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REVIEW ARTICLE

Programmed Death-Ligand 1 Immunohistochemistry Testing: A Review of Analytical Assays and Clinical Implementation in Non–Small-Cell Lung Cancer

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ABSTRACT

Purpose

Three programmed death-1/programmed death-ligand 1 (PD-L1) inhibitors are currently approved for treatment of non–small-cell lung cancer (NSCLC). Treatment with pembrolizumab in NSCLC requires PD-L1 immunohistochemistry (IHC) testing. Nivolumab and atezolizumab are approved without PD-L1 testing, though US Food and Drug Administration-cleared complementary PD-L1 tests are available for both. PD-L1 IHC assays used to assess PD-L1 expression in patients treated with programmed death-1/PD-L1 inhibitors in clinical trials include PD-L1 IHC 28-8 pharmDx (28-8), PD-L1 IHC 22C3 pharmDx (22C3), Ventana PD-L1 SP142 (SP142), and Ventana PD-L1 SP263 (SP263). Differences in antibodies and IHC platforms have raised questions about comparability among these assays and their diagnostic use. This review provides practical information to help physicians and pathologists understand analytical features and comparability of various PD-L1 IHC assays and their diagnostic use.

Methods

We reviewed and summarized published or otherwise reported studies (January 2016 to January 2017) on clinical trial and laboratory-developed PD-L1 IHC assays (LDAs). Studies assessing the effect of diagnostic methods on PD-L1 expression levels were analyzed to address practical issues related to tissue samples used for testing.

Results

High concordance and interobserver reproducibility were observed with the 28-8, 22C3, and SP263 clinical trial assays for PD-L1 expression on tumor cell membranes, whereas lower PD-L1 expression was detected with SP142. Immune-cell PD-L1 expression was variable and interobserver concordance was poor. Inter- and intratumoral heterogeneity had variable effects on PD-L1 expression. Concordance among LDAs was variable.

Conclusion

High concordance among 28-8, 22C3, and SP263 when assessing PD-L1 expression on tumor cell membranes suggests possible interchangeability of their clinical use for NSCLC but not for assessment of PD-L1 expression on immune cells. Development of LDAs requires stringent standardization before their recommendation for routine clinical use.

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INTRODUCTION

The treatment landscape for non–small-cell lung cancer (NSCLC) has rapidly changed after the introduction of immune checkpoint inhibitors, including programmed death-1 (PD-1) and programmed death-ligand 1 (PD-L1) inhibitors. Nivolumab (US Food and Drug Administration [FDA], European Medicines Agency [EMA], and Japanese Ministry of Health, Labour and Welfare [MHLW]) and atezolizumab (FDA) are approved for patients with unresectable, locally advanced, and/or metastatic NSCLC (squamous and nonsquamous) with prior chemotherapy.[1-4] PD-L1 testing is not required for patient selection with nivolumab or atezolizumab, although complementary PD-L1 diagnostics are approved for NSCLC.[5,6] Pembrolizumab is FDA, EMA, and MHLW approved for first-line treatment of

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patients with metastatic NSCLC (squamous and nonsquamous) whose tumors demonstrate PD-L1 staining in ≥ 50% of tumor cells (the so-called tumor proportion score [TPS]), or patients with locally advanced and/or metastatic NSCLC with prior chemotherapy and a ≥ 1% TPS.[7-9]

The approvals in NSCLC were based on studies demonstrating longer overall survival and better tolerability with these checkpoint inhibitors compared with docetaxel or platinum-based chemotherapy.[10-15] Two additional PD-L1 inhibitors, durvalumab and avelumab, are being evaluated as monotherapy or combination therapy for first- or second-line treatment of NSCLC.[16-20]

For each PD-1/PD-L1 inhibitor, a specific PD-L1 immunohistochemistry (IHC) assay was developed to assess PD-L1 expression levels on malignant NSCLC tumor and/or immune cells (Table 1). The FDA-approved, European Conformity–In Vitro Diagnostic (CE-IVD)-marked, and MHLW-approved PD-L1 IHC 22C3 pharmDx assay (22C3) and CE-IVD-marked PD-L1 IHC SP263 assay (SP263) are approved as companion diagnostics for pembrolizumab, with PD-L1 testing required to determine patient eligibility.[23,24,27] The FDA-cleared, CE-IVD-marked, and MHLW-approved PD-L1 IHC 28-8 pharmDx assay (28-8) and CE-IVD-marked SP263 used with nivolumab, and the FDA-cleared PD-L1 IHC SP142 assay (SP142) used with atezolizumab are approved as complementary diagnostics; they are not required for treatment but may support clinical decisions.[5,6,21,22]

