Inter-reader Reliability of Programmed Death Ligand-1 (PD-L1) Scoring Using the VENTANA PD-L1 (SP263) Assay in Non-Small Cell Lung Cancer (NSCLC)

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Background

- Multiple immunotherapies targeting the immune checkpoint programmed cell death-1/programmed cell death ligand-1 (PD-1/PD-L1) pathway are approved for the treatment of patients with various cancers, including non-small cell lung cancer (NSCLC) and urothelial carcinoma (UC)[1]
- Clinical data from multiple studies across several indications have demonstrated that PD-L1 expression level can be used to enrich for responders to PD-1/PD-L1 targeted monotherapy[2-4]
- The VENTANA PD-L1 (SP263) Assay is an immunohistochemical diagnostic assay for anti-PD-L1 immune checkpoint inhibitors[5]
  - It is approved by the US FDA as a complementary diagnostic for use with durvalumab in UC[6]
  - In Europe, it is CE marked for use with durvalumab in NSCLC and UC, and with pembrolizumab and nivolumab in NSCLC[7]
- There is a need to assess the inter-observer reproducibility between pathologists for the VENTANA PD-L1 (SP263) Assay, to determine PD-L1 expression level at multiple clinically relevant PD-L1 cut-offs and scoring algorithms used with anti-PD-1/PD-L1 therapies in NSCLC

Study objectives

- The primary objective is to investigate inter-observer reliability, applied to PD-L1 scoring of tumour cells (TCs) in NSCLC tumour samples, using the VENTANA PD-L1 (SP263) Assay
- The exploratory objective is the inter-observer reliability of PD-L1 scoring in immune cell (IC) infiltrates in NSCLC tumour samples using the VENTANA PD-L1 (SP263) Assay

Methods

- Six expert pulmonary pathologists from different European sites independently scored 200 NSCLC tumour samples stained using the VENTANA PD-L1 (SP263) Assay (Ventana Medical Systems, Inc.)
  - Archival, commercially sourced, formalin-fixed, paraffin-embedded resections were selected to represent the dynamic range of PD-L1 expression
- The proportion of TCs with PD-L1 membrane staining (TC score) was scored based on pre-defined intervals (<1%, 1–4%, 5–9%, 10–15%, etc.). The proportion of ICs present as a proportion of the tumour environment (PIC value) and the percentage of ICs staining positive for PD-L1 (IC score) were recorded as continuous variables
  - PD-L1 expression level was evaluated at cut-offs of ≥1%, ≥25% and ≥50% for TC score and at a cut-off of ≥25% for IC score
- PD-L1 staining in alveolar macrophages was excluded from assessment by all pathologists

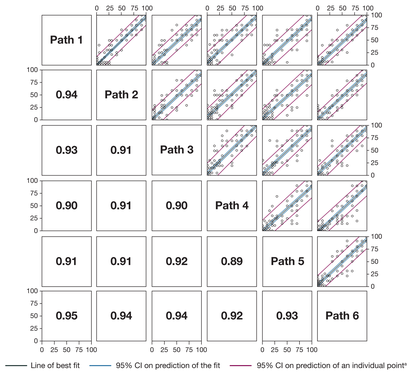
Statistical analyses

- Pathologist concordance for PD-L1 classification across TC ≥1%, ≥25% and ≥50% was determined by generating tables of agreement in PD-L1 expression level between pathologists and calculating the overall percent agreement (OPA), average positive agreement (APA) and average negative agreement (ANA) and the lower bound of the 95% confidence interval (CI)
  - Percent agreement data for IC ≥25% cut-off were not included as very few samples were above the cut-off, rendering the analysis less informative
- Reliability across pathologists for the TC score, PIC value and IC score was determined using the lower bound of the 95% CI on the intra-class correlation coefficient[8] (ICC; single-observer, absolute-agreement, 2-way random effects model)
  - Reliability was defined as poor (<0.5), moderate (≥0.5 to <0.75), good (≥0.75 to <0.9) or excellent (≥0.9)[8]
- Pathology reliability in classifying patient samples as PD-L1 high or low was assessed using Fleiss' kappa
  - Reliability was interpreted using kappa as poor (<0), slight (0.01–0.20), fair (0.21–0.40), moderate (0.41–0.60), substantial (0.61–0.80) or almost perfect (0.81–1.00)[9]
- All statistical analyses were performed using R version 3.5.1 (R Foundation for Statistical Computing, Vienna, Austria)

