A PD-L1 IHC 28-8 PharmDx ring trial on metastatic melanoma: practical aspects

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Conflicts of interest

This ring trial was funded by Bristol-Myers Squibb Belgium
**Background & objectives**

- Evaluation of PD-L1 IHC staining is challenging

- A Belgian ring trial for PD-L1 IHC staining in melanoma was organized by the pathology department of Antwerp University Hospital

- **Aim:**
  - evaluation of reproducibility of PD-L1
  - give feedback in order to standardize the interpretation of PD-L1 staining protocols for melanoma testing
Melanoma PD-L1 ring trial (RT)

Organized between Dec 2017 – Jul 2018

Contained 6 samples (metastasized melanoma)
- 3 cases with $<5\%$ PD-L1
- 3 cases with $\geq 5\%$ PD-L1

Participation of 14 different Belgian laboratories (1 lab participated with 2 methods)
Set-up:

1. First and last slide of all samples were stained with the reference method (PD-L1 28-8 pharmDx protocol on an Autostainer Link 48).
   - Inclusion of control cell line to confirm technical performance of the run
   - Inclusion of blanc control for each sample

2. Blank slides were sent to participating sites for staining. PD-L1 stained slides + interpretation of pathologist were sent back for evaluation.

3. Stained slides were compared with slides stained with reference method.
   Evaluations of participating site was compared with evaluation of 2 (certified) reference pathologists.
Criteria for evaluation of the slides / Scoring system

• Tumor percentage score (TPS)

• Criteria for interpretation of the PD-L1 staining: manual of the PD-L1 IHC 28-8 pharmDx assay

• Cut-off:
  <5%
  ≥5%

• Average range:
  <1%, 1–5%, 5–15%, 15–30%, 30–50%, and ≥50%
## RESULTS

<table>
<thead>
<tr>
<th>Sample</th>
<th>PD-L1 expression with PD-L1 IHC 28-8 pharmDx assay (%)</th>
<th>Average range*</th>
<th>Sites** with good staining***, n (%) (n = 15)</th>
<th>Sites** with good staining****, n (%) (n = 15)</th>
<th>Common mistakes (FP/FN)**</th>
<th>Remark</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Low</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18S71</td>
<td>&lt;1%</td>
<td>&lt;1% (cutoff: &lt;5%)</td>
<td>4 (27%)</td>
<td>15 (100%)</td>
<td>11 FP / 0 FN (1–5%, n = 11)</td>
<td>11 FP because of melanin</td>
</tr>
<tr>
<td>18S72</td>
<td>&lt;1%</td>
<td>&lt;1% (cutoff: &lt;5%)</td>
<td>9 (60%)</td>
<td>15 (100%)</td>
<td>6 FP / 0 FN (1–5%, n = 6)</td>
<td></td>
</tr>
<tr>
<td><strong>Moderate</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18S74</td>
<td>5%</td>
<td>5–15% (cutoff: ≥5%)</td>
<td>10 (67%)</td>
<td>10 (67%)</td>
<td>0 FP / 5 FN (1–5%, n = 5)</td>
<td>Low intensity of staining</td>
</tr>
<tr>
<td>18S93</td>
<td>4%</td>
<td>1–5% (cutoff: &lt;5%)</td>
<td>14 (93%)</td>
<td>15 (100%)</td>
<td>0 FP / 1 FN (&lt;1%, n = 1)</td>
<td>Low intensity of staining</td>
</tr>
<tr>
<td><strong>High</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18S73</td>
<td>10%</td>
<td>5–15% (cutoff: ≥5%)</td>
<td>6 (40%)</td>
<td>15 (100%)</td>
<td>9 FP / 0 FN (15–30%, n = 9)</td>
<td>9 FP because of melanin</td>
</tr>
<tr>
<td>18S76</td>
<td>20%</td>
<td>15–30% (cutoff: ≥5%)</td>
<td>0 (0%)</td>
<td>8 (53%)</td>
<td>0 FP / 15 FN (&lt;1%, n = 3; 1–5%, n = 4; 5–15%, n = 8)</td>
<td>Educational sample</td>
</tr>
</tbody>
</table>

FN, false negative; FP, false positive; PD-L1, programmed death ligand 1.

*Based on CheckMate 067.

**One site participated using two protocols and is counted as two sites for the purposes of this analysis.

**Based on average range.

***Based on cutoff.

**Based on cutoff.

**Conclusion:** Overall, the staining of most sites is within the correct cutoff
# results

<table>
<thead>
<tr>
<th>Sample</th>
<th>Sites* with discrepant** scoring, % (n = 15)</th>
</tr>
</thead>
<tbody>
<tr>
<td>18S71</td>
<td>33% (5 FP, 0 FN)</td>
</tr>
<tr>
<td>18S72</td>
<td>13% (2 FP, 0 FN)</td>
</tr>
<tr>
<td>18S74</td>
<td>47% (3 FP, 4 FN)</td>
</tr>
<tr>
<td>18S93</td>
<td>27% (4 FP, 0 FN)</td>
</tr>
<tr>
<td>18S73</td>
<td>20% (0 FP, 3 FN)</td>
</tr>
<tr>
<td>18S76</td>
<td>40% (2 FP, 4 FN)</td>
</tr>
</tbody>
</table>

FN, false negative; FP, false positive.
*One site participated using two protocols and is counted as two sites for the purposes of this analysis.
**Discrepant refers to the assigned score with respect to the 5% cut-off.