In CheckMate 017 [Study of BMS-936558 (Nivolumab) Compared to Docetaxel in Previously Treated Advanced or Metastatic Squamous Cell Non–Small-Cell Lung Cancer] and CheckMate 057 [Study of BMS-936558 (Nivolumab) Compared to Docetaxel in Previously Treated Metastatic Nonsquamous NSCLC], nivolumab demonstrated a survival benefit compared with docetaxel in patients with previously treated NSCLC, although hazard ratios were more favorable for patients with nonsquamous NSCLC with high versus low PD-L1 expression (assessed using 28-8).[10,11] For patients with squamous NSCLC, there was a benefit with nivolumab regardless of PD-L1 levels.[11] In patients with nonsquamous NSCLC, nivolumab efficacy was enhanced in patients with PD-L1 expression in ≥ 1% of tumor cells, and the survival benefit was further enhanced with increasing PD-L1 levels.[10] Nevertheless, for patients with < 1% PD-L1 staining, nivolumab treatment demonstrated comparable survival and objective response rates, more durable responses, and a favorable safety profile compared with docetaxel.[10] KEYNOTE-010 [Study of Two Doses of Pembrolizumab (MK-3475) Versus Docetaxel in Previously Treated Participants With Non–Small-Cell Lung Cancer] and KEYNOTE-024 [Study of Pembrolizumab (MK-3475) Compared to Platinum-Based Chemotherapies in Participants With Metastatic Non–Small-Cell Lung Cancer] assessed PD-L1 levels using 22C3 and showed improved clinical outcomes with pembrolizumab in previously treated patients with a ≥ 1% TPS and previously untreated patients with a ≥ 50% TPS.[12,13] POPLAR [phase 2; Randomized Phase 2 Study of Atezolizumab (an Engineered Anti-PD-L1 Antibody) Compared With Docetaxel in Participants With Locally Advanced or Metastatic Non–Small-Cell Lung Cancer Who Have Failed Platinum Therapy] and OAK [phase 3; Study of Atezolizumab Compared With Docetaxel in Participants With Locally Advanced or Metastatic Non–Small-Cell Lung Cancer Who Have Failed Platinum-Containing Therapy] studies both showed a correlation between clinical improvement with atezolizumab and PD-L1 expression (assessed using SP142) in patients with previously treated squamous or nonsquamous NSCLC, with the greatest benefit from atezolizumab in patients with high tumor and immune-cell PD-L1 levels (tumor cell [TC]3 [≥ 50%]/immune cell [IC]3 [≥ 10%]), especially in the OAK study.[14,15] The OAK study also reported improved clinical outcomes with atezolizumab versus docetaxel in patients with low tumor PD-L1 levels (TC0/IC0 [< 1%]).[15]

Because each PD-1/PD-L1 inhibitor has its own PD-L1 assay and clinical trials have assessed varying levels of PD-L1 expression for correlation with clinical outcomes, there is confusion among physicians about how to use PD-L1 levels for their patients. The commonly asked questions include the following: (1) Are results between different assays interchangeable? (2) What PD-L1 level is clinically relevant and does it vary depending on the assay selected? (3) Is an actual biopsy specimen required or can an archived sample be used? (4) Is all diagnostic material suitable for PD-L1 testing? (5) Are there specific requirements for the laboratory performing the test? Several recent studies[28-41] have begun to answer some of these questions by analyzing concordance among the different assays[28,29,36,38,41,42] and the effect that diagnostic materials and laboratory-specific factors have on assay results.[32-34,37,39,41] This review describes comparability of PD-L1 IHC assays across antibodies and platforms; discusses practical issues that can influence assay results, such as tumor sampling and histologic versus cytologic assessments; and emphasizes the importance of having specialized laboratories and trained staff to perform the analyses.

