review articles

Analytical Concordance of PD-L1 Assays Utilizing Antibodies From FDA-Approved Diagnostics in Advanced Cancers: A Systematic Literature Review

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abstract

PURPOSE Four programmed death ligand 1 (PD-L1) immunohistochemistry assays (28-8, 22C3, SP263, and SP142) have been approved for use by the US Food and Drug Administration (FDA). Analytical concordance between these assays has been evaluated in multiple studies. This systematic review included studies that investigated the analytical concordance of immunohistochemistry assays utilizing two or more PD-L1 antibodies from FDA-approved diagnostics for evaluation of PD-L1 expression on tumor or immune cells across a range of tumor types and algorithms.

METHODS Literature searches were conducted in MEDLINE (via PubMed) and EMBASE to identify studies published between January 1, 2010, and March 31, 2019, that evaluated analytical concordance between two or more assays based on antibodies from FDA-approved assays. Proceedings of key oncology and pathology congresses that took place between January 2016 and March 2019 were searched for abstracts of studies evaluating PD-L1 assay concordance.

RESULTS A total of 42 studies across a range of tumor types met the selection criteria. Concordance between 28-8-, 22C3-, and SP263-based assays in lung cancer, urothelial carcinoma, and squamous cell carcinoma of the head and neck was high when used to assess PD-L1 expression on tumor cells (TCs). SP142-based assays had overall low concordance with other approved assays when used to assess PD-L1 expression on TCs. Analytical concordance for assessment of PD-L1 expression on immune cells was variable and generally lower than for PD-L1 expression on TCs.

CONCLUSION A large body of evidence supports the potential interchangeability of 28-8-, 22C3-, and SP263-based assays for the assessment of PD-L1 expression on TCs in lung cancer. Further studies are required in tumor types for which less evidence is available.

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INTRODUCTION

Programmed death-1 (PD-1) and programmed death ligand 1 (PD-L1) inhibitors have been approved in the United States and globally for the treatment of a range of tumor types. PD-(L)1 inhibitors approved by the US Food and Drug Administration (FDA) include atezolizumab, avelumab, cemiplimab, durvalumab, nivolumab, and pembrolizumab.[1-6] PD-L1 expression on tumor cells (TCs) and immune cells (ICs) is a mechanism of tumor immune escape through engagement and activation of the PD-1 receptor.[7,8] The expression of PD-L1 on TCs or ICs is associated with enhanced response to PD-(L)1 inhibitor therapy in some tumor types.[7] As of December 2020, four PD-L1 diagnostic immunohistochemistry (IHC) assays have been approved by the US FDA for assessment of PD-L1 expression on TCs or ICs in clinical practice (Table 1).

PD-L1 assay approvals are specific to the tumor types and therapeutic regimens for which the FDA authorizes their use and are variable with regard to the scoring algorithms used and the cell types on which PD-L1 expression is evaluated (ie, TCs, ICs, or both). Currently, there is a lack of data supporting assay harmonization. Not all laboratories can provide multiple PD-L1 assays corresponding to the approved indication for several reasons, including high cost or limited access to IHC staining platforms. Consequently, not having the approved assay may hinder PD-L1 testing and/or result interpretation and potentially a physician's recommendation for treatment guidance. Defined assay performance criteria are critical to guide pathologists and oncologists in identifying the most appropriate assay for an intended use and for interpreting test results. A variety of factors should be incorporated into such decisions, including

ASSOCIATED CONTENT

Appendix

Author affiliations and support information (if applicable) appear at the end of this article.

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CONTEXT

Key Objective

Evaluate analytical concordance between programmed death ligand 1 (PD-L1) immunohistochemistry assays utilizing antibodies from US Food and Drug Administration–approved diagnostics across a range of tumor types, scoring algorithms, and PD-L1 expression cutoffs.

