Maastricht UMC+

PDL1: een simpele kleuring?

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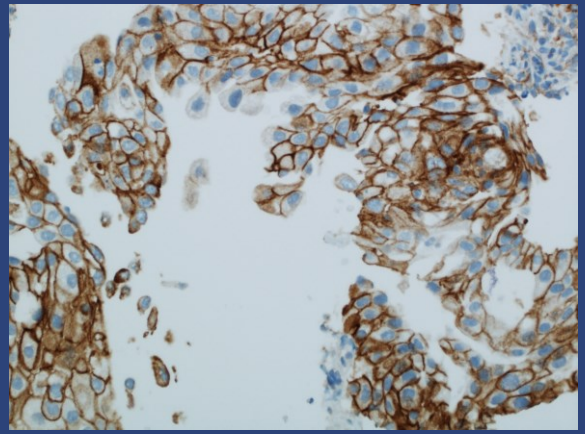


Disclosure of speaker's interests	
(Potential) conflict of interest	See below
Potentially relevant company relationships in connection with event	Company names
• Research funding • Attending advisory boards • Speaker fee	• Novartis, MSD, BMS • Pfizer, MSD, BMS, Roche • Roche

PDL1: een simpele kleuring?

Approved name/symbol (HGNC, OMIM):
Programmed cell death 1 ligand 1 (PDCD1LG1), gene: *CD274*

alternative names: PDL1, programmed death ligand 1
PDCD1L1, PDCD1 ligand 1
B7H1, B7 homolog 1
CD274

Simpel: als iets niet moeilijk is, eenvoudig, kunsteloos, onnozel, dom, onschuldig suf, zwak van hersenen, niet goed wijs, zonder veel complicaties, weinig ontwikkeld, argeloos, flauw, licht, naïef, niet samengesteld, onbetekenend

Kleuring: het kleuren, kleur is eigenschap van licht bepaald door verschillende golflengtes
Immunohistochemische kleuring: aankleuren van weefsels/celstructuren (lichtmicroscoop)

Which of the following correctly summarizes differences between anti-programmed cell death ligand 1 (PD-L1) antibodies used for immunohistochemistry (IHC) detection of PD-L1?

A Each antibody requires differently prepared tissue samples

B Each antibody is read using a different instrument

C Each antibody is scored based on a unique population of cells

D Each antibody is associated with a different immunotherapy

What is the lowest cutoff of PD-L1 expression on tumor cells that is used to define PD-L1-positive patients in clinical trials?

- 1%
- 5%
- 10%
- 25%
- 50%

In ongoing clinical trials, what biomarker cut-off defines PD-L1-positive tumors for durvalumab therapy?

A PD-L1 expression of at least 50% as measured by 22C3

B PD-L1 expression of at least 1% as measured by 28-8

C PD-L1 expression of at least 5% as measured by SP142

D PD-L1 expression of at least 25% as measured by SP263

Overview

- Approved PDL1 IHC diagnostic assays in NSCLC
- Interassay and interobserver comparison (tumor/immune cells)
- Utility of diagnostic materials
  - Histology vs cytology
  - Tumor heterogeneity
- Laboratory developed assays, standardization, EQA, training
- Other biomarkers
- Conclusions

Table 1. Approved and Investigational PD-L1 Diagnostic Assays in NSCLC

	Nivolumab		Pembrolizumab		Atezolizumab	Durvalumab	Avelumab
Antibody clone	28-8	SP263	22C3	SP263	SP142	SP263	73-10
Assay developer	Dako[5,25]	Ventana[24]	Dako[22,23]	Ventana[24]	Ventana[6]	Ventana[16]	Dako[55]
PD-L1 immunohistochemistry scoring*	TC	TC	TC	TC	TC and/or tumor-infiltrating IC	TC	TC
PD-L1 levels evaluated in clinical trials	TC: ≥ 1%, ≥ 5%, ≥ 10%[5]	TC: ≥ 1%, ≥ 5%, ≥ 10%[5]	TC: ≥ 1%, ≥ 50%[22]	TC: ≥ 1%, ≥ 50%[22]	TC: ≥ 50% (TC3)† IC: ≥ 10% (IC3)†[6,15]	TC: ≥ 25%[16]	TC: ≥ 1%[56]
PD-L1 level in first-line therapy	NA	NA	TC ≥ 50%	TC ≥ 50%	NA	NA	NA
PD-L1 level in second-line therapy	None	None	TC ≥ 1%	TC ≥ 1%	None	NA	NA
Diagnostic status	Complementary: testing not required US/EU: NSQ NSCLC Japan: SQ and NSQ NSCLC	Complementary: testing not required EU: NSQ NSCLC	Companion: testing required US/EU/Japan: SQ and NSQ NSCLC	Companion: testing required EU: SQ and NSQ NSCLC	Complementary: testing not required US/EU: SQ and NSQ NSCLC	Not yet approved for durvalumab	Not yet approved for avelumab
Approved IVD PD-L1 expression levels	US/EU/Japan: all patients eligible	EU: all patients eligible	US/EU/Japan: ≥ 50% (previously untreated); ≥ 1% (previously treated)		US/EU: all patients eligible	Not available for NSCLC	Not available for NSCLC

