Role of Immunohistochemistry in Mutation Testing.......and one or two other biomarkers

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Personalised approach to treating cancer

• Understanding the molecular basis of cancer has identified specific drivers and allowed for sub-classification of the disease, revealing the potential for targeted agents

‘ONE SIZE FITS ALL’

PERSONALISED THERAPY

• Personalised treatment consists of three essential components:
  – Oncogenic target that drives cancer growth
  – Predictive biomarker that detects presence of the target
  – Well conducted clinical studies that confirm treatment efficacy in the identified patient group
Methods of biomarker analysis

- Change in DNA sequence
  - Transcription
  - mRNA transcript
    - Translation
    - Protein
      - Biological Activity
      - Oncogenesis
      - Drug target

Other methods:
- DNA mutational analysis
- FISH/CISH
- Multiplexed genotyping
- Microarray
- Next-generation sequencing
- RT-PCR
- Immunohistochemistry
Biomarker Testing Guidelines


- All non-squamous tumours in patients with advanced/recurrent disease should be tested for EGFR mutation and ALK rearrangement
- Selected squamous tumours (from patients with minimal or remote smoking history) should strongly be considered for testing

Other therapeutic choices driven by histology

- Pemetrexed
  - efficacy
- Bevacizumab
  - safety
- Nivolumab
  - licence
Securing a histological subtype in NSCLC

• Around 25% of small biopsy samples
• Around 40% of cytology samples
• Uncertainty about NSCLC histotype

• Predictive IHC reduces NSCLC-NOS to under 10%

• Predict adenocarcinoma (TTF1)
• Predict Squamous cell carcinoma (p40, p63, CK5/6)
Refining the diagnosis of NSCLC-NOS

NSCLC-NOS
25-40% of cases

TTF1

p63

NSCLC – probably Squamous Ca
NSCLC-Probably Adenocarcinoma

Or
P40
CK5/6
Immunohistochemical Subtyping of NSCLC

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

Loo PS et al, JTO 2010
<table>
<thead>
<tr>
<th>Cell type</th>
<th>EGFR mutation</th>
<th>KRAS mutation</th>
<th>BRAF mutation</th>
<th>ALK fusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adeno-Carcinoma</td>
<td>15%</td>
<td>30.9%</td>
<td>2.5%</td>
<td>12.5%</td>
</tr>
<tr>
<td>Probable Adenoca (by IHC)</td>
<td>5.9%</td>
<td>41.8%</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Squamous Carcinoma</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Probable Squamous ca (by IHC)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>NSCLC-NOS</td>
<td>7.3%</td>
<td>31%</td>
<td>2.7%</td>
<td>0</td>
</tr>
<tr>
<td>Small cell carcinoma</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

**Histology vs Mutation**

<table>
<thead>
<tr>
<th></th>
<th>TTF-1 positive</th>
<th>TTF-1 negative</th>
<th>Mutations missed if only TTF-1 +ve cases tested</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>EGFR mutation</strong></td>
<td>15%</td>
<td>2.5%</td>
<td>P&lt;0.0001</td>
</tr>
<tr>
<td><strong>KRAS mutation</strong></td>
<td>33%</td>
<td>36%</td>
<td>NS</td>
</tr>
<tr>
<td><strong>BRAF mutation</strong></td>
<td>2.5%</td>
<td>2.1%</td>
<td>NS</td>
</tr>
<tr>
<td><strong>ALK fusion</strong></td>
<td>4.2%</td>
<td>1.3%</td>
<td>NS</td>
</tr>
</tbody>
</table>

Kret A et al. Lung Cancer Jan, 2015; suppl 1
IHC and EGFR mutations

- Mutation specific antibodies
- L858R (43B2)
  - Sens 86% Spec 99%
- Ex19 E746-A750del (6B6)
  - Sens 100% Spec 98% for specific mutation
  - Sens 61% for any Ex19del

  Cooper et al, J Clin Path 2013

- General experience variable
  - L858R – 36 - 100% (meta analysis 0.76)
  - Ex19 – Sensitivity 40 – 100% (meta analysis 0.60)
  - Meta-analysis Chen Z et al. PLoS1 2014; 9, e105940

  Not comprehensive but a substitute if mutation testing not possible? Bone Biopsy? Speed?
FLEX survival: ITT population

<table>
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<tr>
<th>Survival</th>
<th>Median</th>
<th>1-year</th>
</tr>
</thead>
<tbody>
<tr>
<td>CT + cetuximab</td>
<td>11.3 mo</td>
<td>47%</td>
</tr>
<tr>
<td>CT</td>
<td>10.1 mo</td>
<td>42%</td>
</tr>
</tbody>
</table>