INTERASSAY COMPARISON AND INTEROBSERVER VALIDATION

Several studies assessed analytic performance (eg, staining characteristics) of FDA-cleared PD-L1 IHC assays using same sets of resected or biopsied NSCLC specimens. Concordant with their evaluation in respective clinical trials, three assays (28-8, 22C3, and SP263) assessed PD-L1 levels as membrane staining of tumor cells only, using percentage of cells staining at any intensity,[5,22,23] whereas SP142 independently assessed PD-L1 expression levels on tumor cell membranes and tumor-infiltrating immune cells.[6] Three studies summarized later in this review also assessed interobserver differences within and between assays.[29,36,38]

Tumor Cells

Studies comparing PD-L1 IHC assays used in clinical trials are summarized in Table 2. The Blueprint PD-L1 IHC Assay Comparison Project[28] analyzed 38 formalin-fixed, paraffin-embedded (FFPE) NSCLC tumor samples (most from surgical resections) to assess the analytical and clinical comparability of four of these assays. Tumor sections were stained using Dako PD-L1 IHC (28-8 and 22C3) assays on the Autostainer Link 48 system (Agilent Technologies, Santa Clara, CA) and Ventana PD-L1 IHC (SP142 and SP263) assays (Ventana Medical Systems, Tucson, AZ) on the Benchmark ULTRA staining system (Ventana). PD-L1 expression was assessed on tumor cell membranes and immune cells (membrane or cytoplasm) by three pathologists who were experts

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: 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%.

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]	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]	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]	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]	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]	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]	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]	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]	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]	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.

in scoring 28-8 and 22C3 (pathologist 1), SP142 (pathologist 2), or SP263 (pathologist 3). Three assays (28-8, 22C3, and SP263) detected similar PD-L1 levels on tumor cells, whereas lower PD-L1 levels were detected with SP142.[28] The Blueprint study also compared clinical classification across all four assays by having the pathologists assess whether PD-L1 expression was above or below the selected treatment-determining level for each assay.[28] The results showed that clinical classification could vary significantly, emphasizing the importance of matching the assays with the PD-L1 level for their intended therapeutic use.[28]

In the German harmonization trial, 15 FFPE, resected NSCLC tumor specimens (eight squamous, seven nonsquamous) were stained per clinical trial protocols and PD-L1 was assessed by nine pathologists using a six-step score (< 1%, ≥ 1%, ≥ 5%, ≥ 10%, ≥ 25%, ≥ 50%) or dichotomous PD-L1 levels (≥ 1%, ≥ 5%, ≥ 10%, ≥ 50%).[29] Overall, PD-L1 expression detected on tumor cell membranes was similar with 28-8 and 22C3; lower with SP142 than 28-8, 22C3, and SP263; and higher with SP263 than 28-8, 22C3, and SP142. Good concordance was observed using integrated dichotomous PD-L1 expression levels (κ = 0.59 to 0.80; mean, 0.72).[29]

An AstraZeneca study assessed concordance among 28-8, 22C3, and SP263 in 493 FFPE NSCLC samples by one trained pathologist, and reported a high correlation between percentage PD-L1 staining on tumor cell membranes, with overall percent agreement (OPA) > 90% among assays across 10% incremental cutoffs.[36]

The National Comprehensive Cancer Network involved 13 pathologists and analyzed 90 archival, resected NSCLC tumor specimens stained using three clinical trial PD-L1 IHC assays (28-8, 22C3, and SP142) and a laboratory-developed assay (LDA; E1L3N). The results showed that the SP142 assay detected lower PD-L1 levels on tumor and immune cells than the other assays.[38] The overall concordance among assays was excellent (κ = 0.813), and was even higher if SP142 was excluded (κ = 0.971).[38]

The French harmonization study analyzed 41 resected NSCLC specimens and assessed comparability of four clinical trial assays: 28-8, 22C3, SP142, and SP263.[30] Seven thoracic pathologists trained on assessing PD-L1 reported that results with 28-8, 22C3, and SP263 were highly concordant for tumor cell membrane staining (κ > 0.75; OPA > 90%).