Abbreviations: IC, immune cells; IVD, in vitro diagnostic; NA, not applicable; NSCLC, non–small-cell lung cancer; NSQ, non-squamous; PD-L1, programmed death-ligand 1; SQ, squamous; TC, tumor cells.
*All assays score cells at any intensity.
†TC0 < 1%, TC1 1% to < 5%, TC2 5% to < 50%, TC3 ≥ 50%, IC0 < 1%, IC1 1% to < 5%, IC2 5% to < 10%, IC3 ≥ 10%.

- 5 antibodies, 5 therapies
- 2 platforms (Dako, Ventana)
- Many different evaluation criteria
- PDL1 companion: Pembrolizumab; complementary nivolumab, atezolizumab

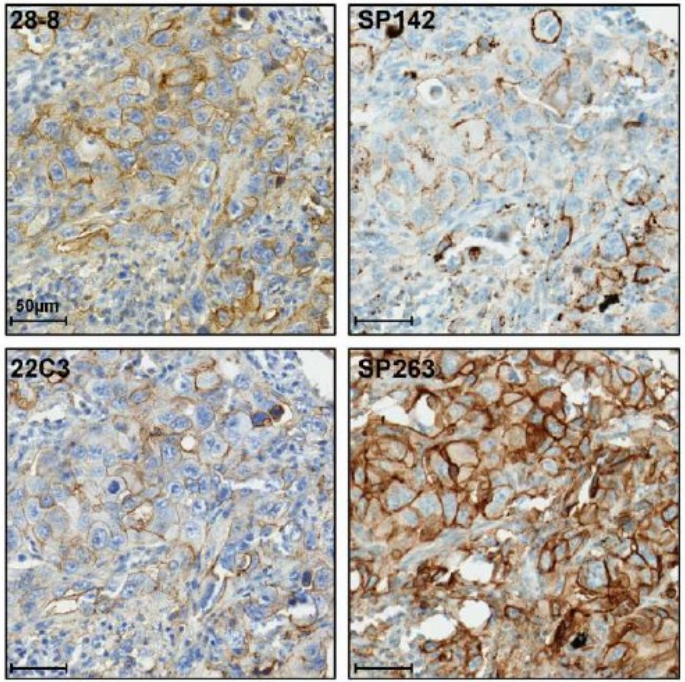
Harmonization studies required!

Buettner et al., JCO 2017 PDL1 immunohistochemistry testing review

Studies comparing approved PDL1 IHC assays: German harmonization and IASCL Blueprint study

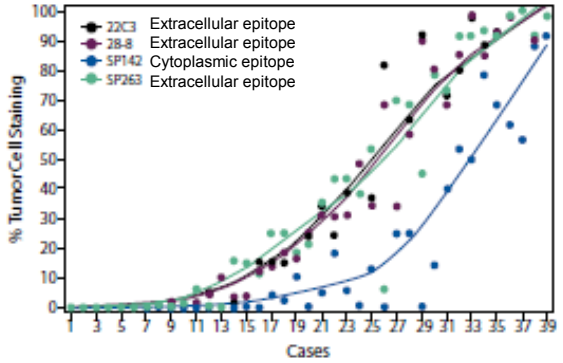
PD-L1 IHC variation

Different PD-L1 clones on consecutive sections of one tumor

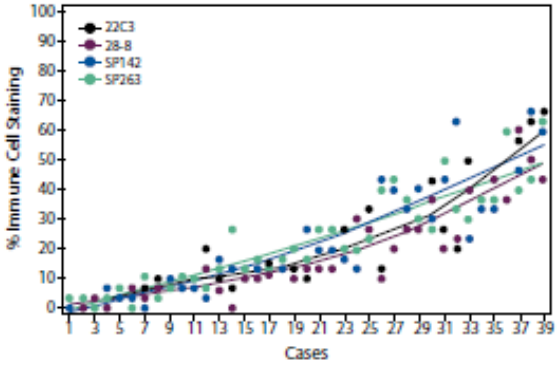


Scheel et al. Modern Pathol 2016

Figure 1 Staining patterns of clinical trial assays for PD-L1 immunohistochemistry. Example micrographs of four clinical trial assays for PD-L1 immunohistochemistry: matched regions on consecutive slides stained with the indicated assays. The case was scored PD-L1 positive (score 5, ≥50%) by all assays.



