: &lt;1, 1-4, 5-49, ≥50; % of tumour infiltrated by PD-L1+ve ICs 0-3: &lt;1, 1-4, 5-9, ≥10</td>
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Interpretation

Dako 22C3 anti-PD-L1
Heterogeneity of PD-L1 expression

Dako 22C3 anti-PD-L1
Suitable specimens

- Endoscopic tissue biopsy
- Aspirate with structured elements
- Cutting needle tissue biopsy
- Aspirate with dispersed cells
’Cytology’ specimens

Dako 22C3 anti-PD-L1

Dako 22C3 anti-PD-L1
Bringing it together
What and when to test: automatic (reflex) testing?

**ADVANTAGES**

- Saves time
- Provides clinicians with comprehensive information
- Permits ‘forward planning’
- Aids integration of information
- Establishes principle that predictive profiling is routine

**DISADVANTAGES**

- Costs more
- Information may be inappropriate to immediate management
- Information may be inappropriate when disease relapses
Gene profiling
Analysis of blood samples
Protein expression in tissue sections

- ALK fusion protein
  - crizotinib, ceritinib, alectinib
- PD-L1
  - nivolumab, pembrolizumab, atezolizumab, durvalumab, avelumab
- EGFR
  - necitumumab
Have a nice day!