UMC Utrecht

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

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Disclosure Information

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

Enterprise | Interest

AstraZeneca, MSD and Roche Diagnostics: receival of research grants.

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]

1. Reck et al. *N Engl J Med* 2016;375(19):1823-33.
2. Planchard et al. ESMO Guideline. *Ann Oncol* 2018;29(Supplement_4):iv192-iv237.
3. EMA. Imfinzi (Durvalumab) 2018. Available from: https://www.ema.europa.eu/en/medicines/human/EPAR/imfinzi.
4. Skov et al. *Apmis* 2015;123(2):108-15.
5. Russell-Goldman et al. *Cancer Cytopathol* 2018;126(4):253-63.
6. Skov BG, Skov T. *Appl Immunohistochem Mol Morphol* 2017;25(7):453-9.
7. Ilie et al. *Cancer Cytopathol* 2018;126(4):264-74.
8. Hernandez et al. *Am J Clin Pathol* 2019;151(4):403-15.
9. Nambirajan A, Jain D. *Cytopathology* 2018;29(6):505-24.
10. Miller et al. *AIMM* 2000; 8(3):228-35.

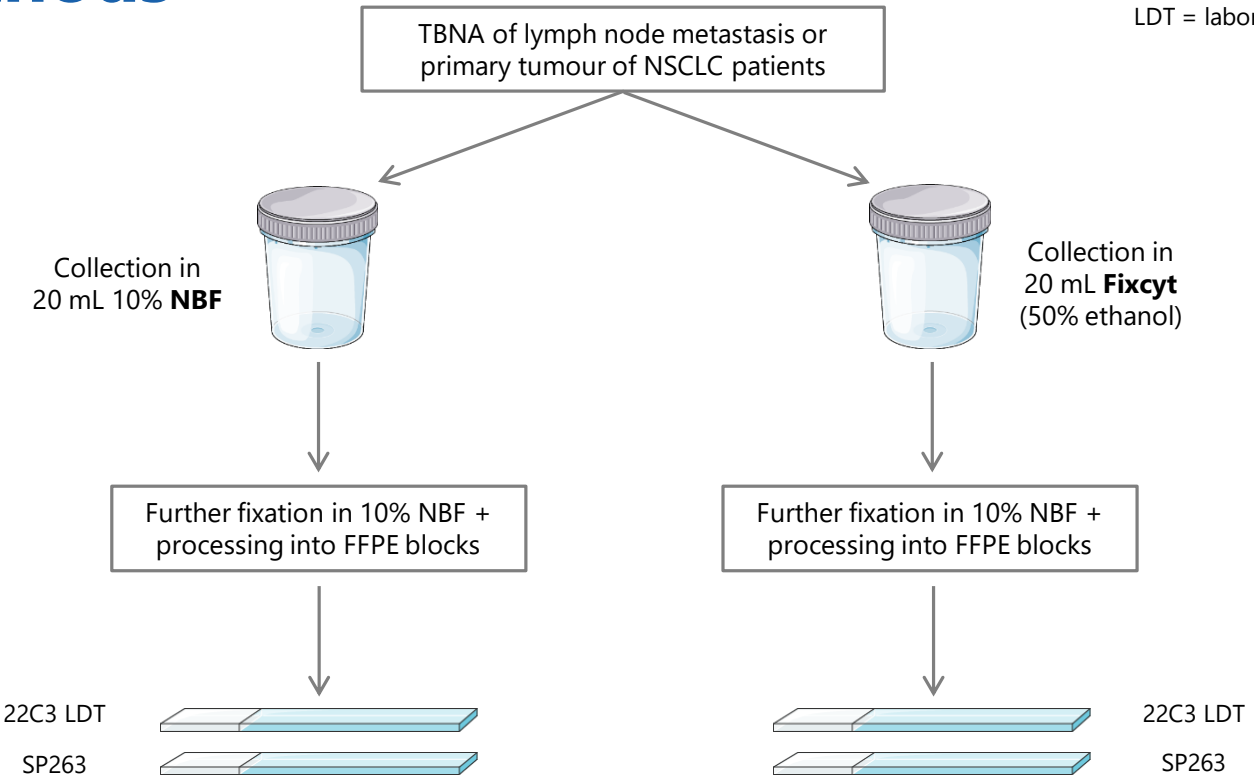
Aim

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

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



Methods

PD-L1 immunostaining:

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

PD-L1 scoring:

