



PD-L1 TESTING IN NSCLC

OVERVIEW OF PD-L1 TESTING¹

Antibody	28-8	22C3	SP142	SP263
Instrument and detection systems required	Dako EnVision Flex on AutostainerLink 48	Dako EnVision Flex on AutostainerLink 48	Ventana OptiView detection and amplification on Benchmark ULTRA	Ventana OptiView detection on Benchmark ULTRA
Product for which assay was validated	nivolumab	pembrolizumab	atezolizumab▼	durvalumab▼
Associated scoring algorithm	TPS	TPS	TC, IC	TC

CONCORDANCE BETWEEN ASSAYS BY SCORING ALGORITHM AND CUTOFFS

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HIRSCH *ET AL.* 2016

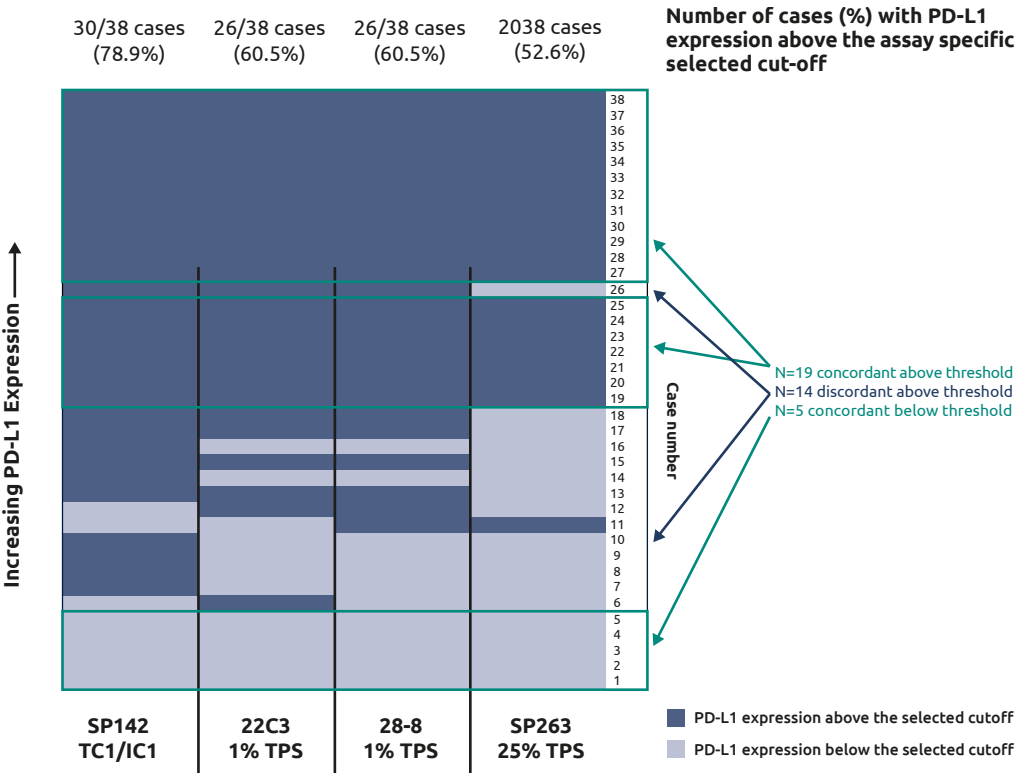
The Blueprint PD-L1 IHC Assay Comparison Project.

- ▶ All four assays (28-8, 22C3, SP142, SP263) were tested¹
- ▶ All four assays stained TC and IC cells¹



1 THE BLUEPRINT PD-L1 IHC ASSAY COMPARISON PROJECT. HIRSCH *ET AL.* 2016

Heat map to show concordance between assays (test samples expressing PD-L1 at levels above or below each assay’s validated cutoff threshold).¹



