UK NEQAS ICC & ISH: Non-Small Cell Lung Cancer EQA Surveillance for ALK and PD-L1

Suzanne Parry
7th June 2017
UK NEQAS ICC & ISH Headquarters
Outline of Presentation

- **UK NEQAS IHC ALK EQA Assessments over 2 years of surveillance**

- **Findings from the UK NEQAS IHC pre-pilot assessment for PD-L1 in NSCLC**
NSCLC ALK IHC EQA

• More laboratories now using IHC as the front-line method for ALK testing in NSCLC

• Many studies and papers highlighting the importance of ALK IHC:

  “ALK IHC standardized assessment achieved high inter-observer reproducibility among an international panel of participants and high correlation with ALK FISH. A screening strategy using ALK IHC should be considered.”


  “All ALK-IHC-positive patients responded to crizotinib except three with primary resistance
No tumor response was observed in 13 ALK-FISH-positive but ALK-IHC-negative patients This was confirmed in an external cohort of 16 patients. ROC curves for ALK-IHC and ALK-FISH compared to treatment outcome, showed that dichotomous ALK IHC outperforms ALK-FISH”
NSCLC ALK IHC EQA

- More laboratories now using IHC as the front-line method for ALK testing in NSCLC

- Important to set up a robust EQA module – to identify and assist laboratories who may be having technical issues

- Module to incorporate both the Technical and Interpretive:
  - Is the staining as expected?
  - Are participants interpreting ALK IHC samples as expected?
UK NEQAS EQA for ALK includes Cell lines and Patient Cancer Samples

Example of samples used in assessment

<table>
<thead>
<tr>
<th>Sample</th>
<th>Sample</th>
<th>FISH status (Vysis)</th>
<th>IHC status (Roche D5F3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Cell line: 50% knock in + 50% adenocarcinoma</td>
<td>-ve</td>
<td>Approx. 50% +ve &amp; 50% -ve</td>
</tr>
<tr>
<td>B</td>
<td>Cell line: 100% adenocarcinoma</td>
<td>-ve</td>
<td>100% -ve</td>
</tr>
<tr>
<td>C</td>
<td>Cell line: 50% isogenic + 50% adenocarcinoma</td>
<td>+ve (Break apart: Inversion)</td>
<td>Approx. 50% +ve &amp; 50% -ve</td>
</tr>
<tr>
<td>D</td>
<td>Cell line: 50% isogenic + 50% adenocarcinoma</td>
<td>+ve (Break apart: Inversion)</td>
<td>Approx. 50% +ve &amp; 50% -ve</td>
</tr>
<tr>
<td>E</td>
<td>NSCLC tumour adenocarcinoma</td>
<td>+ve (Break apart: Inversion + deletion)</td>
<td>+ve</td>
</tr>
<tr>
<td>F</td>
<td>NSCLC tumour adenocarcinoma</td>
<td>-ve</td>
<td>-ve</td>
</tr>
</tbody>
</table>
Expected Level of Staining

+ve cell line -ve cell line

+ve cell line -ve cell line

+ve tumour -ve tumour

All stained with the Roche D5F3 assay
## Early Assessments: Combinations of Antibodies and Platforms Used (Data from 2\textsuperscript{nd} EQA)