Knowledge Generated

Analytical concordance between 28-8-, 22C3-, and SP263-based assays was high when used to assess PD-L1 expression on tumor cells (TCs) in lung cancer, urothelial carcinoma, and squamous cell carcinoma of the head and neck. SP142-based assays had low concordance with other assays for assessment of PD-L1 expression on TCs. Analytical concordance for assessment of PD-L1 expression on immune cells was variable and generally lower than for PD-L1 expression on TCs.

Relevance

As the immune checkpoint inhibitor treatment landscape continues to become increasingly complex, PD-L1 assay analytical concordance, in context with data on the predictive performance, sensitivity, and specificity of assays, informs decisions around assay choice and interpretation.

analytical concordance, predictive performance, and the sensitivity and specificity of available assays around the relevant clinical cutoffs.[9]

Three previous literature reviews have evaluated PD-L1 assay concordance in lung cancers. A review by Büttner et al[10] found high concordance and reproducibility for

TABLE 1. Summary of US Food and Drug Administration–Approved PD-L1 Assays and Associated Scoring Algorithms

Assay	Dako PD-L1 IHC 28-8 pharmDx Assay[51]	Dako PD-L1 IHC 22C3 pharmDx Assay[53]	Ventana PD-L1 (SP142) Assay[52]	Ventana PD-L1 (SP263) Assay[73]
For use with (drug)	Nivolumab ± ipilimumab (Bristol Myers Squibb)	Pembrolizumab (Merck)	Atezolizumab (Roche or Genentech)	Durvalumab (AstraZeneca)
Manufacturer	Dako[a]	Dako[a]	Ventana[b]	Ventana[b]
Approved PD-L1 scoring algorithm(s)	% TC	TPS,[c] CPS[d]	% IC, % TC, or % IC[e]	% TC or % IC[f]
Approval status and cutoffs	Companion 1L NSCLC: ≥ 1%[g] Complementary 2L NSQ NSCLC: ≥ 1%, ≥ 5%, ≥ 10% 2L SCCHN: ≥ 1% 2L UC: ≥ 1%	Companion 1L or 2L NSCLC: TPS ≥ 1% 1L UC: CPS ≥ 10 3L+ gastric or GEJ: CPS ≥ 1 2L+ CC: CPS ≥ 1 2L+ ESCC: CPS ≥ 10 1L SCCHN: CPS ≥ 1 1L TNBC: CPS ≥ 10	Companion 1L UC[h]: ≥ 5% IC 1L TNBC: ≥ 1% IC 1L NSCLC: ≥ 50% TC or ≥ 10% IC Complementary 2L NSCLC: ≥ 50% TC or ≥ 10% IC	Complementary[i] 2L UC: ≥ 25% TC or ICP > 1% and IC+ ≥ 25% or ICP = 1% and IC+ = 100%

NOTE. Approvals are companion or complementary diagnostics to PD-(L)1 inhibitor monotherapy except where noted.

Abbreviations: 1L, first line; 2L, second line; 3L, third line; CC, cervical cancer; CPS, combined positive score; ESCC, esophageal squamous cell carcinoma; GEJ, gastroesophageal junction; IC, immune cell; ICP, immune cell present; IHC, immunohistochemistry; NSCLC, non–small-cell lung cancer; NSQ, nonsquamous; PD-L1, programmed death ligand 1; SCCHN, squamous cell carcinoma of the head and neck; TC, tumor cell; TNBC, triple-negative breast cancer; TPS, tumor proportion score; UC, urothelial carcinoma.

[a]Dako, an Agilent Technologies Inc company.

[b]Ventana Medical Systems, a member of the Roche group.

[c]TPS = % of viable TCs showing partial or complete membrane staining relative to all viable TCs present in the sample (positive and negative).

[d]CPS = number of PD-L1–staining cells (TCs, lymphocytes, and macrophages) divided by the total number of viable TCs in the region assessed, multiplied by 100.

[e]PD-L1 status is determined on either the percentage of PD-L1–expressing TCs of any intensity or the proportion of tumor area occupied by PD-L1–expressing tumor-infiltrating ICs of any intensity.