Results

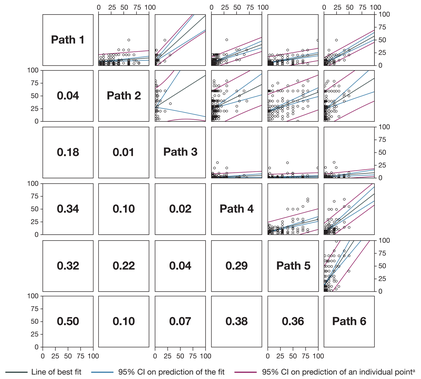
- TC scoring showed strong concordance between pathologists across cut-offs (**Table 1**)
  - OPA was ≥90% for TC ≥1% and >94% for both TC ≥25% and ≥50%
- TC scoring showed strong 1:1 pair-wise correlations (R^2 >0.89), with no systematic bias between pathologists (**Figure 1**)
- Systematic and substantial differences in PIC values (R^2 range: 0.01–0.50) and IC scores (R^2 range: 0.26–0.46) were noted between pathologists with poor pair-wise correlations (**Figures 2** and **3**)
- ICC indicated 'excellent' reliability for TC scoring between pathologists (ICC = 0.96) (**Table 2**)
  - Reliability was 'poor' between pathologists for both PIC values (ICC = 0.21) and IC scores (ICC = 0.48)
- Fleiss' kappa indicated 'substantial' agreement between pathologists for TC ≥1% and 'almost perfect' agreement for TC ≥25% and ≥50% cut-offs in classifying patients by PD-L1 expression level as either high or low (**Table 3**)

Figure 1. Pair-wise scatterplots for PD-L1 TC scoring by pathologists



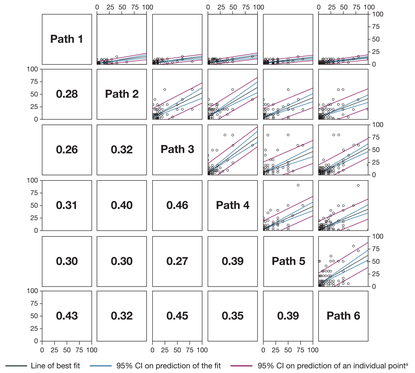
[a]95% of the data are expected to lie within these boundaries
CI, confidence interval; Path, pathologist; PD-L1, programmed cell death ligand-1; TC, tumour cell; TC score, proportion of TCs with PD-L1 membrane staining

Figure 2. Pair-wise scatterplots for PD-L1 PIC scoring by pathologists



[a]95% of the data are expected to lie within these boundaries
CI, confidence interval; Path, pathologist; PD-L1, programmed cell death ligand-1; PIC value, proportion of immune cells present as a proportion of the tumour environment

Figure 3. Pair-wise scatterplots for PD-L1 IC scoring by pathologists



[a]95% of the data are expected to lie within these boundaries
CI, confidence interval; IC score, percentage of immune cells staining positive for PD-L1; Path, pathologist; PD-L1, programmed cell death ligand-1

Table 1. Overall agreement between pathologists for PD-L1 TC scores across cut-offs

PD-L1 cut-off	OPA (95% CI)	APA (95% CI)	ANA (95% CI)
TC ≥1%	90.6% (89.6%)	92.1% (91.2%)	88.5% (87.3%)
TC ≥25%	95.0% (94.3%)	92.8% (91.7%)	96.2% (95.6%)
TC ≥50%	94.4% (93.6%)	90.2% (88.8%)	96.1% (95.5%)

Lower bound of the 95% CI for all calculations is >85%, the value typically required to be exceeded for ILR studies
ANA, average negative agreement; APA, average positive agreement; CI, confidence interval; ILR, inter-laboratory reproducibility; OPA, overall percent agreement; PD-L1, programmed cell death ligand-1; TC, tumour cell; TC score, proportion of TCs with PD-L1 membrane staining

Table 2. ICC for reliability of scores between pathologists

Class	ICC (95% CI)	Interpretation of ICC lower bound
TC scores	0.96 (0.95–0.97)	Excellent
PIC values	0.21 (0.11–0.31)	Poor
IC scores	0.48 (0.40–0.56)	Poor

CI, confidence interval; IC score, percentage of immune cells staining positive for PD-L1; ICC, intra-class correlation coefficient; PD-L1, programmed cell death ligand-1; PIC value, proportion of immune cells present as a proportion of the tumour environment; TC, tumour cell; TC score, proportion of TCs with PD-L1 membrane staining

Table 3. Fleiss' kappa for reliability in classification of patients based on TC scores between pathologists

PD-L1 cut-off	Kappa	Interpretation of reliability
TC ≥1%	0.806	Substantial
TC ≥25%	0.890	Almost perfect
TC ≥50%	0.863	Almost perfect

Kappa values are for single cut-offs (i.e. the pathologists score as high or low)
PD-L1, programmed cell death ligand-1; TC, tumour cell; TC score, proportion of TCs with PD-L1 membrane staining

Conclusions

- Assessment of TC scores by expert pathologists was highly reproducible in NSCLC tumour samples using the VENTANA PD-L1 (SP263) Assay, building confidence in the performance of this assay in patient selection for anti-PD-L1 therapy
- PD-L1 staining on ICs is not included in the assays that aim to identify patients eligible for immuno-oncology monotherapy treatment in NSCLC.[10,11] PIC and IC scoring were not reproducible in NSCLC tumour samples, suggesting that the assessment methodology is unreliable for this tumour type and assay
- The results for NSCLC are in contrast with those for UC, where inter-laboratory reproducibility studies have shown strong agreement in both TC and IC scoring[12]
- Further studies are required to investigate this difference in the pathology of tumour types

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