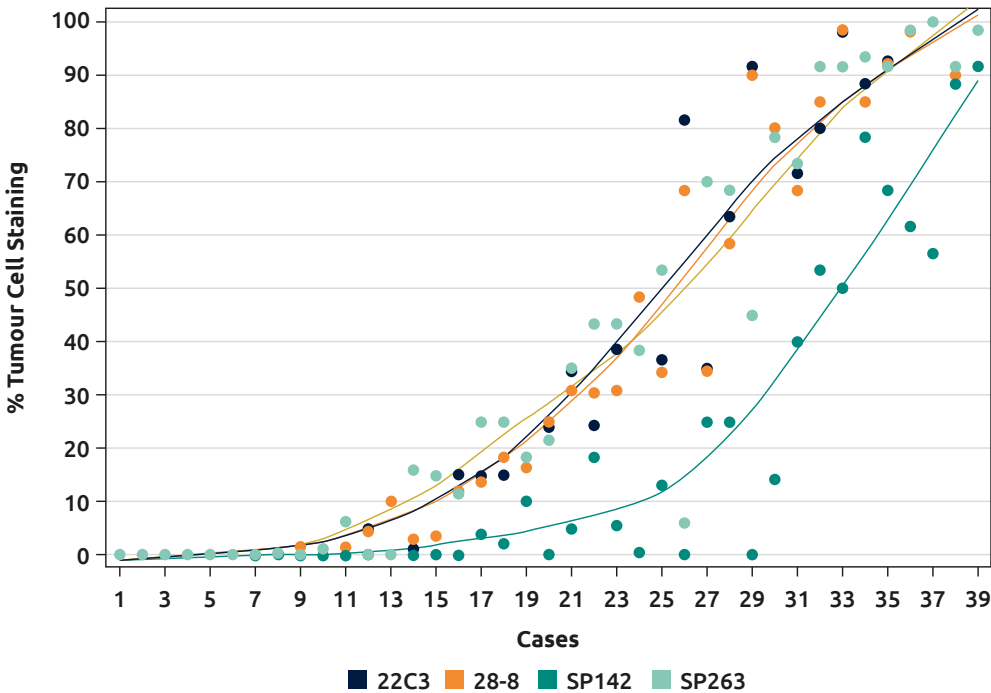
- Five cases out of 38 show concordance below all threshold values for all assay/algorithm combinations¹
- 19/38 cases show expression above all threshold values for all assay/algorithm combinations¹
- The remaining 14 cases show a combination of discordant outcome across the various assay/algorithm combinations¹

Adapted from Hirsch *et al.* 2016.¹ Sample number is shown on the right-hand side.

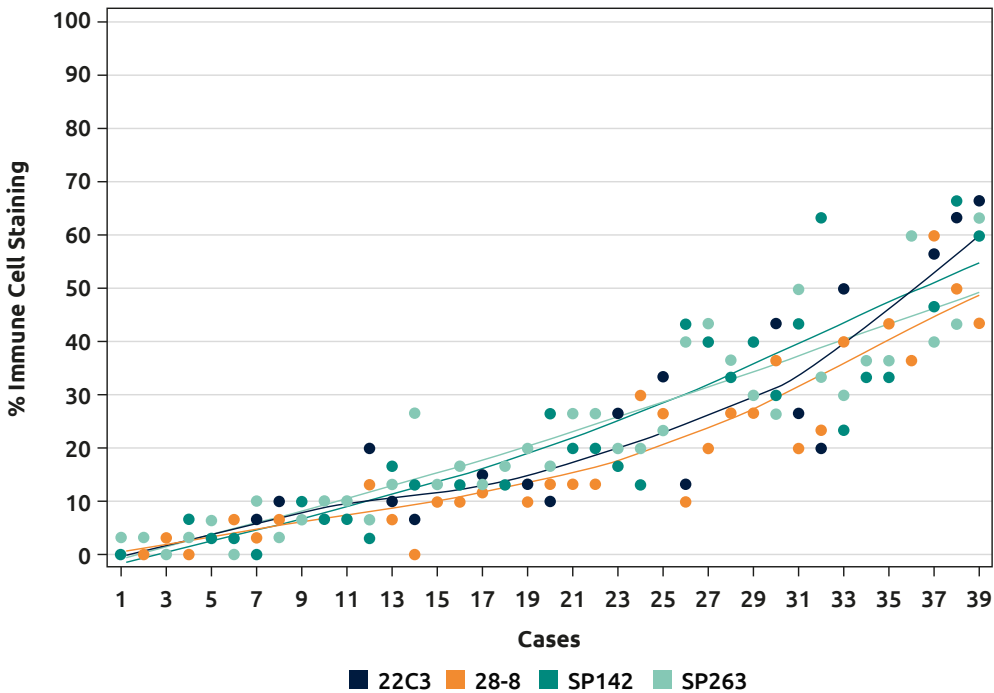


1 THE BLUEPRINT PD-L1 IHC ASSAY COMPARISON PROJECT. HIRSCH *ET AL.* 2016

Analytical comparison of percentage tumour cell and immune cell staining, by case, for each assay



Adapted from Hirsch *et al.* 2016.¹



Adapted from Hirsch *et al.* 2016.¹



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RATCLIFFE *ET AL.* 2017

▶ Three assays were tested: SP263, 22C3, 28-8 using 500 archival NSCLC samples²

The correlation coefficients comparing TC staining between assays were highly correlated for all comparisons:²

- ▶ Ventana SP263 versus Dako 22C3, Spearman correlation coefficient = 0.925
- ▶ Ventana SP263 versus Dako 28-8, Spearman correlation coefficient = 0.948
- ▶ Dako 28-8 versus Dako 22C3, Spearman correlation coefficient = 0.954

An overall percentage agreement of >90% was achieved between assays at multiple PD-L1 expression cutoffs.²

Overall percentage agreement between assays at multiple expression cutoffs²

Expression cutoff	Ventana SP263 vs. Dako 28-8 OPA (lower 95% CI), %	Dako 22C3 vs. Dako 28-8 OPA (lower 95% CI), %	Ventana SP263 vs. Dako 22C3 OPA (lower 95% CI), %
≥1%	91.7 (89.3)	93.7 (91.7)	91.1 (88.7)
≥10%	92.9 (90.7)	94.9 (93.0)	92.7 (90.5)
≥25%	94.9 (93.0)	96.6 (94.9)	94.3 (92.3)
≥50%	95.9 (94.2)	97.2 (95.6)	93.5 (91.4)

The positive percentage agreement (PPA) and negative percentage agreement (NPA) mostly showed >90% agreement, with more variability in the PPA.

