Technical validation of PD-L1 22C3 immunohistochemistry for cell block preparations: cyto-histological concordance and fixative effects

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Objective

Programmed cell death ligand-1 (PD-L1) expression assessed by immunohistochemistry (IHC) is used as a predictive biomarker for anti-PD-1/PD-L1 therapies. Initial work by our group (JASLC WLCG 2018: abstract P5.09.28) provided evidence of a good concordance between matched formalin-fixed paraffin-embedded (FFPE) cell blocks (CB) and lung tumor resections (LTR).

We here report the results of:
1. an expanded cohort of matched FFPE-CB and LTR specimens
2. studies on dual-processed materials performed to exclude an adverse effect of pre-analytical variables (CytoLyt® preparation, delayed formalin fixation [DF])

Methods

1. Matched FFPE-CB and LTR specimens
   - Paired specimens of fine needle biopsy-derived cells blocks and subsequent lung tumor resections of the same anatomic site were obtained from the archives of the Department of Pathology, University Health Network.
2. Control tissue processing
   - Benign tonsillar and placental tissues were subjected to variable pre-analytical conditions prior to CB preparation
   - Immediate 15% neutral-buffered formalin (NBF) fixation
   - Delayed fixation: 24 h
   - Delayed formalin fixation (DF) after storage in 0.9% saline (1-24 hours)
3. Dual processed malignant effusion and fine needle specimens
   - Specimens were equally split and fixed with CytoLyt® vs. 10% NBF prior to CB preparation

Cell block preparation, PD-L1 IHC and evaluation
- CB were prepared using Histolab®. CB sections were reviewed for a minimum of 100 tumor cells. PD-L1 IHC was performed using 22C3 pharmDX™ assay (Dako).
- IHC was evaluated by microscopy (tumor proportion scores determined by pathologists) and digitally (HALO Membrane v1.4 algorithm; PerkinElmer). Analysis was performed with standard statistical methods. Ethics approval was obtained.

Image analysis
- Slides scanned at high resolution (≥200) on Aperio ScanScope AT2 Whole slide scanner (Leica Biosystems Inc).

Patient demographics

<table>
<thead>
<tr>
<th>Matched CB and LTR specimens</th>
<th>Dual processed specimens</th>
<th>N. of positive cases:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median age (years)</td>
<td>72 (range: 42-89)</td>
<td>72 (range: 58-90)</td>
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<tr>
<td>Gender (male/female)</td>
<td>38/34</td>
<td>38/34</td>
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<tr>
<td>Total no. of cases</td>
<td>81</td>
<td>81</td>
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<tr>
<td>Adenocarcinoma</td>
<td>70</td>
<td>70</td>
</tr>
<tr>
<td>Squamous cell carcinoma</td>
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<tr>
<td>Adenosquamous carcinoma</td>
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<td>1</td>
</tr>
</tbody>
</table>

Results

1. PD-L1 tumor proportion score (TPS) concordance between lung tumor resections and cell blocks
   - 25% TPS cut-off
   - LTR: Lung Tumor Resections
   - CB: Cell Block (Fine Needle Biopsy-derived Cell Blocks)

2. PD-L1 TPS and Image analysis of control tissues subjected to variable pre-analytical conditions
   - Tonsil (deep crypt epithelium)
   - Placenta (syncytiotrophoblast)

3. PD-L1 45% spositivity between dual processed effusions and fine needle aspirates

Summary

There is high correlation of PD-L1 TPS between:
1. Matched FFPE cell blocks and lung tumor resections
2. Dual-processed specimens (CytoLyt® pre-fixation vs. 10% NBF)

Cytology specimens - both after 10%NBF fixation and CytoLyt® pre-fixation - are useful for PD-L1 evaluation by 22C3 immunohistochemistry in lung carcinoma.

Clinical validation of PD-L1 IHC with cytology specimens is required.