A US study assessed PD-L1 expression using 28-8 and 22C3 on 158 lung cancer biopsy specimens and observed a strong correlation across specimens, with OPA ranging from 96% to 97% for PD-L1 levels of ≥ 1%, ≥ 5%, ≥ 10%, ≥ 25%, and ≥ 50%.[42] A Danish study compared PD-L1 expression using 28-8 and 22C3 on 87 lung cancer histologic specimens (60 resections and 27 biopsies) and 86 cytologic specimens, and reported excellent interassay concordance across cytologic (R^2 = 0.95) and histologic specimens (R^2 = 0.95).[41]

Although data from these studies are generally consistent, recent data presented at the European Society for Medical Oncology's ESMO Asia 2016 and the International Association for the Study of Lung Cancer 2016 highlight some potential discrepancies that can occur between laboratories (Table 2).

Tumor Cells: Interobserver Comparison

Three studies that analyzed interpathologist concordance all reported high agreement across assays (> 86%) when assessing tumor cell membrane staining (Table 3).[36-38] When analyzed on the basis of specified PD-L1 levels in the AstraZeneca study, the OPA with an independent pathologist dropped to 75% to 77% with PD-L1 ≥ 1% and 85% to 89% with ≥ 10%, yet remained > 90% with PD-L1 ≥ 25% and ≥ 50%.[36]

The DREAM study (Intra- and Interobserver Reproducibility Study of PD-L1 Biomarker in Non–Small-Cell Lung Cancer) assessed intra- and interobserver reproducibility of PD-L1–expression scoring in NSCLC using 22C3, and the impact of training on reproducibility.[31] PD-L1 expression levels of 60 specimens, as

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, 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

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.

determined by 10 pathologists, were compared with gold standard assessment (by two Dako-trained and certified pathologists). OPA values were 90% and 91% for intraobserver reproducibility and 82% and 84% for interobserver reproducibility for PD-L1 expression levels of ≥ 1% and ≥ 50%, respectively. However, training appeared to have little effect on reproducibility, with no change in OPA after training for PD-L1 expression levels ≥ 1% (82%) and only slight improvement for PD-L1 expression levels ≥ 50% (78% *v* 82%).

Immune Cells

Unlike the other assays, SP142 was developed to detect PD-L1 expression levels on tumor cell membranes and tumor-infiltrating immune cells. However, all PD-L1 validated antibodies and assays are also able to stain immune cells and allow evaluation of the contingent of positive immune cells. In contrast to PD-L1 expression levels on tumor cells, assessment of PD-L1 expression levels on immune cells showed greater variability and low interobserver concordance ($\kappa < 0.2$) despite comparable immune cell staining.[28,29,38] The poor concordance in assessment of PD-L1 expression on immune cells may be due to different approaches adopted by the pathologists in scoring. The variability can result from (1) lack of prespecified criteria for assessment of PD-L1 staining on immune cells; (2) PD-L1 positivity, which, unlike tumor cells, can be both cytoplasmic and membranous; and (3) areas of staining rather than individual count (in percentage) of positive cells was applied when scoring PD-L1 positivity on immune cells.

UTILITY OF DIAGNOSTIC MATERIALS

Histologic Versus Cytologic Assessment

A major shortcoming in the development of companion or complementary diagnostic assays is the exclusion of cytologic materials for PD-L1 assessment in clinical trials. Consequently, the available PD-L1 tests are approved for histologic specimens only. This has made some pathologists cautious about the clinical use of cytology samples for PD-L1 testing. About one-third of patients with lung cancer are diagnosed via cytologic material only, which can be obtained from less-invasive procedures, such as computed tomography-guided fine-needle aspiration and endobronchial ultrasound (EBUS)-guided aspiration biopsies.[39,41] Skov and Skov compared PD-L1 expression levels in 86 paired FFPE samples of cytologic cell block and histologic material from lung malignancies, using 28-8 and 22C3, and observed a high degree of agreement between histologic and cytologic specimens for each assay (85% to 95% agreement depending on prespecified PD-L1 expression level).[41] In cases demonstrating disagreement between the two sample types, the tumor tended to demonstrate PD-L1 heterogeneous staining in the histologic material, especially for PD-L1 expression ≥ 5% and ≥ 10%.[41]

These data suggest that a reliable assessment of PD-L1 expression on tumor cells can also be obtained using cytologic material processed to obtain a cell block, providing an acceptable alternative when histologic specimens are not available, at least when PD-L1 expression is detected. However, before recommending routine clinical use of cytology specimens, a standardized process should be established because of the wide range of processing methods, including cell collection (eg, aspiration, stamp, liquid based, cell block) and fixation (eg, alcohol based, formalin).