**Conclusion:**
- melanin causes an over-estimation
- cases close to the 5% cut-off: difficult interpretation
- average disconcordance: 34.5%
# Melanoma PD-L1 ringtrial – AB and platforms

## RESULTS

<table>
<thead>
<tr>
<th>Site*</th>
<th>Score based on average range</th>
<th>Score based on cutoff</th>
<th>Antibody clone</th>
<th>Platform</th>
<th>Protocol</th>
<th>Detection kit</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1 FP / 1 FN</td>
<td>1 FN</td>
<td>28-8</td>
<td>Omnis</td>
<td>In house</td>
<td>DAB</td>
</tr>
<tr>
<td>2</td>
<td>1 FN</td>
<td>1 FN</td>
<td>22C3</td>
<td>BenchMark ULTRA</td>
<td>In house</td>
<td>DAB</td>
</tr>
<tr>
<td>3</td>
<td>3 FP / 1 FN</td>
<td>1 FN</td>
<td>22C3</td>
<td>Omnis</td>
<td>In house</td>
<td>DAB</td>
</tr>
<tr>
<td>4</td>
<td>1 FP</td>
<td>–</td>
<td>22C3</td>
<td>BenchMark ULTRA</td>
<td>In house</td>
<td>DAB</td>
</tr>
<tr>
<td>5</td>
<td>2 FP</td>
<td>–</td>
<td>SP263</td>
<td>BenchMark ULTRA</td>
<td>Kit</td>
<td>DAB</td>
</tr>
<tr>
<td>6</td>
<td>1 FN</td>
<td>1 FN</td>
<td>22C3</td>
<td>Autostainer</td>
<td>Kit</td>
<td>DAB</td>
</tr>
<tr>
<td>7</td>
<td>3 FP</td>
<td>–</td>
<td>SP263</td>
<td>Autostainer</td>
<td>In house</td>
<td>ALP</td>
</tr>
<tr>
<td>8</td>
<td>1 FP / 1 FN</td>
<td>–</td>
<td>22C3</td>
<td>Omnis</td>
<td>In house</td>
<td>DAB</td>
</tr>
<tr>
<td>9</td>
<td>3 FP</td>
<td>–</td>
<td>22C3</td>
<td>BenchMark ULTRA</td>
<td>In house</td>
<td>DAB</td>
</tr>
<tr>
<td>10</td>
<td>2 FP</td>
<td>–</td>
<td>22C3</td>
<td>BenchMark ULTRA</td>
<td>In house</td>
<td>DAB</td>
</tr>
<tr>
<td>11</td>
<td>1 FP</td>
<td>–</td>
<td>22C3</td>
<td>BenchMark ULTRA</td>
<td>In house</td>
<td>DAB</td>
</tr>
<tr>
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<td>2 FP</td>
<td>–</td>
<td>22C3</td>
<td>BenchMark ULTRA</td>
<td>In house</td>
<td>DAB</td>
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<td>–</td>
<td>22C3</td>
<td>BenchMark ULTRA</td>
<td>In house</td>
<td>ALP</td>
</tr>
<tr>
<td>14</td>
<td>2 FP / 1 FN</td>
<td>1 FN</td>
<td>22C3</td>
<td>Omnis</td>
<td>In house</td>
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<td>DAB</td>
</tr>
</tbody>
</table>

ALP, alkaline phosphatase; DAB, 3,3'-diaminobenzidine; FN, false negative; FP, false positive.

*One site participated using two protocols and is counted as two sites for the purposes of this analysis.
CONCLUSION:

- 80% used the 22C3
- Benchmark most popular platform with 60%
- 92% of the laboratories used an in-house protocol
- Only 2 laboratories used ALP as detection kit
- Overestimation again due to intense hyperpigmentation
PD-L1 28-8 IHC: detection kit ALP
PD-L1 28-8 IHC: negative control and staining
• PD-L1 IHC staining resulted in similar conclusions in about 65% of cases, independent of the platforms and clones used

• Abundant melanin deposition causes overestimation
  → use ALP or magenta as detection kit
  → use negative control slide

• Histiocytic reaction
  → use HE staining
Most challenging cases are around 5% cut-off
→ evaluate the whole slide and not only the hot spots
→ score each field separately
→ ask for a second opinion from another experienced pathologist

THANK YOU FOR YOUR ATTENTION
We thank the Belgian laboratories and pathologists who participated in this ring trial

- The Institute of Pathology and Genetics (IPG)
- Cliniques Universitaires Mont-Godinne
- Cliniques Universitaires Saint-Luc
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- Universitair Ziekenhuis Leuven
- Laboratoire National de Santé Luxembourg
- AZ Sint-Jan
- Klina Ziekenhuis Antwerpen
- AZ Delta Roeselare
- Universitair Ziekenhuis Brussel
- Universitair Ziekenhuis Antwerp
- CHU Liège
- Virga Jessa Ziekenhuis Hasselt
- Institut Jules Bordet

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