TC



IC

Blueprint: clinical classification varies across all 4 assays

Match assays + PDL1 expr level for intended therapy

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Scheel et al., Moder n Pathol 2016; Hirsch et al., JTO 2017

Tsao et al., IASLC Atlas of PD-L1 immunohistochemistry testing in lung cancer, 2017

ETOP Lungscape tumour cohort:

- Surgically resected, stage I-III NSCLC tumors
- Fully annotated clinical information
- Tissue microarrays (TMA)

N=2709, 17 centers (mostly European)

→

PD-L1 Project

Aim: Characterize the prevalence and clinical significance of PD-L1 positivity

n=2181, 14 centers

In the framework of the PD-L1 project:

- Harmonized ETOP laboratories' PD-L1 scoring on TMAs, by an external quality assessment (EQA) program
- Cross-validated the TMA approach versus Whole Sections in the ETOP Lungscape cohort

Assay: DAKO PD-L1 IHC 28-8 pharmDx™ (CDx to Nivolumab)

Setup:

- Centralized IHC staining at 2(-3) laboratories
- Local reading of slides
- DAKO mandated 2-day PD-L1 IHC pathologist scoring training

External Quality Assessment (EQA): Methodology

1st EQA round

- All 14 centers evaluated 20 TMA cases:
  - 4 cell lines
  - 8 tissues with 2 cores each
- Scoring of % PD-L1 positive neoplastic cells (by core + overall) in **13** levels:
  - 0
  - <1%
  - 1-<5%
  - 5-10%
  - 11-20%
  - 21-30%
  - 31-40%
  - 41-50%
  - 51-60%
  - 61-70%
  - 71-80%
  - 81-90%
  - 91-100%

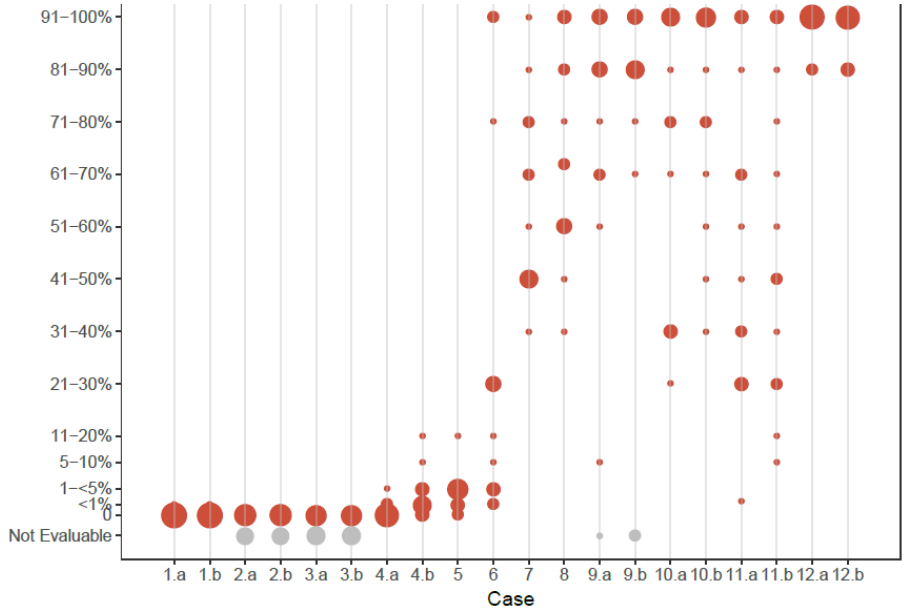
2nd EQA round

- to further harmonize the scoring behavior of centers:
- 12 out of the 14 centers scored
  - The 20 TMA cases (same as 1st round)
  - 65 digital cases (also evaluated by DAKO pathologist)
- Scoring of % PD-L1 positive neoplastic cells (by core + overall) in **6** levels
  - <1%
  - 1-<5%
  - 5-<10%
  - 10-<25%
  - 25-<50%
  - ≥50%

Benchmark: Mode value (by case) based on all scorings

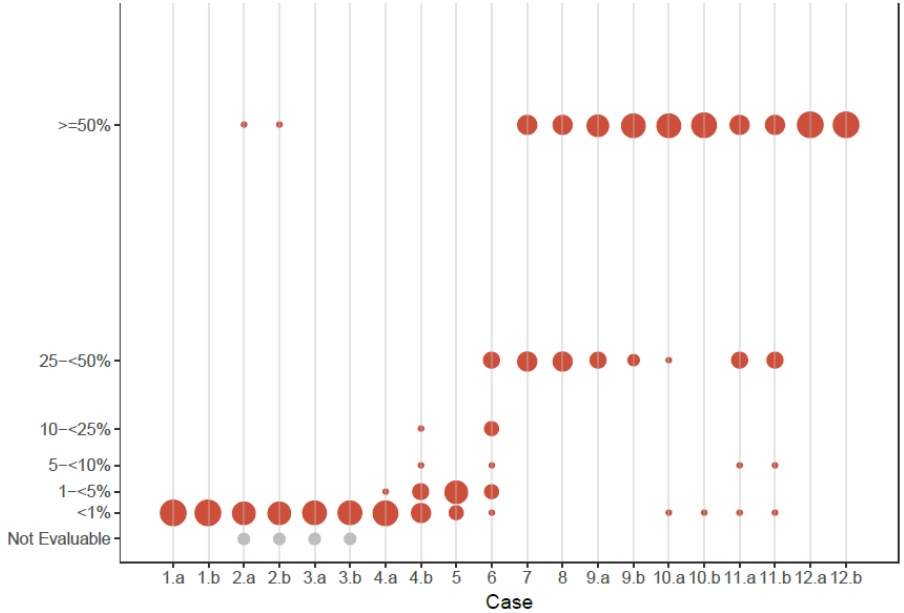
External Quality Assessment (EQA) Results for TMA cases