Tumor Heterogeneity

Archival versus fresh biopsy. In the KEYNOTE-010 study of previously treated NSCLC, assessment of PD-L1 expression as measured by 22C3 levels from archival samples was initially allowed, but a protocol amendment required assessment from new samples obtained before enrollment.[32] In a reanalysis of the study, outcomes were assessed on the basis of whether PD-L1 expression was assayed in archival (n = 456) or rebiopsy (n = 578) samples.[32] The prevalence (or rate) of PD-L1 tumor expression levels ≥ 50% was similar in archival (40%) and new samples (45%), and clinical outcomes associated with PD-L1 expression levels ≥ 50% were also similar for archival and new samples.

In the ATLANTIC trial (Global Study to Assess the Effects of MEDI4736 in Patients With Locally Advanced or Metastatic Non–Small-Cell Lung Cancer), a phase 2 study of durvalumab in patients with platinum-refractory NSCLC, PD-L1 expression was assessed for 112 patients with recently acquired (< 3 months) and older (3 months to 1 year, 1 year to 3 years, and > 3 years) tumor samples.[34] When compared with recently acquired samples, concordance was highest for archival samples < 3 years old (76.2%). These data, derived from a limited number of cases, suggest that rebiopsy in patients who have received prior anticancer treatment may not be required for clinical assessment of PD-L1 expression, and that archival samples of ≤ 3 years old are suitable for PD-L1 assessment with the caveat that blank slides for PD-L1 staining should not be older than 6 months.

Intertumoral and intratumoral heterogeneity. Surgical resection samples and biopsy specimens can be taken from different sites within the tumor or from primary versus metastatic sites. PD-L1 expression may demonstrate intra- and intertumoral heterogeneity, and it is important to understand the effect different sample sites have on PD-L1 expression levels to assess their suitability for testing.

Rehman et al[37] assessed PD-L1 expression on three separate blocks obtained from each of 35 resected NSCLC tumor samples, using SP142. PD-L1 levels were similar across all three blocks from each tumor when analyzed by tumor cell–membrane staining (interclass correlation coefficient, 94%). However, when assessed on immune cells, the correlation between blocks was only 75%. Studies including larger sample sizes should confirm these results. No data for 28-8, 22C3, and SP263 regarding this issue are available.

Ilie et al[33] assessed PD-L1 staining in 160 patients with operable NSCLC on both whole surgical tissue sections and matched lung biopsy specimens, using SP142. PD-L1 staining was discordant between surgically resected and biopsy specimens in 48% of cases ($\kappa = 0.218$), with biopsy specimens showing lower PD-L1 staining than whole-tissue samples (TC1/2/3 and/or IC1/2/3, 26% *v* 74%, respectively). The authors noted that most (75%) discordant cases were based on assessment of PD-L1 staining on immune cells.

Gniadek et al[45] stained tissue microarray cores (four per tumor) from various areas of 150 FFPE, resected primary cancers

(79 squamous cell carcinomas and 71 adenocarcinomas), using SP142. Sensitivity of one microarray core for scoring PD-L1 expression > 50% and > 1% was 85% and 87% for adenocarcinoma and 95% and 90% for squamous cell carcinoma, respectively.

Sakakibara et al[39] assessed the utility of EBUS-transbronchial needle aspiration to evaluate PD-L1 expression in lung tumor cells. EBUS-transbronchial needle aspiration showed good concordance with resected lymph node metastases (r = 0.93; n = 5), transbronchial biopsy (r = 0.75; n = 16) and corresponding resected primary tumors (r = 0.75; n = 6). Concordance was lower between resected primary tumors and resected lymph node metastases (r = 0.49; n = 47) or transbronchial biopsy (r = 0.52; n = 41). This study was limited by the small size and needs to be confirmed by additional, larger sample studies. The ATLANTIC trial assessed intertumoral heterogeneity and reported similar PD-L1 tumor cell staining between primary and metastatic samples (35% *v* 33%), and concordance of 89%.[34]

The varying concordance between different sites within the tumor as well as between primary and metastatic lesions may reflect differences between biopsy methods and in tumor heterogeneity. This is possible with cytology and biopsy specimens.