PPA and NPA between assays at multiple expression cutoffs²

Reference assay at specified expression cutoff	Dako 28-8	
	PPA (lower 95% CI), %	NPA (lower 95% CI), %
Dako 28-8 ≥1%		
Dako 28-8 ≥10%		
Ventana SP263 ≥25%	90.1 (85.5)	97.5 (95.6)
Dako 22C3 ≥50%	97.5 (93.7)	97.0 (95.2)

Adapted from Ratcliffe *et al.* 2017.²



2 RATCLIFFE *ET AL.* 2017

PPA and NPA between assays at multiple expression cutoffs ² (continued)		
	Ventana SP263	
Reference assay at specified expression cutoff	PPA (lower 95% CI), %	NPA (lower 95% CI), %
Dako 28-8 ≥1%	90.4 (87.1)	93.5 (89.9)
Dako 28-8 ≥10%	91.4 (87.6)	94.0 (91.1)
Ventana SP263 ≥25%		
Dako 22C3 ≥50%	91.7 (86.4)	94.1 (91.7)
PPA and NPA between assays at multiple expression cutoffs ² (continued)		
	Dako 22C3	
Reference assay at specified expression cutoff	PPA (lower 95% CI), %	NPA (lower 95% CI), %
Dako 28-8 ≥1%	92.5 (89.4)	95.5 (92.3)
Dako 28-8 ≥10%	94.8 (91.5)	95.1 (92.4)
Ventana SP263 ≥25%	86.0 (80.8)	98.8 (97.2)
Dako 22C3 ≥50%		

Adapted from Ratcliffe *et al.* 2017.²

3 SCHEEL *ET AL.* 2018

- ▶ All four assays (28-8, 22C3, SP142, SP263) were tested³
- Interassay concordance for TCs**
- ▶ Assays 22C3 and 28-8 showed similar concordance coefficients for the ≥1% and ≥50% cut-offs in the near-perfect range (k=0.82–0.89)³
- ▶ Assay SP263 showed perfect agreement for the ≥50% cut-off (k=1), whereas the coefficients of the ≥1% cut-off had limited validity, owing to the high ratio of positive cases (k=0.76)³
- ▶ Assay SP142 showed substantial concordance for the ≥1% cut-off (k=0.71) and near-perfect concordance for the ≥50% cut-off (k=0.95)³
- ▶ At the ≥50% cut-off for TCs, assays 22C3 and 28-8 detected five of 15 positive cases, assay SP263 six of 15, and assay SP142 three of 15³

4 ADAM *ET AL.* 2017

- ▶ Three assays were tested: 28-8, 22C3 and SP263⁴
- ▶ The 28-8, 22C3 and SP263 assays were highly concordant for tumour cell and immune cell staining across the Dako or Ventana platforms (R²=0.886 to 0.953)⁴



SUMMARY

There is a reasonable agreement of PD-L1 assays in NSCLC¹⁻⁴

FURTHER INFORMATION

- ▶ SP263 can be used to identify patients suitable for pembrolizumab and nivolumab, based on a method comparison study carried out by AstraZeneca, which compares data from currently available PD-L1 assays, PD-L1 IHC 22C3 pharmDx (used in the clinical studies of pembrolizumab), PD-L1 IHC 28-8 pharmDx (used in the clinical studies of nivolumab) and VENTANA PD-L1 (SP263) Assay⁵
- ▶ Laboratory-developed tests (LDTs) were also tested, showing more variable results⁴

Adverse events should be reported.
Reporting forms and information can be found at
<https://yellowcard.mhra.gov.uk/> or search for MHRA Yellow Card in the Google Play or Apple
App Store. Adverse events should also be reported to MSD (Tel: 0208 154 8000)

REFERENCES

1. Hirsch FR *et al.* *J Thoracic Oncol.* 2017;12(2): 208–222.
2. Ratcliffe MJ *et al.* *Clin Cancer Res.* 2017;23(14): 3585–3591.
3. Scheel AH *et al.* *Mod Pathol.* 2016;29(10): 1165–1172.
4. Adam J *et al.* *J Thoracic Oncol.* 2017;12(1): S11–S12.
5. VENTANA PD-L1 (SP263) Assay (CE IVD). Available at: <https://diagnostics.roche.com/global/en/products/tests/ventana-pd-l1-sp263-assay2.html> Accessed December 2020.

ABBREVIATIONS

- CI**, confidence interval
IC, immune cells
IHC, immunohistochemistry
LDT, laboratory developed test
- NPA**, negative percent agreement
OPA, overall percent agreement
NSCLC, non small cell lung cancer
PD-L1, programmed death ligand-1
- PPA**, positive percent agreement
TC, tumour cells
TPS, tumour proportion score.