1st EQA round



- For the initial 13-level grouping of PD-L1 expression :
  - 56.4% agreements to the mode values
  - 8.9% not evaluable cases
- 12 out of 14 sites →2nd EQA round

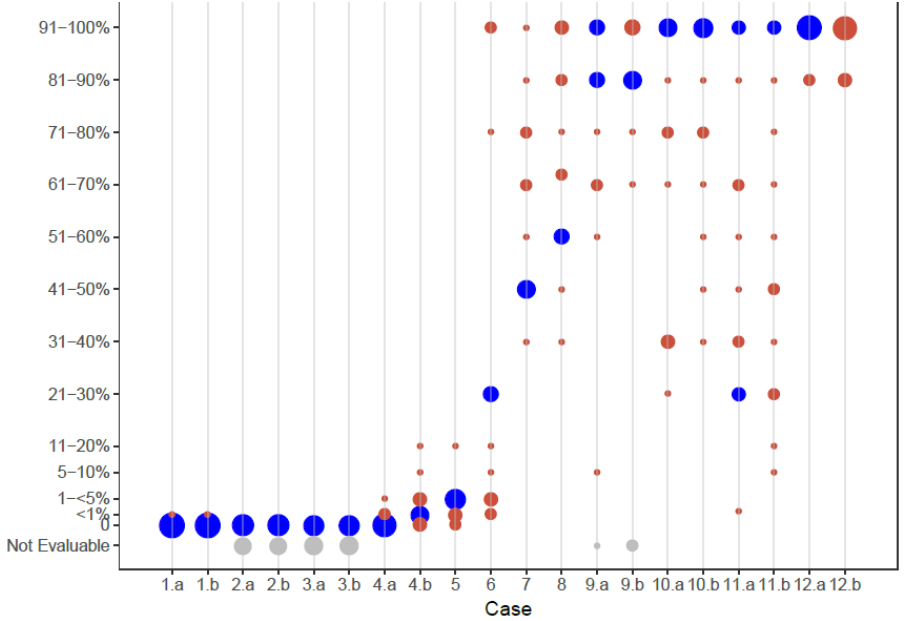
2nd EQA round



- For the 6-level grouping of PD-L1 expression:
  - 79.6% agreements to the mode values
  - 3.3% not evaluable cases

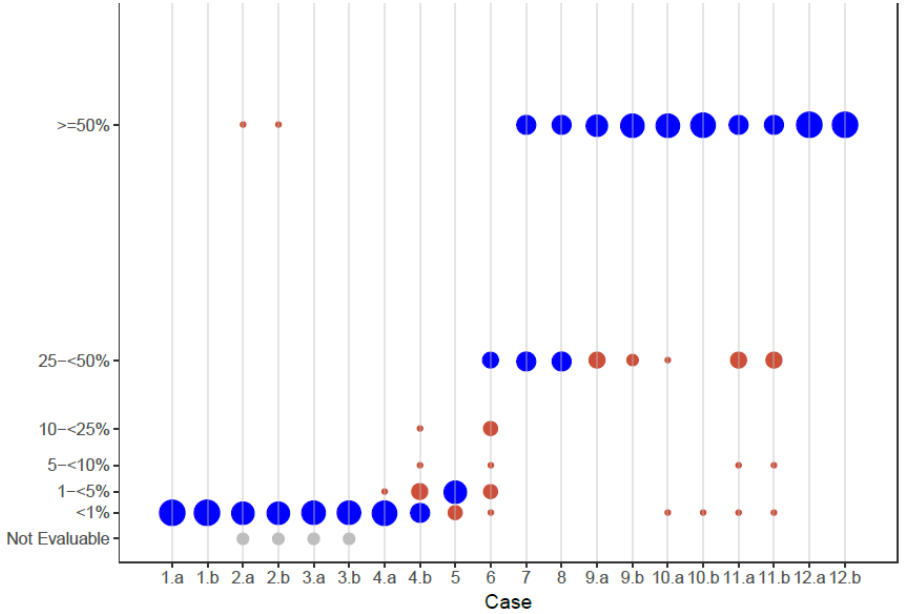
External Quality Assessment (EQA) Results for TMA cases

1st EQA round



2nd EQA round

Mode values in blue



- For the initial 13-level grouping of PD-L1 expression :
  - 56.4% agreements to the mode values
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- 12 out of 14 sites → 2nd EQA round