[f]PD-L1 status is determined by the percentage of TC with any membrane staining above background or by the percentage of tumor-associated IC with staining at any intensity above background. If > 1% of the sample is composed of ICs, then PD-L1–expressing ICs must be ≥ 25%. If ≤ 1% of the sample is composed of ICs, then PD-L1–expressing ICs must equal 100%.

[g]Companion diagnostic to nivolumab plus ipilimumab in patients with 1L metastatic NSCLC expressing PD-L1 on ≥ 1% of TCs, with no epidermal growth factor receptor or anaplastic lymphoma kinase genomic tumor aberrations.

[h]UC ineligible for cisplatin-containing therapy.

[i]The 2L UC indication for durvalumab was withdrawn in February 2021.

assessment of PD-L1 expression on TCs in non–small-cell lung cancer (NSCLC) with the 28-8, 22C3, and SP263 assays, while the detection of PD-L1 expression on TCs with the SP142 assay was lower than with other assays.[10] There was poor concordance between assays when measuring PD-L1 expression on ICs.[10] Similar findings were reported in a review by Udall et al,[11] which found that the 28-8, 22C3, and SP263 assays produced comparable results when used to evaluate PD-L1 expression on TCs. However, the authors concluded that there was a lack of standardization among PD-L1 assays in terms of expression cutoffs and scoring algorithms, and that information on the interchangeability of PD-L1 assays was limited.[11] PD-L1 assay interchangeability was further evaluated in a meta-analysis of PD-L1 assay concordance by Torlakovic et al,[9] which concluded that FDA-approved assay kits were generally more interchangeable with a well-developed, fit-for-purpose, laboratory-developed test (LDT) than with another FDA-approved kit developed for a different purpose.

This systematic review was undertaken to update previous literature reviews, with the goal of assessing analytical concordance between assays utilizing antibodies from FDA-approved diagnostics for assessment of PD-L1 expression on TCs and/or ICs across a range of tumor types, algorithms, and PD-L1 expression cutoffs.

METHODS

The methodology of this study adhered to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses guidelines.[12] Systematic searches were conducted in MEDLINE (via PubMed) and EMBASE (Elsevier) to identify studies published between January 1, 2010, and March 31, 2019, that evaluated concordance between two or more assays based on antibodies from FDA-approved diagnostics. The search string employed was ("PD-L1" OR "Programmed death ligand 1" OR "PDL1" OR "Programmed death ligand") AND ("IHC" OR "immunohistochemistry") AND (concordance OR validation OR sensitivity OR specificity OR correlat* OR reproducib* OR valid* OR agree*). Search results were limited to English-language publications only.

Proceedings of key oncology and pathology congresses that took place between January 1, 2016, and March 31, 2019, were searched for abstracts of studies evaluating concordance between assays utilizing antibodies from FDA-approved diagnostics. The congresses searched were the annual meetings of the American Association for Cancer Research (AACR), the Association for Molecular Pathology (AMP), the American Society of Clinical Oncology (ASCO), the College of American Pathologists (CAP), the European Society for Medical Oncology (ESMO), the Society for Immunotherapy of Cancer (SITC), and the United States and Canadian Academy of Pathology (USCAP). The ASCO Gastrointestinal Cancers Symposium (ASCO GI), the ASCO Genitourinary Cancers Symposium (ASCO GU), the ASCO-SITC Clinical Immuno-Oncology Symposium, the European Congress of Pathology (ECP), and the International Association for the Study of Lung Cancer World Conference on Lung Cancer (IASLC WCLC) were also searched.