HR=0.87* (95% CI 0.76–1.00), p=0.044

Number of patients at risk
CT + cetuximab 557 383 251 155 53 3
CT 568 383 225 134 48 0

*Stratified
HR, hazard ratio; mo, months
Identification of discriminating EGFR IHC score threshold for cetuximab efficacy by response rate

Increased tumor response for CT + cetuximab with EGFR IHC score ≥200¹

Response rate estimated for overlapping intervals of IHC score range²

¹O’Byrne K, et al. CMSTO, 2010
High EGFR expression (≥200)  
n=345

Low EGFR expression (<200)  
n=776

Response rate by EGFR expression level

CT + cetuximab

CT

Response rate (%)  

29.6  32.6  p=0.36

28.1  44.4  p=0.002

Treatment interaction test: p=0.040

O’Byrne K, et al. CMSTO, 2010
High and low EGFR expression

Low EGFR
IHC score <200

High EGFR
IHC score ≥200

IHC score=0
IHC score=112
IHC score=270
Calculating the EGFR IHC score

EGFR IHC score = \[1 \times (\% \text{ cells } 1+) + 2 \times (\% \text{ cells } 2+) + 3 \times (\% \text{ cells } 3+)\]

EGFR IHC score ranges from 0–300

EGFR IHC scoring instructions

- Four intensities: 0 = no staining, 1+ = weak, 2+ = moderate, 3+ = strong staining
- Intensities defined by “magnification rule” (Rüschoff et al. 2010):

  - **Staining intensity: 3+**
    - Visible by eye or under low power examination: 4x or 5x
  - **Staining intensity: 2+**
    - Needs a more detailed magnification: 10x – 20x
  - **Staining intensity: 1+ or 0**
    - Needs high magnification: 40x
Calculating the EGFR IHC score: Using the “magnification rule”

Modified from Prof. Rüschhoff; Targos, Kassel
IHC and BRAF^{V600E} mutations

- **VE1 clone**
  - 90% V600E cases positive (n=21)
  - 0% non-V600E cases positive *Ilie et al., 2013*

- **VE1 clone**
  - 100% V600E cases positive (n=5)
  - 4.8% non-V600E cases positive (1/21) *Sasaki et al., 2013*

- **VE1 clone vs anti-B-raf clone (various tumours)**
  - VE1: 98% sens, 97% spec *Routhier et al., 2013*
  - Anti-B-raf: 95% sens, 83% spec

- **Can we ignore the non-V600E mutations?** *Smit E, 2014*
MET as an oncogene

- HGF Ligand binding
  - c-MET dimerization
- GAB1 the major substrate of activated ‘pMET’
- Oncogenesis
  - ‘Reactivation’ of embryogenetic mechanisms
  - Disrupted tissue homeostasis
  - Motility and ‘invasiveness’
- Activation
  - Not mutation
  - Upregulation, exaggerated physiology
  - Inducable

Comoglio et al. Nat Rev Drug Discov 2008;7:504-16
**MET assessment:**
Gene copy number or protein IHC?

**Anti- MET agents**

Small molecule MET TKIs – ARQ197 (Tivantinib)

Anti-MET monoclonal antibodies - MetMab
Development of Met IHC as a Diagnostic

- Intensity of Met staining on tumor cells scored on 0–3+ scale
  
  ![1+](image1.png) ![2+](image2.png) ![3+](image3.png)

- Estimated that ~50% of patients would have ‘Met High’ tumors
- Met by IHC was assessed after randomization

‘Met High’ was defined prior to unblinding as:
≥50% tumor cells with a staining intensity of 2+ or 3+

- Tissue was obtained from 100% of patients.
- 95% of patients had adequate tissue for evaluation of Met by IHC.
- 54% patients had ‘Met High’ NSCLC.

Spigel et al, ESMO 2010
MET HIGH = Positive

MET LOW = Negative

Spigel et al, J Clin Oncol 2013
T cell activation can be augmented by targeting immune checkpoints

- T-cell responses are regulated though a complex balance of inhibitory (“checkpoints”) and activating signals
- Tumours can dysregulate checkpoints and activating pathways, and consequently the immune response
- Targeting checkpoints and activating pathways is an innovative approach to cancer therapy, designed to promote an immune response

High levels if PD1 or PDL1 protein expression (IHC) may inhibit Immune response

Block PD1 or PDL1
Immune damage to tumour

Biomarkers for Immunotherapy?