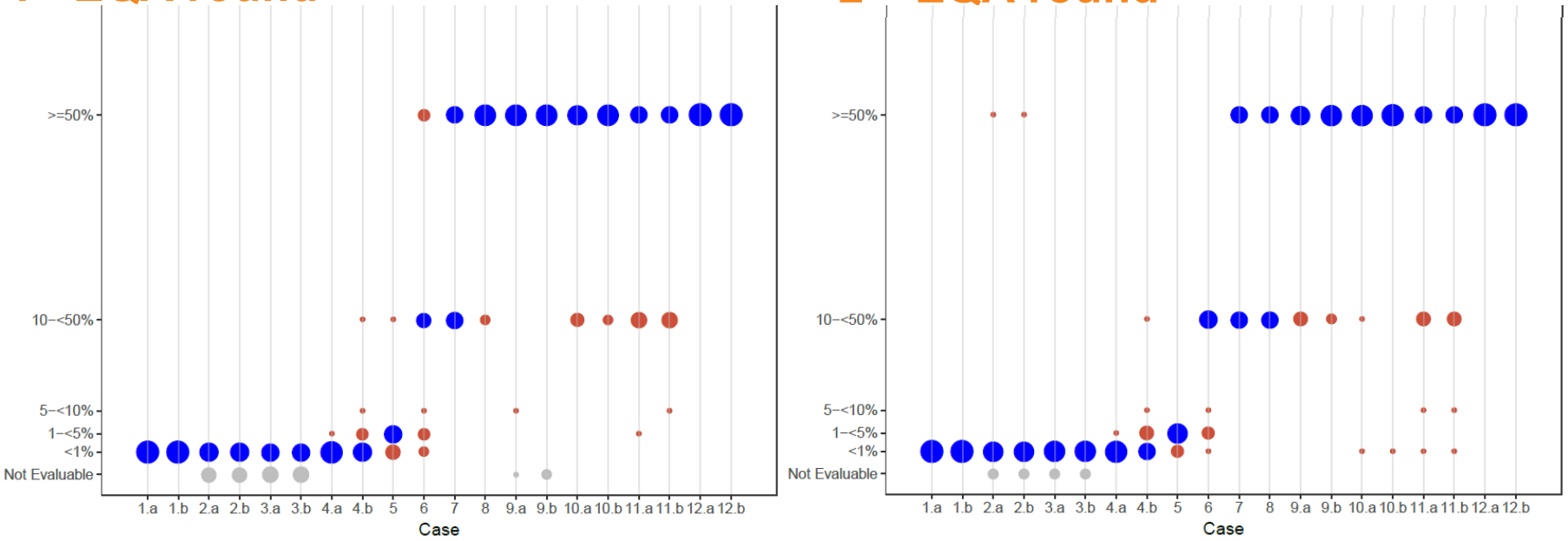
- For the 6-level grouping of PD-L1 expression:
  - 79.6% agreements to the mode values
  - 3.3% not evaluable cases

External Quality Assessment (EQA) Results for TMA cases

1st EQA round

2nd EQA round

Mode values in blue



Using (post-hoc) a common 5-level grouping, the agreements to the mode values were:

	Round 1	Round 2
All 14 centers:	75.4%	
12 centers	73.3%	80.8%

Cross-validation of PD-L1 scoring: TMAs vs Whole Sections

Table 1: Agreement in the % of PD-L1 positive neoplastic cells using TMAs vs Whole Sections (No of cases, Total N=237)

PD-L1 in TMAs	PD-L1 in Whole Sections						
	<1%	1-<5%	5-<10%	10-<25%	25-<50%	≥50%	Not Evaluable
<1%	89	17	2	1	1	2	1
1-<5%	5	13	2	1	1	1	0
5-<10%	3	2	2	4	0	1	0
10-<25%	1	0	2	1	6	1	0
25-<50%	0	1	0	3	3	4	0
≥50%	1	2	0	0	0	34	0
Not Evaluable	16	4	3	0	0	6	1

Complete agreement: 60.3%

Discrepant cases:

- Underestimation: 18.6%
- Overestimation: 8.4%

  (by TMA w.r.t. Whole Section)

  - Not evaluable by TMA while evaluable by Whole Section: 12.2%

Extent of discrepancy:

- Only 1 level: 19.0%
- >1 level: 8.0%

Whole Section' result as 'Gold Standard':

Cut-off for PD-L1 positivity	TMA's	
	Sensitivity	Specificity
1%	78.5%	89.9%
5%	84.7%	92.5%
50%	79.1%	98.2%

Table 2. Studies Comparing Clinical Trial or pharmDx PD-L1 Immunohistochemistry Assays