LABORATORY-DEVELOPED ASSAYS

Studies reported thus far have mainly compared results obtained with clinical trial assays performed in Clinical Laboratory Improvement Amendments–certified laboratories on dedicated platforms. Pathology laboratories that only use one IHC platform will be unable to perform PD-L1 IHC assays that were developed using a different platform (eg, the 28-8 or 22C3 assay for a laboratory with a Ventana or Leica stainer).[35] To make PD-L1 testing more widely available, LDAs have been tried using multiple IHC platforms. To assess the clinical utility of these LDAs for routine PD-L1 testing, it is crucial first to determine if they can achieve similar analytical performance as the clinical trial assays. We reviewed five studies comparing analytical performance of LDAs (Table 4).

The French harmonization study compared PD-L1 levels using five PD-L1 antibodies (28-8, 22C3, E1L3N, SP142, and SP263) and three IHC platforms (Table 4),[49-51] and compared results obtained with Dako (Agilent Technologies) and Ventana reference assays and LDAs.[30] Among 27 LDAs performed across seven centers, 14 (51.8%) demonstrated acceptable concordance with 28-8, 22C3, or SP263 reference assays on dedicated platforms for tumor cell staining. Concordance varied for LDAs, with the most concordant results observed with SP263 on both tumor (κ = 0.81) and immune cells (κ = 0.64), high concordance with 28-8 (κ = 0.73), 22C3 (κ = 0.73), and E1L3N (κ = 0.78) on tumor cells, and poor concordance with SP142 (κ = 0.64) except with the Leica Bond platform (SP142 κ = 0.78 to 0.81). This study showed that all antibodies and platforms may potentially be used to create LDAs, but validation is required. Antibody SP263 was the most concordant across platforms and centers, indicating this antibody may be the easiest to harmonize.

Similar results were reported in phase 2 of the German harmonization trial.[40] This study assessed PD-L1 expression using four clinical trial assays (28-8, 22C3, SP142, and SP263) and 11

Table 4. Studies Comparing Laboratory-Developed PD-L1 Immunohistochemistry Assays

First Author	Antibodies Compared	Samples Analyzed	Scoring Method	Observer	Antibody Comparison of PD-L1 Expression on TC	Antibody Comparison of PD-L1 Expression on IC
Adam[30]	28-8, 22C3, SP142, SP263, E1L3N	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	14 of 27 LDAs (51.8%) concordant with reference assays (weighted κ ≥ 0.75 for ≥ 1% and ≥ 50% thresholds)	OPA, 60%-92.5%
Scheel[40]	28-8, 22C3, SP142, SP263, E1L3N, QR1	Tissue microarray containing 21 lung cancer specimens	PD-L1 expression levels ≥ 1%, ≥ 50%	Pathologists trained on scoring Dako or Ventana assays	LDA showed moderate concordance (κ = 0.50); six of 11 (55%) LDAs passed quality control	Not reported for LDA
Neuman[35]	22C3 (on three platforms)	41 FFPE NSCLC specimens	TPS < 1%, 1%-49%, ≥ 50%	Two pathologists	Same results obtained with different platforms in > 85% of samples	Not reported
Cogswell[46]	28-8, E1L3N	20 FFPE NSCLC specimens	PD-L1 expression scored in 1% increments in the 1%-10% range	Three pathologists	28-8 more sensitive than E1L3N (detected PD-L1 in 22 *v* six tumors)	28-8 more sensitive than E1L3N (detected PD-L1 in 13 *v* seven tumors)
Smith[47]	SP263, E1L3N	100 FFPE NSCLC microarray case cores	H-score for TCs and % PD-L1 staining cells for ICs	Two board-certified pathologists	SP263 more sensitive than E1L3N (SP263 detected PD-L1 in 29 additional tumors)	Similar sensitivity with SP263 and E1L3N
Rimm[38]	28-8, 22C3, SP142, E1L3N	90 archival surgically resected NSCLC tumor samples (45 NSQ, 45 SQ)	Mean TC or IC score	13 pathologists	Mean score for E1L3N (3.20) similar to 28-8 (3.26) and higher than 22C3 (2.96) and SP142 (1.99)	Mean score for E1L3N (2.28) similar to 28-8 (2.28) and higher than 22C3 (2.15) and SP142 (1.62)
Conde[48]	E1L3N, SP142, SP263	40 FFPE NSCLC specimens (SQC carcinomas)	PD-L1 expression levels ≥ 1%, ≥ 5%, ≥ 25%, ≥ 50%	Two pathologists	Similar results with the three clones	Worse correlation among the three clones