<table>
<thead>
<tr>
<th>Primary antibody</th>
<th>Automation Instrument</th>
<th>Detection kit</th>
<th>Excellent</th>
<th>Acceptable</th>
<th>Borderline</th>
<th>Unacceptable</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cell Signalling Technologies. (D5F3)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LabVision Autostainer</td>
<td>Dako Envision HRP/DAB (K5007)</td>
<td></td>
<td>-</td>
<td>1 (100%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leica Bond-III</td>
<td>Leica Bond Polymer Refine (DS9800)</td>
<td></td>
<td>-</td>
<td>1 (100%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ventana Benchmark XT</td>
<td>Ventana UltraView Kit (760-500)</td>
<td></td>
<td>-</td>
<td>-</td>
<td>1 (100%)</td>
<td></td>
</tr>
<tr>
<td><strong>Dako (ALK1)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dako Autostainer Link 48</td>
<td>DAKO Envision FLEX+ mouse Linker</td>
<td></td>
<td>-</td>
<td>-</td>
<td>1 (100%)</td>
<td></td>
</tr>
<tr>
<td>Leica Bond-III</td>
<td>Leica Bond Polymer Refine (DS9800)</td>
<td></td>
<td>-</td>
<td>-</td>
<td></td>
<td>1 (100%)</td>
</tr>
<tr>
<td>Ventana Benchmark XT</td>
<td>Ventana OptiView Kit (760-700)</td>
<td></td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1 (100%)</td>
</tr>
<tr>
<td><strong>Novocastra (5A4) (Concentrate)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dako Autostainer Link 48</td>
<td>Dako EnVision FLEX+ (K8002/12)</td>
<td></td>
<td>2 (100%)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Leica Bond Max</td>
<td>Bond Polymer Refine Red (DS9390)</td>
<td></td>
<td>1 (100%)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Leica Bond-III</td>
<td>Leica Bond Polymer Refine (DS9800)</td>
<td></td>
<td>1 (100%)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Ventana Benchmark ULTRA</td>
<td>Ventana OptiView Kit (760-700)</td>
<td></td>
<td>-</td>
<td>-</td>
<td>1 (100%)</td>
<td>-</td>
</tr>
<tr>
<td>Ventana Benchmark XT</td>
<td>Ventana OptiView Kit (760-700)</td>
<td></td>
<td>1 (100%)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><strong>Novocastra (5A4) (RTU)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leica Bond Max</td>
<td>Leica Bond Polymer Refine (DS9800)</td>
<td></td>
<td>-</td>
<td>1 (100%)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Leica Bond-III</td>
<td>Leica Bond Polymer Refine (DS9800)</td>
<td></td>
<td>-</td>
<td>1 (100%)</td>
<td>-</td>
<td>1 (100%)</td>
</tr>
<tr>
<td><strong>Thermo/Neomarkers (5A4)</strong></td>
<td>Ventana Benchmark XT</td>
<td>Ventana OptiView Kit (760-700)</td>
<td>1 (50%)</td>
<td>-</td>
<td>1 (50%)</td>
<td>-</td>
</tr>
<tr>
<td><strong>Ventana Confirm (ALK01)</strong></td>
<td>Ventana Benchmark XT</td>
<td>Ventana OptiView Kit (760-700)</td>
<td>2 (67%)</td>
<td>-</td>
<td>1 (33%)</td>
<td>-</td>
</tr>
<tr>
<td><strong>Ventana/Roche (D5F3)</strong></td>
<td>Ventana Benchmark ULTRA</td>
<td>Ventana OptiView Kit (760-700)</td>
<td>1 (100%)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><strong>Zytomed (p80)</strong></td>
<td>None (Manual)</td>
<td>Zytomed ZytoChem Plus (HRP)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1 (100%)</td>
</tr>
</tbody>
</table>
Method used may change interpretation!
(2\textsuperscript{nd} EQA Assessment)

Tested as: +ve cell line (C) +ve tumour (E) -ve tumour (F)

Roche D5F3

Novocastra/
Leica 5A4

Dako ALK1
Pass Rates 2 Years of EQA

<table>
<thead>
<tr>
<th>Assessment Run</th>
<th>Pre-pilot (n=36)</th>
<th>109 (n=53)</th>
<th>110 (n=40)</th>
<th>111 (n=42)</th>
<th>112 (n=47)</th>
<th>113 (n=47)</th>
<th>114 (n=50)</th>
<th>115 (n=57)</th>
<th>116 (n=54)</th>
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</thead>
<tbody>
<tr>
<td>Acceptable</td>
<td>83%</td>
<td>70%</td>
<td>98%</td>
<td>85%</td>
<td>87%</td>
<td>94%</td>
<td>80%</td>
<td>80%</td>
<td>89%</td>
</tr>
<tr>
<td>Borderline</td>
<td>14%</td>
<td>17%</td>
<td>0%</td>
<td>10%</td>
<td>9%</td>
<td>6%</td>
<td>14%</td>
<td>18%</td>
<td>4%</td>
</tr>
<tr>
<td>Unacceptable</td>
<td>3%</td>
<td>2%</td>
<td>5%</td>
<td>4%</td>
<td>0%</td>
<td>6%</td>
<td>2%</td>
<td>2%</td>
<td>7%</td>
</tr>
</tbody>
</table>