First Author	Antibodies Compared	Samples Analyzed	Scoring Method	Observer	Interassay Comparison of PD-L1 Expression on Tumor Cells	Interassay Comparison of PD-L1 Expression on Immune Cells
Hirsch[28] Blueprint	28-8, 22C3, SP142, SP263	39 FFPE NSCLC tumor samples (most from surgical resections)	Percentage of tumor cell staining (TPS)	Three pathologists trained on 28-8 and 22C3 (n = 1), SP142 (n = 1), SP263 (n = 1) assays	28-8, 22C3, SP263 analytically similar for PD-L1 staining; fewer TCs expressing PD-L1 with SP142	For all assays, IC staining was more variable than TC staining
Scheel[29] German harmonization	28-8, 22C3, SP142, SP263	Training set: 15 resected FFPE NSCLC (eight SQ, seven NSQ) tumor samples. Validation set: 15 resected FFPE NSCLC (four SQ, 11 NSQ) tumor samples	Six-step proportion score or dichotomous PD-L1 expression levels (≥ 1%, ≥ 5%, ≥ 10%, ≥ 25%, ≥ 50%)	Nine pathologists	Good concordance with dichotomous expression levels for training set (κ = 0.75) and validation set (κ = 0.72). Similar PD-L1 expression with 28-8 and 22C3, lower with SP142, higher with SP263	Low concordance for dichotomous expression levels (κ < 0.2 for training and validation sets)
Rimm[38] NCCN	28-8, 22C3, SP142	90 archival surgically resected NSCLC tumor samples (45 NSQ, 45 SQ)	PD-L1 expression levels ≥ 1% and ≥ 50%	13 pathologists	High correlation across all assays (κ = 0.86). Similar PD-L1 expression with 28-8 and 22C3, lower PD-L1 expression detected with SP142	Low correlation across all assays (κ = 0.19)
Adam[30] French harmonization	28-8, 22C3, SP263	41 NSCLC surgical specimens	PD-L1 expression levels ≥ 1%, ≥ 5%, ≥ 25%, ≥ 50% for TCs; PD-L1 expression levels ≥ 1%, ≥ 5%, ≥ 10% for ICs	Seven thoracic pathologists trained on PD-L1 scoring in expert courses	High correlation across all assays (weighted κ ≥ 0.75 for thresholds ≥ 1% and ≥ 5%) and OPA ≥ 90%	OPA 75%-90% between assays
Ratcliffe[36] AZ	28-8, 22C3, SP263	500 (n = 493 evaluable) FFPE, archival NSCLC samples	PD-L1 expression levels ≥ 1%, ≥ 10%, ≥ 25%, ≥ 50%	One pathologist trained on all methods	OPA 91%-97% between assays	Not reported
Batenchuk[42] USA	28-8, 22C3	158 lung cancer biopsy specimens	PD-L1 expression levels ≥ 1%, ≥ 5%, ≥ 10%, ≥ 25%, ≥ 50%	Pathologists trained and certified on scoring PD-L1 assays	OPA 96%-97% between assays	Not reported
Skov[41] Denmark	28-8, 22C3	86 FFPE lung cancer specimens (46 NSQ, 28 SQ, 12 other)	PD-L1 expression levels ≥ 1%, ≥ 5%, ≥ 10%, ≥ 50%	Pathologist trained on Dako assays	OPA 93%-99% between assays	Not reported
Yeh[43] ESMO Asia 2016	22C3, SP142, SP263	219 surgically resected NSQ NSCLC samples	PD-L1 expression levels ≥ 1% and < 1%	Not specified	Concordance with 22C3 and SP142 (94%); SP263 showed higher PD-L1 expression levels and lower concordance with 22C3 (76%) and SP142 (74%)	Not reported
Soo[44] WCLC 2016	28-8, 22C3, SP142, SP163	20 NSCLC samples (five each of resection, core biopsy specimens, cytologic, and pleural fluid)	PD-L1 expression levels ≥ 1%	Not specified	Similar PD-L1 expression with 22C3 and SP263 (65%-70%); lower expression with 28-8 (15%), and higher expression with SP142 (95%)	Not reported

Abbreviations: FFPE, formalin-fixed, paraffin-embedded; IC, immune cell; NSCLC, non–small-cell lung cancer; NSQ, nonsquamous; OPA, overall percent agreement; PD-L1, programmed death-ligand 1; SQ, squamous; TC, tumor cell; TPS, tumor proportion score.

Comparison of PDL1 IHC assays

- 22C3, 28-8 mostly used, resected tissue
- High concordance and overall % agreement between 22C3, 28-8, SP263 PDL1 expression on tumor cells
- Low concordance and high variability PDL1 expression on immune cells