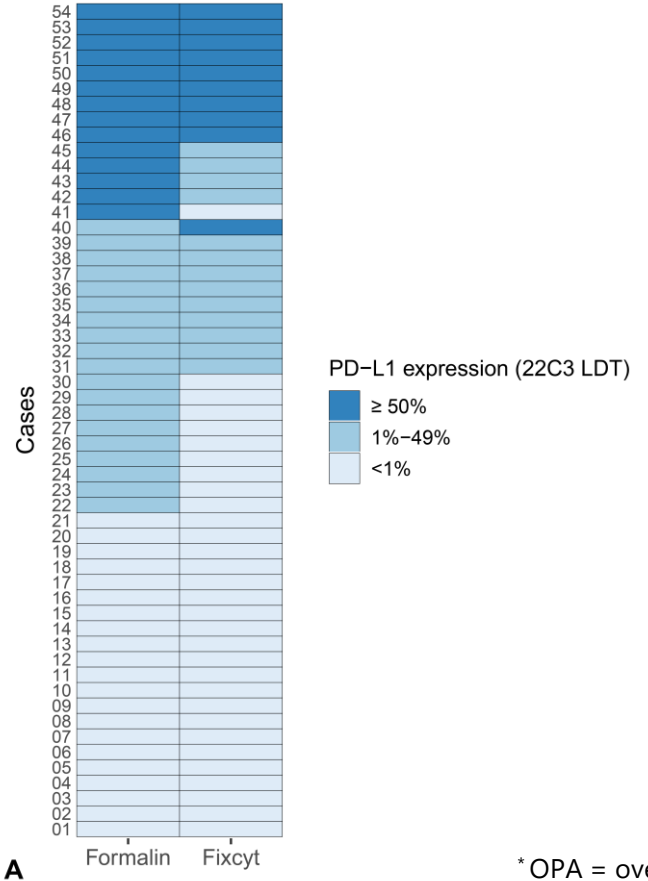
- Experienced pathologist and lung cancer researcher
- Membranous staining on viable tumour cells (≥100)

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)



A

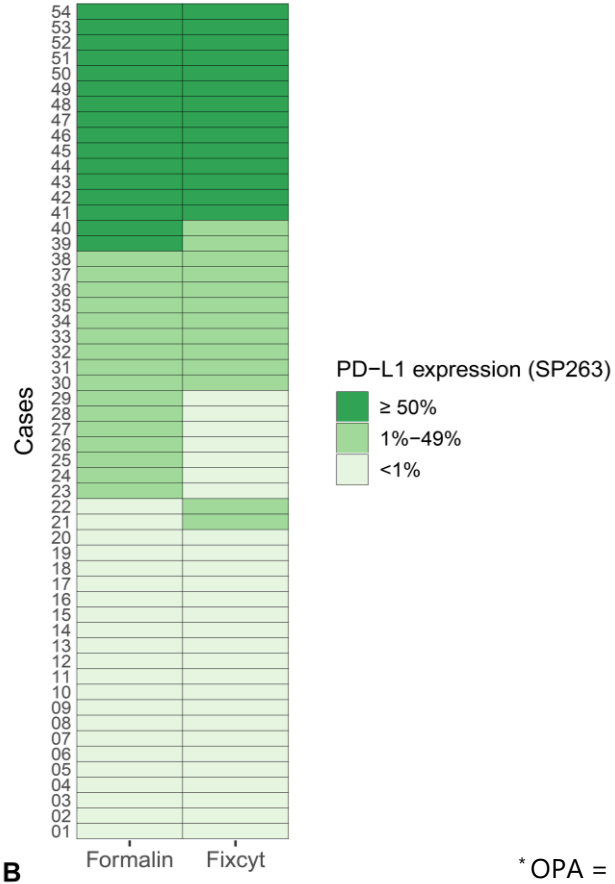
- Analysis of 54 NSCLC patients
- 15 (28%) discordant cases
- 93% of discordant cases show lower TPS in Fixcyt than in formalin

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%).

	Concordance at 1% cut-off				Concordance at 50% cut-off			
	OPA (%)	PPA (%)	NPA (%)	Cohen's kappa	OPA (%)	PPA (%)	NPA (%)	Cohen's kappa
22C3 LDT	81%	70%	100%	0.64	89%	64%	98%	0.68

* OPA = overall percent agreement; PPA = positive percent agreement; NPA = negative percent agreement

Results (SP263)