The study inclusion and exclusion criteria are shown in Table 2. Publications and congress abstracts were compiled, and duplicates were removed manually. Congress abstracts were checked for subsequent publication, with those abstracts that had been subsequently published as full manuscripts excluded. Publications and abstracts were initially screened against two key inclusion criteria: evaluation of PD-L1 expression with at least two assays utilizing antibodies from FDA-approved diagnostics and evaluation of comparability or concordance between two or more assays. Publications meeting these criteria were read in full and scored against the remaining selection criteria. Studies that explicitly stated that assays were performed using materials and equipment other than those specified in the manufacturers' instructions (ie, an LDT) were excluded. Studies that did not explicitly state whether the FDA-approved assay or an LDT was used were assumed to have met the inclusion criteria. In addition, studies that evaluated analytical concordance in a tumor type for which the assay is not approved were included. Disagreements were resolved by majority opinion of the reviewing authors. A standardized template was used to extract key information, including the type of study, location, number of patients or samples evaluated, tumor types included, antibodies or tests used, PD-L1 scoring algorithms used, cell types assessed for PD-L1 expression, key concordance and agreement statistics, and training status of the scoring pathologist. Key data from the identified studies were analyzed descriptively with the aim of identifying trends in concordance statistics. Studies reporting concordance or agreement frequently qualified their results subjectively. Therefore, there is no convention for descriptive reporting of concordance between assays. To assist with evaluation, data were grouped into the following subjective categories: poor, fair, and strong. Concordance was described as poor for k values ≤ 0.4, fair for k values > 0.4 to < 0.7, and strong for k values ≥ 0.7; agreement was described as poor for overall percentage agreement (OPA) $\leq 60\%$, fair for OPA $> 60\%$ to $< 75\%$, and strong for OPA $\geq 75\%$; correlation was described as poor for Pearson correlation coefficient (r^2) and Spearman correlation coefficient (ρ) for r^2 and $\rho \leq 0.6$, fair for r^2 and $\rho > 0.60$ to < 0.85, and strong for r^2 and $\rho \geq 0.85$. Meta-analyses or other statistical analyses of the results were not performed because of the heterogeneity of the studies identified in the search.

RESULTS

Part I: Screening of Reports and Studies' Details

Searches of MEDLINE (PubMed) and EMBASE identified 819 and 2,203 records, respectively, published between

TABLE 2. Inclusion and Exclusion Criteria

Inclusion	Exclusion
• **Evaluation of PD-L1 expression with at least two assays that utilized antibodies from FDA-approved diagnostics from the following list: 28-8, 22C3, SP142, and SP263**[a]	• Concordance on LDTs,[b] RUO antibody clones, or kits not commercially available in the United States
• **Comparability or concordance between two assays that utilized antibodies from FDA-approved diagnostics**	• Concordance between types of samples (FNA, surgical resection, and core needle) using only one antibody clone
• PD-L1 testing via IHC only	• PD-L1 testing via technologies other than IHC
• Evaluation of PD-L1 expression using glass slide scoring only	• Evaluation of analytical concordance using tissue microarrays, digital scoring, or scoring of scanned images
• Randomized trials, observational trials, and diagnostic or clinical validation studies	• PD-L2 assessment or multiplex with other factors
• All tumor types, including hematologic tumors	• Animal samples, case reports, editorials; ongoing clinical trials; meta-analyses
• Studies published between January 1, 2010, and March 31, 2019, inclusive	• Studies published before January 1, 2010, or after March 31, 2019
• Articles published in English	

NOTE. Key inclusion criteria used for the initial screening of studies are shown in bold text.

Abbreviations: FDA, US Food and Drug Administration; FNA, fine-needle aspiration; IHC, immunohistochemistry; LDT, laboratory-developed test; PD-L1/2, programmed death ligand 1/2; RUO, research use only.

[a]Studies investigating assays based on antibodies from a diagnostic that is FDA-approved for use in one or more tumor types were included. Unless use of an LDT was explicitly stated, studies were assumed to have met the inclusion criterion. Studies that provided insufficient detail of assay methodology to confirm that the manufacturer's kit was used were included. Studies were included regardless of whether assays were FDA-approved for use in the tumor type in which the study was performed.

[b]LDTs were defined as assays that were not performed with the reagents, methods, and/or equipment specified in the manufacturer's instructions for FDA-approved diagnostics.

