PD-L1 scoring in cytological samples

Spasenija Savic Prince
SSPath/SGPath seminar
Bern, November 16th 2019
NSCLC
70% advanced

40% diagnosed by cytology

Need for PD-L1 testing on cytology specimens
PD-L1 testing on cytology

Question from the audience:

• Is it still true, that no PD-L1 assay was validated for cytology by the different manufacturers?

Answer:

• Yes. No clinical trial validated PD-L1 assay was validated for cytology, neither for cell block nor for conventional cytology by the manufacturers.
Immune cell scoring: Not reliably possible in cytology

- Tumor area cannot be defined
<table>
<thead>
<tr>
<th>Drug</th>
<th>Indication</th>
<th>Type of testing</th>
<th>PD-L1 assay</th>
<th>PD-L1 scoring</th>
<th>Clinically relevant PD-L1 expr. levels</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pembrolizumab</td>
<td>1./2. L adv. stage</td>
<td>Companion</td>
<td>22C3 SP263</td>
<td>TPS</td>
<td>≥ 50% (1. L) ≥ 1 (2. L)</td>
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<tr>
<td>Nivolumab</td>
<td>2. L adv. stage</td>
<td>Complementary *</td>
<td>28-8 SP263</td>
<td>TPS</td>
<td>≥1%, ≥5%, ≥10%</td>
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<td>Atezolizumab</td>
<td>2. L adv. stage</td>
<td>Complementary</td>
<td>SP142</td>
<td>TC/IC</td>
<td>≥ 50% TC or ≥ 10% IC</td>
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<td>Durvalumab</td>
<td>Adjuvant after ChemoradioTx</td>
<td>Complementary (EMA: Companion)</td>
<td>SP263</td>
<td>TPS</td>
<td>≥ 1%</td>
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* only for non-squamous
Immunocytochemistry (ICC) on cell blocks: Use of histology standardized FFPE protocols

FFPE cell block

However, pre-analytic variability:

- > 10 CB preparation methods
- variable pre-fixation (ethanol, formalin, methanol)
  CytoLyt® (methanol-based) reduces immuno-reactivity
**PD-L1 ICC on cell blocks with histology standardized FFPE protocols compared to paired biopsies**

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PD-L1 ICC on cell blocks with histology standardized FFPE protocols: 
Same TPS scoring criteria as for histology
PD-L1 ICC on cell blocks with histology standardized FFPE protocols: **Find the tumor cells**

HE

SP263 assay BMU
PD-L1 ICC on cell blocks with biopsy standardized FFPE protocols: 
Find the tumor cells

HE

SP263 assay BMU

Macrophages

TPS <1
ICC on non cell blocks cytology:
Common practice – diagnostic and predictive

- Europe (EFCS survey 2008, 28 laboratories): 80%
- US (CAP survey 2009-2010, 345 laboratories): 51%
ICC on PAP-stained cytology:
Pre-analytic procedures significantly different from FFPE samples

- Different sources of tumor material
- Different preservatives and transport media
- Different preparation techniques
- Different fixatives: air dried, ethanol-fixed
- Mostly on PAP-stained specimens
ICC on PAP-stained cytology:
Pre-analytic procedures significantly different from FFPE samples

- Adjusted immunochemistry protocols (LDT)
  - Compared to FFPE IHC protocols:
    - No or less pre-treatment
    - Antibody dilution often different
  - +/- controls:
    - Cell cultures, touch prep. placenta, excess pleural effusions
  - Validation:
    - Cytology - histology comparison
PD-L1 ICC on PAP-stained cytology:
Scoring criteria: Diffuse staining included

Shoe box effect

Diffuse ("cytoplasmic") staining counts

SP263 LDT, Leica BOND MAX

Diffuse staining ("cytoplasmic")

Membranous & diffuse
PD-L1 ICC on PAP-stained cytology with histology standardized FFPE assays compared to paired FFPE specimens

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Noll B et al Cancer Cytopathol 2018; Lozano MD et al Cancer Cytopathol 2019
Case 5

Bronchial secretion: TTF+ adenocarcinoma

PD-L1 ICC with SP263 LDT on Leica BOND MAX
Case 5
Bronchial secretion: TTF+ adenocarcinoma

Transfer of marks from photocopy of the screened PAP-stained cytology
Result of immunocytochemistry:

PD-L1 (SP263):

**Tumor cells**: 70%

Immune cells: not evaluable

EGFR, KRAS, BRAF, HER2 WT; no ALK, ROS, RET or NTRK rearrangement; no MET amplification, no MET EXON 14 skipping mutation

➢ 1 L Pembrolizumab monotherapy
Take home messages

- **PDL1 ICC on cell blocks is valid** using histology standardized FFPE protocols
- **PD-L1 ICC on non-cell block cytology works, but are less standardized**
  - Require rigorous protocol optimization, validation and QC
    - Prospective monitoring of PD-L1 results
    - Development of QC programs for PD-L1 ICC needed
  - Needs experience both in PD-L1 scoring and cytology
    - Scoring (TPS: membranous and “cytoplasmic” staining; IC: not evaluable)
Thank you!

Betty Baschiera
Lukas Bubendorf
Obinna Chijioke
Tatjana Vlajnic

Michael Tamm
Sacha Rothschild

The Power of Diagnostics
Pathology Basel

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