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

LDAs. When analyzed using PD-L1 levels ≥ 1% and ≥ 50%, the clinical trial assays achieved good concordance (κ = 0.73 to 0.89), and the LDAs showed moderate concordance with the 22C3 reference assay (κ = 0.50). LDAs yielded variable results, with six assays showing staining patterns similar to the 28-8 and 22C3 clinical trial assays and passing quality control, and five assays detecting less PD-L1 membrane staining than the clinical trial assays and failing quality control.

Neuman et al[35] assessed PD-L1 expression with 22C3 on three platforms or staining systems (Table 4) to determine if it is possible to adapt the Dako assay (Agilent Technologies) for use on the Ventana platform. Two pathologists scored 41 FFPE NSCLC samples on all staining systems, and concordance was compared between platforms. PD-L1 expression on tumor cells, classified as strongly positive (≥ 50%), weakly positive (1% to 49%), or negative (< 1%), was scored the same with the Dako and Ventana UltraView systems in 87.8% of cases (Pearson correlation coefficient, 0.91), and with the Dako and Ventana OptiView systems in 85.3% of cases (Pearson correlation coefficient, 0.89).

Four additional studies compared the LDA for E1L3N with clinical trial assays.[38,46-48] Two studies reported that the 28-8 and SP263 assays were more sensitive (ie, they detected PD-L1 expression on more tumors) than the E1L3N assay on tumor cells, and the 28-8 assay was more sensitive than the E1L3N assay on immune cells.[46,47] Rimm et al[38] reported that mean PD-L1 expression scores for E1L3N were comparable to 28-8 and significantly higher than 22C3 and SP142 on both tumor and immune cells. Conde et al[48] reported comparable results with E1L3N, SP142, and SP263 on tumor cells but not immune cells.

Although some LDAs show high concordance with clinical trial assays, the variable results indicate there is a need for standardization of PD-L1 LDA methods. With appropriate standardization and followed by external quality assessment (EQA) programs, it may be possible to adapt PD-L1 IHC assays for use on multiple IHC platforms.

NEED FOR TRAINING OR SPECIALIZED PATHOLOGISTS

Expression of PD-L1 is a fragile biomarker and it is important that the inherent biologic uncertainties are not compounded by poor-quality IHC or interpretation by pathologists.[52] Interpretation of PD-L1 IHC assays differs from most other IHC assays in the need for appreciation and understanding of the often-heterogeneous morphology of pulmonary tumors. Therefore, specialized training is important to maintain consistency and quality of interpretation between pathologists.

The International Quality Network for Pathology is a recently established international forum that aims to improve the quality of tissue-based biomarker assessment by bringing together individual quality assurance organizations, such as the United Kingdom National External Quality Assessment Service, Nordic IHC Quality Control, European Society of Pathology Quality Assurance, and the German Society of Pathology's Quality Assurance Initiative Pathology, details of which can be found on their website (http://www.iqnpath.org). These EQA systems generally assess the technical performance of staining and not the performance of the pathologist reporting the case.

To address this variability, it is crucial to have pathologists who are appropriately trained to interpret PD-L1–labeled tissue sections. It is encouraging that all the pharmaceutical companies with immune modulators currently licensed for treatment of NSCLC offer such education; successful completion of training results in certification of the pathologist to score the appropriate PD-L1 IHC assay. This training is currently assay specific and accreditation is not currently mandated. There may be benefit in developing a universal scoring system and moving responsibility for training to academic centers, which could potentially broaden availability of PD-L1 testing beyond specialty diagnostic centers. These decisions may be country specific.