Buettner et al., JCO 2017

Interobserver variation

Table 3. Studies Reporting Interobserver Comparison of PD-L1 Expression Scoring

First Author	Antibodies Compared	Samples Analyzed	Scoring Method	Observer	Interassay Comparison of PD-L1 Expression on Tumor Cells	Interassay Comparison of PD-L1 Expression on Immune Cells
Rimm[38]	28-8, 22C3, SP142, E1L3N	90 archival surgically resected NSCLC tumor samples (45 NSQ, 45 SQ)	PD-L1 expression levels ≥ 1% and ≥ 50%	13 pathologists	Interobserver concordance was 0.86 overall: 0.83 for 28-8, 0.88 for 22C3, 0.87 for SP142, and 0.86 for E1L3N	Interobserver concordance was 0.19 overall: 0.17 for 28-8, 0.21 for 22C3, and 0.19 for SP142, and 0.23 for E1L3N
Rehman[37]	SP142	35 FFPE, resected NSCLC samples (17 NSQ, 18 SQ)	Percentage TC or IC staining	Five pathologists	Correlation coefficient, 94%	Correlation coefficient, 27%
Ratcliffe[36]	28-8, 22C3, SP263	200 FFPE, archival NSCLC samples	PD-L1 expression levels ≥ 1%, ≥ 10%, ≥ 25%, ≥ 50%	CLIA laboratory pathologist review *v* independent pathologist review	OPA > 85% for PD-L1 expression ≥ 10%, ≥ 25%, and ≥ 50% for all assays; 76%-77% for PD-L1 expression ≥ 1% for all assays	Not reported
Cooper[31]	22C3	120 NSCLC samples		Review by two Dako-trained and certified pathologists *v* review by 10 independent pathologists	OPA 84% for PD-L1 ≥ 1% and 82% for PD-L1 ≥ 50%	Not reported

Intraobserver reproducibility 90, 91%

Abbreviations: CLIA, Clinical Laboratory Improvement Amendments; FFPE, formalin-fixed, paraffin-embedded; IC, immune cell; NSCLC, non–small-cell lung cancer; NSQ, nonsquamous; OPA, overall percent agreement; PD-L1, programmed death-ligand 1; SQ, squamous, TC, tumor cell.

Buettner et al., JCO 2017

ETOP, Express, Pepsi studies PDL1 IHC

ETOP-Lungscape: n=2008 resected NSCLC, stage I-III (K. Kerr ASCO 2017), abstract 8516

- PDL1 (Dako 28-8) on TMAs: positivity at 1% and 5% cut-off in >33% tumors
- Correlates with better prognosis (AD), but 50% cut-off does not (histopathol, survival)
- Correlates with never smokers, no history of cancer, larger tumor size

MUMC+: Verification Dako 28-8 and 22C3 on ETOP-Lungscape TMAs (n=83 tumors) and consensus screening with 3 observers: 95.7 and 96.8% agreement, resp with consensus (at ≥50% PDL1 expression level)

Express study (25 countries, PDL1 22C3 expression on previously untreated stage IV NSCLC, correlation histopathol, demographic and EGFR, ALK data (3 level score):
MUMC+, Zuyderland (n=50): both 50% cases <1%, 25% cases 1-49%, 25% cases ≥50% (5/7 KRAS+ cases have PDL1 ≥50%)

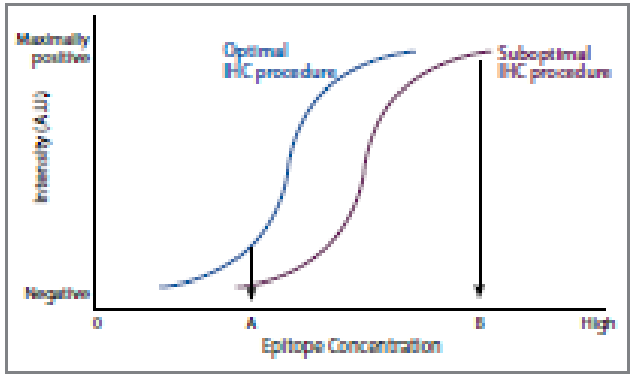
Pepsi study (8 sites, optimization PDL1 IHC NL): ongoing: TMA 1 IHC, digital scoring and TMA 2 IHC (n=30): good correlation, 80-90% agreement per scoring level (mainly 22C3).
MUMC+: 95.5% agreement on a 3 level PDL1 score. *Project:TMA with low antigen cases.*

LDAs, standardization, EQA, training

- LDAs: in literature variable results when comparing LDAs with clinical trial assyas
- Need for standardization and external quality assurance testing (EQA): NordiQC, ESP, UKNEQAS
- Because of often-heterogeneous morphology of NSCLC and reported variations in PDL1 expression: training/certification pathologists important for consistency and quality of PDL1 IHC interpretation
- Experience different in other tumors

Table 1. **Assessment marks for IHC assays and antibodies run C1, PD-L1 IHC**

CE-IVD / FDA approved PD-L1 assays	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	Suff. OPS[2]
22C3 pharmDX, **SK006**	12	Dako/Agilent	10	1	0	1	92%	92%
22C3 pharmDX, **SK006**[4]	2	Dako/Agilent	0	0	1	1	-	-
28-8 pharmDX, **SK005**	7	Dako/Agilent	3	3	1	0	86%	86%
SP263, 790-4905	16	Ventana/Roche	9	2	2	3	69%	77%
SP142, 740-4859	1	Ventana/Roche	0	0	0	1	-	-
Antibodies[3] for laboratory developed PD-L1 assays, conc. antibody	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	Suff. OPS[2]
mAb clone **22C3**	13	Dako/Agilent	1	1	4	7	15%	-
mAb clone **E1L3N**	8	Cell Signaling	1	1	1	5	25%	-
mAb **CAL10**	1	Biocare	0	0	1	0	-	-
rmAb clone **28-8**	6	Abcam	0	1	1	4	17%	-
rmAb clone **ZR3**	1	Zeta Corporation	1	0	0	0	-	-



