Decreased PD-L1 immunostaining in cytological NSCLC specimens after fixation in an ethanol based fixative

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I hereby declare that I have had business or personal interests in the following industrial enterprises since 1 September 2018:

**Name of the enterprise / Nature of the interest**

<table>
<thead>
<tr>
<th>Enterprise</th>
<th>Interest</th>
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<tr>
<td>AstraZeneca, MSD and Roche Diagnostics</td>
<td>receival of research grants.</td>
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Background

- In non-small cell lung cancer (NSCLC) immunohistochemical expression of PD-L1 predicts likelihood of response to PD-(L)1 checkpoint inhibitors\(^1\).

- Clinically relevant cut-offs of PD-L1 expression are 1% (Durvalumab)\(^2\) and 50% (Pembrolizumab)\(^3\).

- Management of many patients with advanced NSCLC is based on cytology instead of histology\(^4\).

- Limited amount of studies assessing concordance of PD-L1 immunostaining between histology and cytology (formalin fixed)\(^5,6,7,8\).

- Routinely used fixatives in cytology are often different from formalin, such as ethanol based fixatives\(^9\). Potential negative effect on PD-L1 immunostaining?\(^10\).

Aim

To determine if pre-fixation of TBNA-derived* NSCLC specimens in an ethanol based fixative leads to a decrease in PD-L1 immunostaining compared to formalin fixation, using a standardised assay (SP263) and a laboratory-developed test (LDT) (22C3).

*TBNA = transbronchial needle aspiration
Methods

TBNA of lymph node metastasis or primary tumour of NSCLC patients

Collection in 20 mL 10% NBF

Collection in 20 mL Fixcyt (50% ethanol)

Further fixation in 10% NBF + processing into FFPE blocks

Further fixation in 10% NBF + processing into FFPE blocks

22C3 LDT
SP263

22C3 LDT
SP263

Abbreviations:
TBNA = transbronchial needle aspiration
NBF = neutral buffered formalin
FFPE = formalin-fixed paraffin-embedded
LDT = laboratory-developed test

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Methods

PD-L1 immunostaining:
❖ 22C3 (LDT) on Dako Omnis platform (dilution 1:50)
❖ SP263 (standardised assay) on VENTANA Benchmark Ultra platform

PD-L1 scoring:
❖ Experienced pathologist and lung cancer researcher
❖ Membranous staining on viable tumour cells (≥100)

Statistical analysis:
❖ Concordance of PD-L1 immunostaining at 1% and at 50% cut-off
❖ OPA, PPA and NPA* and Cohen’s kappa

* OPA = overall percent agreement; PPA = positive percent agreement; NPA = negative percent agreement
Results (22C3 LDT)

- Analysis of 54 NSCLC patients
- 15 (28%) discordant cases
- 93% of discordant cases show lower TPS in Fixcyt than in formalin

Table 2. Concordance of PD-L1 tumour proportion scores (TPS) between specimens fixed in formalin and in Fixcyt for antibodies SP263 and 22C3 LDT, using two different cut-offs for PD-L1 positivity (≥1% and ≥50%).

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<tr>
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<td>OPA (%)</td>
<td>PPA (%)</td>
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<tr>
<td>22C3 LDT</td>
<td>81%</td>
<td>70%</td>
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* OPA = overall percent agreement; PPA = positive percent agreement; NPA = negative percent agreement
### Results (SP263)

- Analysis of 54 NSCLC patients
- 11 (20%) discordant cases
- 82% of discordant cases show lower TPS in Fixcyt than in formalin

#### Table 2. Concordance of PD-L1 tumour proportion scores (TPS) between specimens fixed in formalin and in Fixcyt for antibodies SP263 and 22C3 LDT, using two different cut-offs for PD-L1 positivity (≥1% and ≥50%).

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<td>78%</td>
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*OPA = overall percent agreement; PPA = positive percent agreement; NPA = negative percent agreement
Examples of PD-L1 staining: 1% cut-off

22C3 LDT

A

≥1% cut-off

B

SP263

C

Formalin

D

Fixcyt
Examples of PD-L1 staining: 50% cut-off

22C3 LDT

E

F

≥50% cut-off

SP263

G

H

Formalin

Fixcyt
Conclusions

❖ Use of an ethanol based fixative leads to lower PD-L1 immunostaining compared to fixation in formalin.

❖ 1% cut-off: discordance between Fixcyt-fixed and formalin-fixed material with both antibodies (22C3 LDT and SP263).

❖ 50% cut-off: higher discordance with use of 22C3 LDT compared to SP263.

❖ Clinical implications:
  ▪ risk of assigning patients to a lower PD-L1 TPS category with use of an ethanol based fixative;
  ▪ potentially denying patients valuable treatment options.
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