UK NEQAS
Immunocytochemistry & In-Situ Hybridisation
Methods Used Over 2 Years of EQA

- Leica/Novocastra (5A4)
- Cell Signaling (D5F3)
- Dako (ALK1)
- Abcam
- Roche Confirm (ALK01)
- Diagnostic Biosystems (5A4)
- Thermo/Neomarks (5A4)
- Zytomed (p80)
- Origene (1A4)
- Ventana/Roche D5F3

Assessment Run

% Usage

0% 10% 20% 30% 40% 50% 60% 70% 80% 90%

Methods Used Over 2 Years of EQA

- Leica/Novocastra (5A4)
- Cell Signaling (D5F3)
- Dako (ALK1)
- Abcam
- Roche Confirm (ALK01)
- Diagnostic Biosystems (5A4)
- Thermo/Neomarks (5A4)
- Zytomed (p80)
- Origene (1A4)
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- Thermo/Neomarks (5A4)
- Zytomed (p80)
- Origene (1A4)
- Ventana/Roche D5F3

Assessment Run

% Usage

0% 10% 20% 30% 40% 50% 60% 70% 80% 90%
In-house Controls Submitted a by Participant (2 Years on)

+ve Lung adenocarcinoma  
-ve Lung adenocarcinoma  
Appendix

Perfect!

- Ideally: +ve & -ve lung NSCLC tumours with appendix - good gauge of the sensitivity of the assay

- However, some labs still using lymphoma in-house control – leads to a false sense of security
ALK Summary

- 80% of EQA users now use the Roche (D5F3) approved assay: 90% pass rate
- 12% Leica (5A4)
- 2% still continue to use the Dako (ALK1) even though it is **NOT** recommended for NSCLC


“Choice of methodology directly affected final interpretation of distributed ALK-positive and ALK-negative NSCLC cases, which are correctly identified by 89% and 88% of participants, respectively. Antibody detection method was a contributing factor in false-negative staining results. The choice of laboratory controls was found to be unsuitable, and as such, in-house control recommendations are also provided.”
Outline of Presentation

- UK NEQAS IHC ALK EQA Assessments over 2 years of surveillance

- Findings from the UK NEQAS IHC pre-pilot assessment for PD-L1 in NSCLC
## PD-L1 CDx Landscape

<table>
<thead>
<tr>
<th></th>
<th>Ventana (SP142)</th>
<th>Ventana (SP263)</th>
<th>Dako (28-8)</th>
<th>Dako (22C3)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Type</strong></td>
<td>Rabbit Monoclonal</td>
<td>Rabbit Monoclonal</td>
<td>Rabbit Monoclonal</td>
<td>Rabbit Monoclonal</td>
</tr>
<tr>
<td><strong>Commercial Availability</strong></td>
<td>CE IVD analytical claim</td>
<td>CE-IVD predictive claim</td>
<td>CE IVD predictive claim</td>
<td>CE IVD predictive claim, CDx</td>
</tr>
<tr>
<td><strong>Pharma Partner</strong></td>
<td>Roche / Genentech</td>
<td>AstraZeneca / MedImmune</td>
<td>BMS</td>
<td>Merck</td>
</tr>
<tr>
<td><strong>Pharma</strong></td>
<td><img src="image" alt="Roche" /></td>
<td><img src="image" alt="AstraZeneca" /></td>
<td><img src="image" alt="BMS" /></td>
<td><img src="image" alt="Merck" /></td>
</tr>
<tr>
<td><strong>Drug name</strong></td>
<td>Atezolizumab</td>
<td>Durvalumab</td>
<td>Nivolumab</td>
<td>Pembrolizumab</td>
</tr>
</tbody>
</table>
| **CDx Configuration** | • BenchMark Series  
  • RTU Antibody  
  • OptiView Detection  
  • OptiView Amp  
  • Complementary Dx | • BenchMark Series  
  • RTU Antibody  
  • OptiView Detection  
  • Complementary Dx | • Dako Autostainer  
  • Dako Link  
  • RTU antibody  
  • Complementary Dx | • Dako Autostainer  
  • Dako Link  
  • RTU antibody  
  • Companion Dx |

**PD-1/PD-L1: The target of many drug companies**
Before Assessment: Methodology Was Showing a Range or Protocols Used By Participants