January 1, 2010, and March 31, 2019. Manual searches of the proceedings of key congresses identified an additional 521 abstracts presented between January 1, 2016, and March 31, 2019. A total of 3,477 unique records were screened against the two key inclusion criteria, of which 97 met both criteria and were reviewed in detail by the authors. A total of 42 publications and abstracts met all inclusion and exclusion criteria and were included in the review. Included and excluded manuscripts and abstracts, as well as the number of studies evaluating each assay by tumor type, are shown in Figure 1. Details of the studies included in the review are summarized in Appendix Table A1.

Part II: Analytical Concordance in Studies Assessing PD-L1 Expression on TCs Only

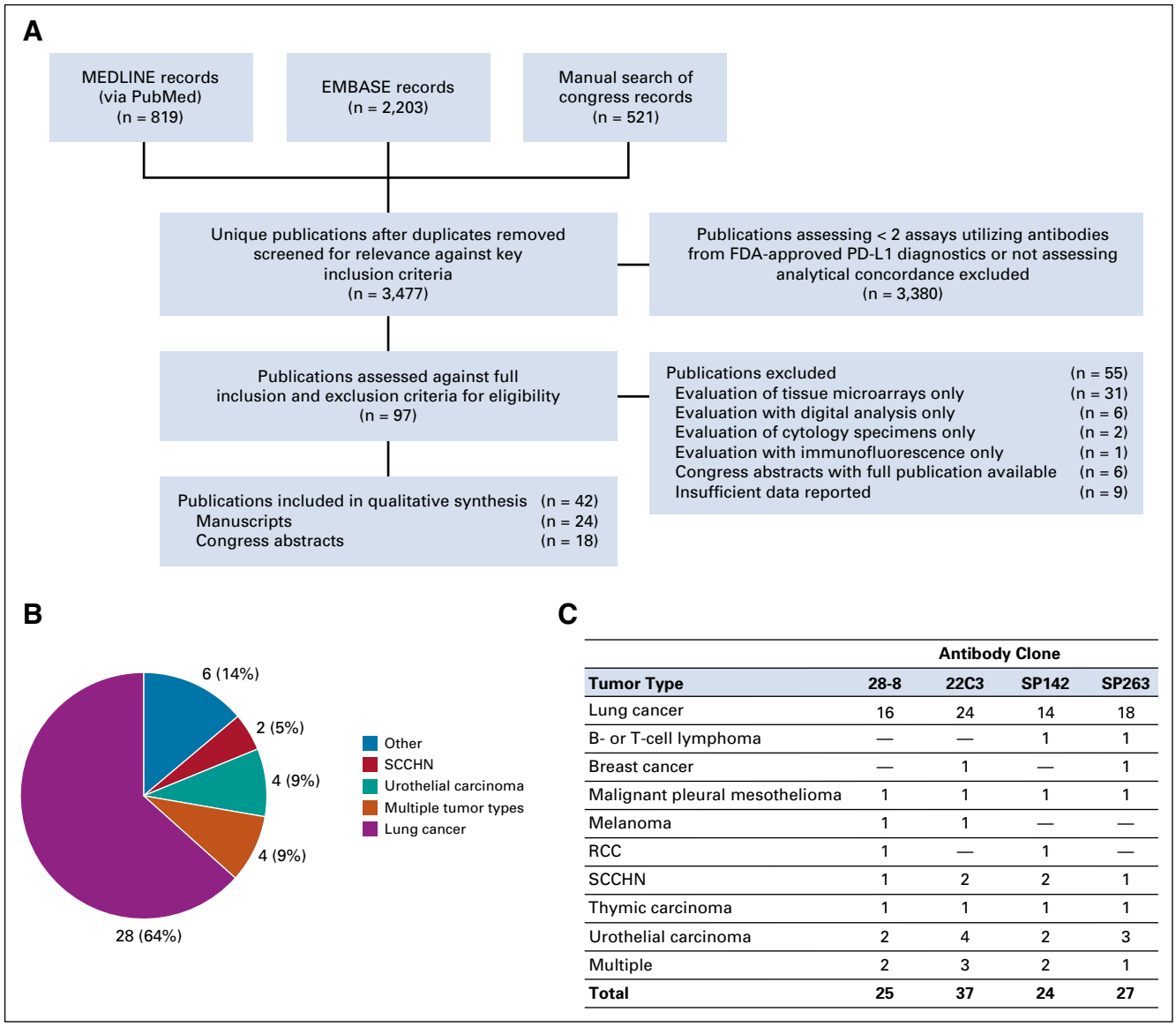
Analytical concordance for assessment of PD-L1 expression on TCs only using 28-8-, 22C3-, SP142-, and SP263-based assays is shown in Table 3. The training status of the pathologists was reported in six studies and was not specified in the remaining studies. The details of pathologist training were not reported in enough studies to enable an assessment of the impact of pathologist training on analytical concordance.