B

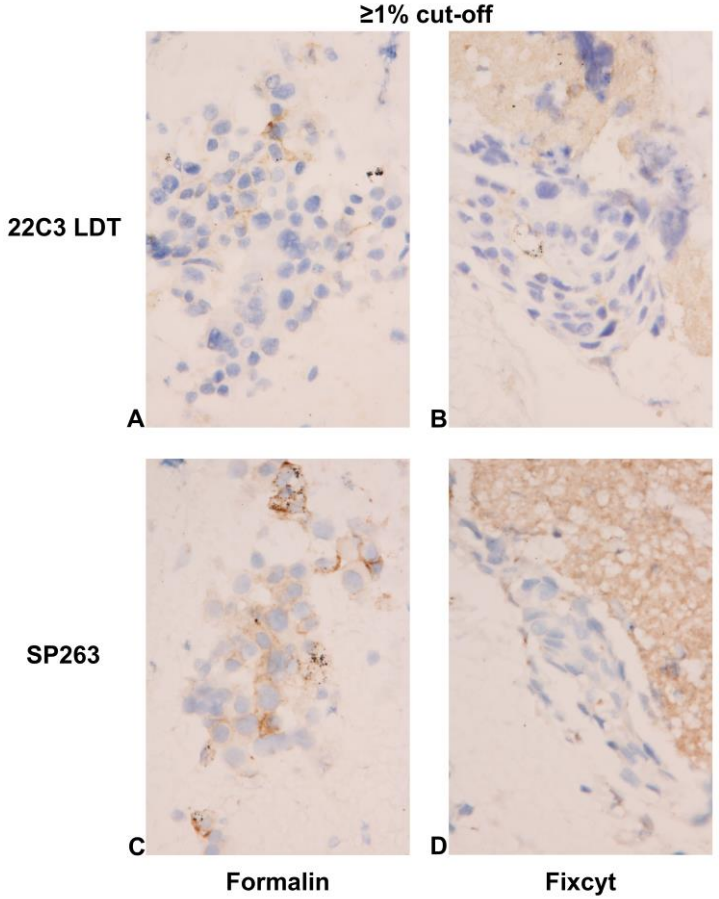
- Analysis of 54 NSCLC patients
- 11 (20%) discordant cases
- 82% of discordant cases show lower TPS in Fixcyt than in formalin

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%).

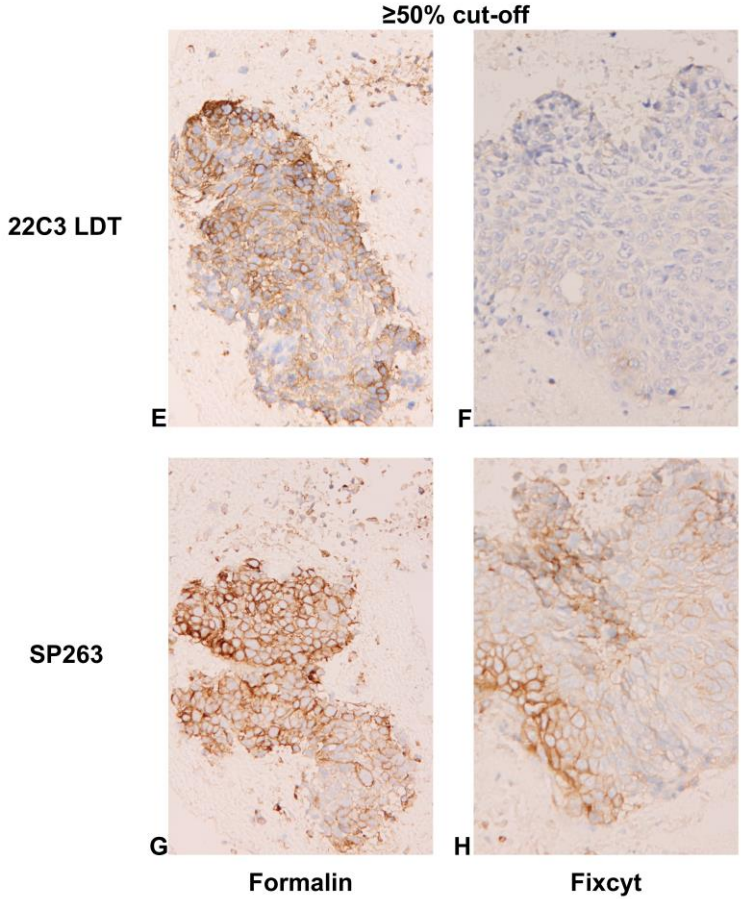
	Concordance at 1% cut-off				Concordance at 50% cut-off			
	OPA (%)	PPA (%)	NPA (%)	Cohen's kappa	OPA (%)	PPA (%)	NPA (%)	Cohen's kappa
22C3 LDT	81%	70%	100%	0.64	89%	64%	98%	0.68
SP263	83%	78%	91%	0.67	96%	88%	100%	0.91

* OPA = overall percent agreement; PPA = positive percent agreement; NPA = negative percent agreement

Examples of PD-L1 staining: 1% cut-off



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



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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