Buettner et al., JCO 2017; NordiQC Assessment run C1 PDL1
Tsao et al., IASLC Atlas of PD-L1 immunohistochemistry testing in lung cancer, 2017

Utility of diagnostic materials

Table 1. Recommended Preanalytic Conditions for Immunohistochemistry (IHC)

Parameter	Recommendation
Cold ischemia time	Fewer than 30 minutes if possible, not exceeding 1 hour
Fixative	10% neutral buffered formalin
Time of fixation (biopsy)	6 to 48 hours
Time of fixation (resection)	24 to 48 hours
Preparation	Paraffin-embedded sections, cut at a thickness of 3 to 5 µm
Specimen storage	Tissue blocks
Storage time for blocks	Fewer than 3 years for PD-L1 IHC
Storage conditions for blocks	Prevented from light, heat, and humidity
Storage time for cut sections	Fewer than 2 months, particularly for testing with SP263 antibody
Decalcification	EDTA, if necessary

PD-L1 = programmed cell death-ligand 1.

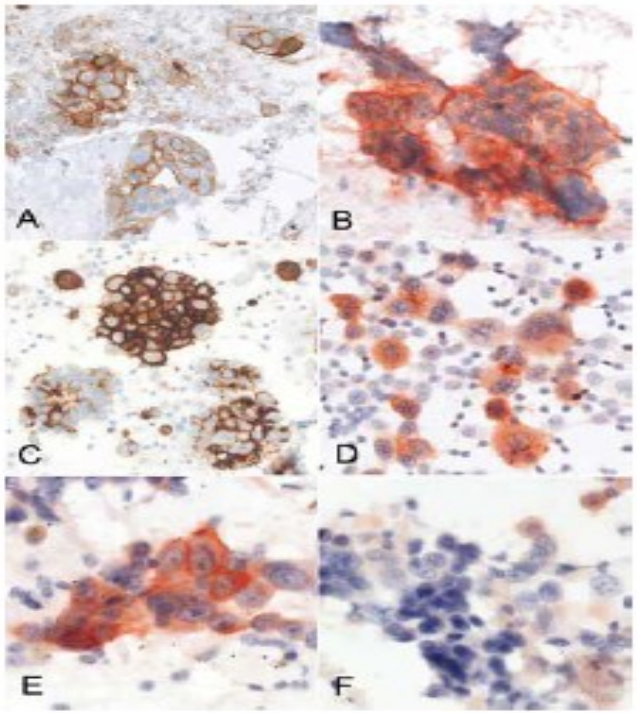
- Archival vs fresh biopsy (previously treated NSCLC):
  - Keynote 010 trial: prevalence and PDL1 TPS similar (40-45% in archival and fresh biopsy material
  - Atlantic trial: rebiopsy not necessary when tissue material is < 3 years old
- Tumor heterogeneity
  - Varying concordance between different sites within a tumor and between primary and metastatic lesions (mainly studies using SP142)
  - May reflect differences between biopsy methods and in tumor heterogeneity.

Buettner et al., JCO 2017
Tsao et al., IASLC Atlas of PD-L1 immunohistochemistry testing in lung cancer, 2017

Utility of diagnostic materials

Histology vs cytology (only PDL1 on histological tissue approved)

- Paired Comparison of PD-L1 Expression on 86 Cytologic (cell blocks) and Histologic Specimens From Malignancies in the Lung Assessed With PD-L1 IHC 28-8 and 22C3
- 85-95% agreement depending on prespecified PDL1 expression level
- In cases of disagreement: heterogeneity in histological tissue (at ≥5% and ≥10%)
- Standardization cell processing necessary: many cell collection and fixation methods
  - Alcohol fixation not recommended (use FFPE)
  - Cytorich red recommended over Cytorich blue?



Skov BG[1], Skov T.Appl Immunohistochem Mol Morphol 2017

Conclusions

- High concordance between 22C3, 28-8 and SP263 PDL1 IHC assays analyzing membrane staining on tumor cells
- Similar results for interobserver concordance, reproducible results when performed in specialized laboratories by trained pathologists/KMBP
- PDL1 may be heterogeneously expressed within tumors and between primaries and metastases. Multiple biopsies to be considered, but still more data needed.
- Owing to variabilitiy between LDAs, standardization is needed before clinical application. ISO accredited labs, trained pathologists and EQA is recommended.
- PDL1 IHC on cytology specimens is desirable and need to be validated.
- Because of limitations, other biomarkers introduced (TILs, TMB, MSI etc.).