Data from studies in which PD-L1 expression agreement was assessed across multiple cutoffs suggested a trend for higher agreement with increasing cutoff in lung cancer and squamous cell carcinoma of the head and neck (SCCHN).[13-16] However, none of the studies formally evaluated the changes in analytical concordance across PD-L1 expression cutoffs.

Overall, concordance between 28-8-, 22C3-, and SP263-based assays in lung cancer, urothelial carcinoma (UC), and SCCHN was high when used to assess PD-L1 expression on TCs. SP142-based assays had overall low concordance with other approved assays when used to assess PD-L1 expression on TCs.

Lung cancer. Most studies reported concordance results in NSCLC only, but a number of studies reported results across heterogeneous types of lung tumors, including small-cell lung cancer and NSCLC, or did not specify the types of lung tumors included. Across studies that evaluated 28-8-based assays, generally strong analytical concordance was seen with 22C3-based assays[15,17-23] and fair-to-strong analytical concordance with SP263-based assays.[15,18,24,25] Analytical concordance between 22C3- and SP263-based assays was variable across studies.[15,16,18,20,26-31] In one study in which a six-category integrated proportion score was used to evaluate PD-L1 expression on TCs, a higher proportion of PD-L1–positive TCs was seen with both 22C3- and SP263-based assays versus 28-8-based assays in nonconcordant samples, and a higher proportion of TCs were stained with SP263-based assays versus 22C3-based assays.[32]

SP142-based assays showed generally poor-to-fair analytical concordance with 28-8-,[15,25] 22C3-,[15,20,33-35] and SP263-based assays[15,25,36-38] for assessment of PD-L1 expression on TCs, with nonconcordant cases showing stronger staining with comparator assays than with SP142-based assays.[32]



	Antibody Clone			
Tumor Type	**28-8**	**22C3**	**SP142**	**SP263**
Lung cancer	16	24	14	18
B- or T-cell lymphoma	—	—	1	1
Breast cancer	—	1	—	1
Malignant pleural mesothelioma	1	1	1	1
Melanoma	1	1	—	—
RCC	1	—	1	—
SCCHN	1	2	2	1
Thymic carcinoma	1	1	1	1
Urothelial carcinoma	2	4	2	3
Multiple	2	3	2	1
Total	**25**	**37**	**24**	**27**

FIG 1. Details of studies included. (A) Disposition of literature search results. (B) Included studies by tumor type (n = 42). (C) The number of studies evaluating each antibody by tumor type. The "Other" category comprises studies in lymphoma, malignant pleural mesothelioma, melanoma, RCC, breast cancer, and thymic carcinoma (all n = 1). The "Multiple tumor types" category refers to studies in which concordance was analyzed in a cohort comprising more than one tumor type. The number of comparisons is equal to 44 because of studies reporting separate concordance results in more than one tumor type, as shown in Appendix Table A1. FDA, US Food and Drug Administration; PD-L1, programmed death ligand 1; RCC, renal cell carcinoma; SCCHN, squamous cell carcinoma of the head and neck.