PD-L1 Negative

PD-L1 Positive (predictive of response)

Less response

1%  5%  10%  50% cell positive

More response

Significance of staining?

Several therapeutics
Several companion diagnostics, for example:

Kerr KM et al. JTO pub online March 2015
# PD-L1 as a predictive immune biomarker: assays, sample collection and analysis in NSCLC studies

<table>
<thead>
<tr>
<th>PD-L1 Assay</th>
<th>Pembrolizumab Merck</th>
<th>Nivolumab Bristol-Myers Squibb</th>
<th>MPDL3280A Roche/Genentech</th>
<th>MEDI4736 AstraZeneca</th>
</tr>
</thead>
<tbody>
<tr>
<td>Source and Collection</td>
<td>Prototype or clinical trial IHC assay (22C3 Ab)(^1,2)</td>
<td>Dako automated IHC assay (28-8 Ab)(^3-4)</td>
<td>Central laboratory IHC assay(^6)</td>
<td>Ventura automated IHC (BenchMark ULTRA using Ventana PD-L1 (SP263) clone)(^8,9)</td>
</tr>
<tr>
<td>Sample Source and Collection</td>
<td>Surface expression of PD-L1 on tumour specimen(^1,2)</td>
<td>Surface expression of PD-L1 on tumour cells(^3,4)</td>
<td>Surface expression of PD-L1 on TILs or tumour cells(^6,7)</td>
<td>Surface expression of PD-L1 on tumour cells(^8,9)</td>
</tr>
<tr>
<td>IHC Staining:</td>
<td>Strong vs weak expression(^1,2)</td>
<td>Strong vs weak expression(^3,4)</td>
<td>IHC Staining Intensity (0, 1, 2, 3):</td>
<td>Unknown</td>
</tr>
<tr>
<td></td>
<td>PD-L1 expression required for NSCLC for enrollment(^1)</td>
<td>Patients not restricted by PD-L1 status in 2nd- &amp; 3rd-line</td>
<td>IHC 3 (≥10% PD-L1(^+))(^6,7)</td>
<td>IHC Staining Intensity:</td>
</tr>
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<td>Note that one arm of KEYNOTE 001 trial requires PD-L1- tumours(^1)</td>
<td>Ph III 1st-line trial in PD-L1(^+)</td>
<td>IHC 2.3 (≥5% PD-L1(^+))(^6,7)</td>
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<td>Archival or fresh tissue(^1,2)</td>
<td>Archival or fresh tissue(^3,4)</td>
<td>IHC 1,2,3 (all patients with evaluable status)(^6,7)</td>
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<td>Tumour PD-L1 expression:</td>
<td>IHC 0,1,2,3 (all patients with evaluable status)(^6,7)</td>
<td>PD-L1 expression required for NSCLC for enrolment in Ph II trials</td>
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<td>≥50% PD-L1(^+) cut-off: 32% (41/129)</td>
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<td>1–49% PD-L1(^+) cut-off: 36% (46/129)</td>
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<td>Tumour PD-L1 expression (all doses): (^9)</td>
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\(^*\)Definition of PD-L1 positivity differs between assay methodologies.

Other markers to consider.....

• HER2
  – No ligand binding domain. Important heterodimerisation
  – Mutation (0.8-4.3%), *Gene Copy # (38-43%),
  – Amplification (~2%), **Over-expression (24-35%)**
  – Gefitinib, Pan-HER inhibitors, Traztuzamab?

  *Hirsch et al. Oncogene 2009
  *Hirsch et al, Br J Can 2002
  *Soh et al, Int J Can 2007
  *Suzuki et al, Lung Cancer 2015
  *Reis et al, Lung Cancer 2015

• PTEN
  – Loss of tumour suppressor gene activity
  – Prognostic effect
  – PI3K/AKT inhibitors?

• RANK and RANK-L
  – Bone metastatic disease
  – Denosumab
Panel of 54 genes with potentially druggable alterations

Govindan et al. Cell. 2012 Sep 14;150:1121-34
IHC and cytotoxic agents

• ERCC1
  – Resistance factor in platinum based chemotherapy
  – Suggestions of a predictive benefit in several studies Olaussen et al. NEJM 2006
  – Specificity of antibody (clone 8F1) Friboulet et al. NEJM 2013

• Tubulin III
  – Associated with responses to taxanes
  – Variable results

• Thymidylate Synthase (TS)
  – Target of pemetrexed
Role of Immunohistochemistry in Mutation Testing and one or two other biomarkers

- Diagnostic IHC
- Predictive markers
  - Mutated proteins
  - WT proteins