<table>
<thead>
<tr>
<th>Primary Antibody Clone</th>
<th>Kit/Code/Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>Roche SP142</td>
<td>740-4859</td>
</tr>
<tr>
<td>Roche SP142</td>
<td>790-4860</td>
</tr>
<tr>
<td>Roche SP142</td>
<td>Concentrate</td>
</tr>
<tr>
<td>Roche SP263</td>
<td>740-4905</td>
</tr>
<tr>
<td>Dako 22C3</td>
<td>PharmDx</td>
</tr>
<tr>
<td>Dako 22C3</td>
<td>Concentrate</td>
</tr>
<tr>
<td>abcam 28.8</td>
<td>Concentrate</td>
</tr>
<tr>
<td>Cell Signalling EIL3N</td>
<td>Concentrate</td>
</tr>
</tbody>
</table>

At least 50% of participants are using Laboratory Developed Tests (LDTs)
Although a technical scheme, the interpretation has to be included.

Suggested to look at each commercial PD-L1 assay separately.

Proposed scoring sheet for each of the different assays [intended!]

Rather than using different scoring criteria for each of the Commercial assays interpretation part of the assessment should be scored by placing tumour percentage positivity (TPS) into BINS.
BIN Scoring System

SP263/22C3/28.8/SP142 Clones

<table>
<thead>
<tr>
<th>TPS (%)</th>
<th>&lt;1% (negative)</th>
<th>1-5%</th>
<th>5-9%</th>
<th>10-24%</th>
<th>25-49%</th>
<th>50-79%</th>
<th>80-100%</th>
</tr>
</thead>
<tbody>
<tr>
<td>BIN</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
<td>7</td>
</tr>
</tbody>
</table>

Roche SP142 Clone Also Requires Reporting Of The Tumour Infiltrating Immune Cells (IC Score)

<table>
<thead>
<tr>
<th>IC (%)</th>
<th>&lt;1% (negative)</th>
<th>1-5%</th>
<th>5-9%</th>
<th>≥10%</th>
</tr>
</thead>
<tbody>
<tr>
<td>BIN</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
</tbody>
</table>

For this clone alone the UK NEQAS PD-L1 pre-pilot did also include an IC score based on the guidelines from Roche.
How To Tackle The Lab-Devised Methods?

• The Lab-Devised Techniques (LDTs) were always going to be difficult for us to assess!

• Consensus from the Advisory Panel was to compare the LDTs with the Gold standard of the corresponding clone assay
Assessment Time!

Discussions with the expert panel of assessors so important to establish a robust EQA service!
# Scores for Clones SP263/22C3/28.8

## Table 1: Expected PD-L1 expression levels in NEQAS TMAs submitted to participants (Phase 1, 2 & 3)

<table>
<thead>
<tr>
<th>Sample Code</th>
<th>Sample</th>
<th>Expected TPS (BIN category) Staining pattern</th>
</tr>
</thead>
<tbody>
<tr>
<td>A (Block 1/2)</td>
<td>Cell line (High)</td>
<td>80-100% Strong membrane staining</td>
</tr>
<tr>
<td>B (Block 1/2)</td>
<td>Cell line (Medium)</td>
<td>50-79% Moderate to strong membrane staining</td>
</tr>
<tr>
<td>C (Block 1/2)</td>
<td>Cell line (Low)</td>
<td>1-4% Weak membrane staining</td>
</tr>
<tr>
<td>D (Block 1/2)</td>
<td>Cell line (negative)</td>
<td>&lt;1% No staining present</td>
</tr>
<tr>
<td>E (Block 1)</td>
<td>Tonsil</td>
<td>A (Acceptable), B (Borderline), U (Unacceptable) Strong membrane staining in intra epithelium Moderate punctate staining in germinal centres</td>
</tr>
<tr>
<td>E (Block 2)</td>
<td>Tonsil</td>
<td></td>
</tr>
<tr>
<td>F (Block 1)</td>
<td>Negative NSCLC</td>
<td>&lt;1% Weak – strong membrane staining in ICs and macrophages</td>
</tr>
<tr>
<td>F (Block 2)</td>
<td>Negative NSCLC</td>
<td></td>
</tr>
<tr>
<td>G (Block 1)</td>
<td>Negative NSCLC</td>
<td>0% No tumour present</td>
</tr>
<tr>
<td>G (Block 2)</td>
<td>Positive NSCLC</td>
<td>50-79% Moderate-strong membrane staining (heterogenous) in TCs and ICs</td>
</tr>
<tr>
<td>H (Block 1)</td>
<td>Positive NSCLC</td>
<td>80-100% Strong membrane staining in TCs and ICs Non-specific staining in fibroblasts</td>
</tr>
<tr>
<td>H (Block 2)</td>
<td>Positive NSCLC</td>
<td></td>
</tr>
</tbody>
</table>
Samples Used in the Assessment