Therefore, it seems sensible to encourage oncologists to seek PD-L1 testing from laboratories with pathologists who have received training and certification for PD-L1 assessment. It would also seem reasonable to expect any pathologist who wishes to interpret PD-L1–stained slides from pulmonary tumors to be familiar with handling and making diagnosis on clinical lung cancer specimens and to be registered with an appropriate pulmonary EQA scheme. It should not be assumed that experience gained from assessing PD-L1 levels in other tumors is transferable to NSCLC, because tumor characteristics and interpretation of PD-L1 expression differ significantly.

LOOKING BEYOND PD-L1 EXPRESSION LEVELS

Although PD-L1 expression has been shown to correlate with clinical outcomes in patients with NSCLC,[10,12,13] some patients with NSCLC who have PD-L1–negative tumors may also derive clinical benefit from PD-1/PD-L1 inhibitors.[11,15] One possible contributor to this is misclassification of PD-L1 status caused by uncontrolled preanalytical variables or sampling bias due to PD-L1 expression heterogeneity. PD-L1 IHC testing is not a perfect biomarker; therefore, research is ongoing to identify new biomarker strategies beyond, or in addition to, PD-L1 IHC, including tumor-infiltrating lymphocytes (TILs), tumor mutation burden (TMB), multiplex IHC (assessment of multiple protein markers on tumor and immune cells), and immune gene signatures that may improve our understanding of the tumor microenvironment and enable better identification of tumors with preexisting immune activity (for a recent review, see Gibney et al[53]). In most patients with NSCLC, such parameters will be evaluated on small diagnostic specimens obtained from the whole tumor, as is the case for PD-L1 IHC testing. It seems reasonable that tumor heterogeneity will also affect these variables, though this remains to be seen.

The presence of TILs in tumor biopsy specimens has been associated with improved overall survival in multiple cancers, including NSCLC, and elevated baseline levels of $CD8^+$ TILs are associated with improved survival in patients with stage 3 NSCLC treated with chemotherapy.[53] It is possible that baseline TILs could also be predictive of outcomes with PD-1/PD-L1 inhibitors.[53] Tumor mutation and neoantigen burden have been linked to improved clinical outcomes with immunotherapy in NSCLC.[53-56] In a feasibility study, assessment of TMB based on comprehensive genomic profiling was significantly associated with improved survival to PD-1/PD-L1 therapy in patients with NSCLC.[57] Recent data from an exploratory, retrospective analysis of CheckMate 026

samples reported that patients with NSCLC who had high TMB had improved progression-free survival and a higher objective response rate with first-line nivolumab compared with chemotherapy,[55] with greatest benefit in patients with high TMB and high PD-L1 expression (≥ 50%).[55] Immune gene signatures and multiplex IHC would allow assessment of multiple additional markers representing tumor microenvironment, which could improve the predictive ability compared with single-marker assessments.[53] More and prospective studies are required to confirm and validate these approaches before they will be available for routine clinical use.

In conclusion high concordance was observed among three of four diagnostic PD-L1 assays (28-8, 22C3, and SP263) when assessing PD-L1 expression on tumor cell membranes[28,29,36,38,41,42] but not on immune cells.[28,29] Similar results were obtained for interobserver concordance,[31,36-38] suggesting that interpretation of PD-L1 levels on tumor cell membranes is reproducible when these three assays are performed in specialized laboratories by trained pathologists. PD-L1 may be heterogeneously expressed within some tumors and may differ between primary and metastatic tissues.[33,37,39,45] Therefore, multiple biopsy specimens from different sites may give a more correct level of PD-L1 expression. However, this conclusion is speculative and remains to be supported by clinical data. Owing to variability between LDAs,[30,35,40] standardization is needed before they can be recommended for routine clinical use. Use of accredited laboratories with expertise in performing PD-L1 IHC assays and appropriately trained pathologists is recommended. To make PD-L1 a reflex test, it is necessary to broaden availability of the test when training of pathologists is completed.

Although PD-L1 IHC testing has value as a biomarker, it has limitations. Research is ongoing to identify novel biomarkers that could be used alone or in combination with PD-L1 expression levels to improve patient selection for immunotherapy.

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