In the Blueprint studies, generally comparable distribution of TC staining with 28-8-, SP263-, and 22C3-based assays was seen across a series of samples.[39,40] In Blueprint phase 1, SP142-based assays showed weaker staining of TCs and fewer positive TCs compared with other assays, while Blueprint phase 2 also found SP142 to have lower sensitivity for detection of PD-L1 expression on TCs than other assays. Although statistical analyses were performed in these studies, formal statistics for comparisons between assays have not been published.[39,40]

Nonlung tumor types. Analytical concordance data for TC scoring were limited in most nonlung tumor types. With the exception of SCCHN and melanoma, the majority of tumor types had a single publication. Generally strong agreement or concordance between 28-8-, 22C3-, and/or SP263-based assays was seen in breast cancer,[41] melanoma,[42] malignant pleural mesothelioma,[43] SCCHN,[13,14] thymic carcinoma,[44] and UC.[45] Analytical concordance for comparisons including SP142-based assays was variable, with fair-to-strong concordance and agreement with 22C3- or SP263-based

TABLE 3. Assay Concordance and Agreement for Assessment of PD-L1 Expression on TCs (All Available Cutoffs)

Study (No.)	Assay Comparison[a]	Cutoff (Reference[b])	OPA (%)	PPA (%)	NPA (%)	Statistical Test Result	Regression Analysis	Pathologist Training
Lung cancer[c]								
Published articles								
Batenchuk et al[17] (412)	28-8 *v* 22C3	≥ 1%[d] (28-8)	97	97	96	Cohen's k = 0.94	—	Pathologists trained and certified by Dako[e]
		≥ 5% (28-8)	95	97	94	Cohen's k = 0.90		
		≥ 10% (28-8)	97	98	96	Cohen's k = 0.94		
		≥ 25% (28-8)	97	98	96	Cohen's k = 0.93		
		≥ 50% (28-8)	98	99	97	Cohen's k = 0.95		
Conde et al[38] (69)	SP263 *v* SP142	Continuous	—	—	—	—	ρ = 0.88 (discovery cohort), ρ = 0.87 (validation cohort)	NR
Fujimoto et al[15] (40)	28-8 *v* 22C3	≥ 1%[d]	78	—	—	Cohen's k = 0.71	—	NR
		≥ 50%	95	—	—			
	28-8 *v* SP263	≥ 1%[d]	78	—	—	Cohen's k = 0.69	—	
		≥ 50%	95	—	—			
	28-8 *v* SP142	≥ 1%[d]	75	—	—	Cohen's k = 0.55	—	
		≥ 50%[f]	90	—	—			
	22C3 *v* SP263	≥ 1%[d]	75	—	—	Cohen's k = 0.64	—	
		≥ 50%	90	—	—			
	22C3 *v* SP142	≥ 1%[d]	73	—	—	Cohen's k = 0.49	—	
		≥ 50%[f]	85	—	—			
	SP263 *v* SP142	≥ 1%	73	—	—	Cohen's k = 0.39	—	
		≥ 50%[f]	90	—	—			
Fujimoto et al[16] (99)	22C3 *v* SP263	1%[d]	88	—	—	—	—	NR
		25%[g]	94	—	—	—	—	
		50%	97	—	—	—	—	
Ilie et al[25] (56)	28-8 *v* SP263	Scoring scale, 0-3[h]	—	—	—	Cohen's k = 0.883	—	NR
		Continuous	—	—	—	—	ρ = 0.996	
	28-8 *v* SP142	Scoring scale, 0-3[h]	—	—	—	Cohen's k = 0.412	—	
		Continuous	—	—	—	—	ρ = 0.860	
	SP263 *v* SP142	Scoring scale, 0-3[h]	—	—	—	Cohen's k = 0.362	—	
		Continuous	—	—	—	—	ρ = 0.852	
Kim et al[27] (97)	22C3 *v* SP263	≥ 1%[d]	—	—	—	Cohen's k = 0.863	—	NR
		≥ 5%	—	—	—	Cohen's k = 0.744	—	
		≥ 10%	—	—	—	Cohen's k = 0.741	—	
		≥ 25%[g]	—	—	—	Cohen's k = 0.823	—	
		≥ 50%	—	—	—	Cohen's k = 0.467	—	
Nakamura et al[21] (137)	28-8 *v* 22C3	Continuous	—	—	—	—	r^2 = 0.86	NR
Pang et al[36] (84)	SP263 *v* SP142	SP142: ≥ 1%; SP263: ≥ 25%[g]	—	—	—	Cohen's k = 0.53	—	NR