Appropriate tissues, and cell lines (from Histiocyte) covering the main decision points for each of the respective assays.
PD-L1 EQA on lung cancer & cell lines

PD-L1 IHC –Dako Agilent Approved
22C3/28.8 CDx

PD-L1 IHC - Ventana Roche Approved
SP263 CDx
Differences between the different assays for PD-L1

- Roche-Ventana SP142
- Roche-Ventana SP263
- Dako Agilent 28-8
- Dako Agilent 22C3
### Mixture of Appropriate Tissues and Cell Lines

<table>
<thead>
<tr>
<th>Strong</th>
<th>Moderate</th>
<th>Weak</th>
<th>-ve cell line (D)</th>
</tr>
</thead>
<tbody>
<tr>
<td>+ve cell line (A)</td>
<td>+ve cell line (B)</td>
<td>+ve cell line (C)</td>
<td>-ve cell line (D)</td>
</tr>
</tbody>
</table>

- **Tonsil (E)**
- **-ve Tumour (F)**
- **Moderate Tumour +ve (G)**
- **Strong +ve cell line (A)**
PD-L1 Pre-Pilot EQA Results Overview:

Median Score

Assessor Score

<table>
<thead>
<tr>
<th>Assay</th>
<th>Median Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>SP263</td>
<td>5</td>
</tr>
<tr>
<td>22C3</td>
<td>4</td>
</tr>
<tr>
<td>SP142</td>
<td>4</td>
</tr>
<tr>
<td>28.8</td>
<td>3</td>
</tr>
<tr>
<td>22C3 LDT</td>
<td>2</td>
</tr>
<tr>
<td>SP142 LDT</td>
<td>2</td>
</tr>
<tr>
<td>28.8 LDT</td>
<td>2</td>
</tr>
<tr>
<td>E1L3N LDT</td>
<td>2</td>
</tr>
<tr>
<td>CAL10 LDT</td>
<td>2</td>
</tr>
</tbody>
</table>

UK NEQAS
Immunocytochemistry & In-Situ Hybridisation
**PD-L1: Approved v LDT**

Approved PD-L1 IHC CDx using 22C3 on strong expressing cell line.  
**80%-100% of cells are positive**

Laboratory Developed Test (LDT) using 22C3 on strong expressing cell line.  
**<20% of cells are positive**
PD-L1: Approved v LDT

Approved CDx using SP263 on Tonsil

Laboratory developed test (LDT) for SP263 on Tonsil
## Acknowledgments

### UK NEQAS ICC & ISH Team:

- Mr Keith Miller
- Mr Andrew Dodson
- Mrs Seema Dhanjal
- Mrs Dawn Wilkinson
- Mrs Amy Newman
- Mr Neil Bilbe
- Mrs Alin Rhodes
- Mrs Clara Lynch
- Dr Merdol Ibrahim

### ALK & PD-L1 NSCLC Assessors & Advisory Panel:

- Dr Paul Cane (London)
- Prof John Gosney (Liverpool)
- Prof Keith Kerr (Scotland)
- Dr Andrew Nicholson (London)
- Dr Phillipe Taniere (Birmingham)
- Dr Erik Thunnissen (Amsterdam)
- Dr Corrado D’Arrigo (Dorset)
- Dr Patrick Pauwels
- Prof Manuel Salto-Tellez (Belfast)
- Dr Birgit Guldhammer Skov
- Dr Vanathi (Australia)

- Mr David Allen (London)
- Mr Steven Forrest (Liverpool)
- Dr Perry Maxwell (Belfast)
- Dr Tony O’Grady (Dublin)
- Dr Jane Starcynski (Birmingham)
- Ms Julia Pagliuso (Australia)
Thank You