(Continued on following page)

TABLE 3. Assay Concordance and Agreement for Assessment of PD-L1 Expression on TCs (All Available Cutoffs) (Continued)

Study (No.)	Assay Comparison[a]	Cutoff (Reference[b])	OPA (%)	PPA (%)	NPA (%)	Statistical Test Result	Regression Analysis	Pathologist Training
Ratcliffe et al[18] (500)	28-8 *v* 22C3	≥ 1%[d] (28-8)	93.7	92.5	95.5	—	—	
		≥ 10% (28-8)	94.9	94.8	95.1	—		
		≥ 25%	96.6	—	—	—		
		≥ 50% (22C3)	97.2	97.5	97.0	—		
		Continuous	—	—	—	—	ρ = 0.954	
	28-8 *v* SP263	≥ 1%[d] (28-8)	91.7	90.4	93.5	—	—	
		≥ 10% (28-8)	92.9	91.4	94.0	—		
		≥ 25%[g] (SP263)	94.9	90.1	97.5	—		
		≥ 50%	95.9	—	—	—		
		Continuous	—	—	—	—	ρ = 0.948	
	22C3 *v* SP263	≥ 1%[d]	91.1	—	—	—	—	
		≥ 10%	92.7	—	—	—		
		≥ 25%[g] (SP263)	94.3	86.0	98.8	—		
		≥ 50% (22C3)	93.5	91.7	94.1	—		
		Continuous	—	—	—	—	ρ = 0.925	
Saito et al[23] (420)	28-8 *v* 22C3	≥ 1% (28-8)[d]	89.0	85.5	91.0	Cohen's k = 0.763	—	
		≥ 25% (28-8)	90.2	98.3	89.0	Cohen's k = 0.677	—	
		≥ 50% (28-8)	91.9	94.9	91.6	Cohen's k = 0.643	—	
		≥ 1% (22C3)[d]	89.0	84.4	91.7	Cohen's k = 0.763	—	
		≥ 25% (22C3)	90.2	58.3	99.7	Cohen's k = 0.677	—	
		≥ 50% (22C3)	91.9	53.6	99.4	Cohen's k = 0.643	—	
Skov and Skov[74] (87)	28-8 *v* 22C3	≥ 1%[d]	97	96[j]	97[k]	—	—	
		≥ 5%	99	98[j]	99[k]			
		≥ 10%	95	93[j]	97[k]			
		≥ 50%	93	80[j]	96[k]			
Velcheti et al[75] (6,024)	28-8 *v* 22C3	< 1%	—	—	—	*P* = .96	—	
		1%-49%	—	—	—			
		≥ 50%	—	—	—			
Villaruz et al[31] (302)	22C3 *v* SP263	Continuous	—	—	—	—	Correlation coefficient = 0.88	
Xu et al[34] (135)	22C3 *v* SP142	TPS < 1%, 1%-49%, ≥ 50% (22C3)	—	—	—	Cohen's k = 0.481	—	
		< 1%, 1% to < 5%, 5% to < 50%, ≥ 50% (SP142)	—	—	—	Cohen's k = 0.324	—	
Congress abstracts								
Beck et al[26] (80)	22C3 *v* SP263	≥ 1%[d]	—	93.2	—	Cohen's k = 0.878	—	
		≥ 25%[g]	—	100.0	—	Cohen's k = 0.698	—	
		≥ 50%	—	95.2	—	Cohen's k = 0.790	—	
Cho et al[30] (109)	22C3 *v* SP263	< 1%, 1%-49%, ≥ 50%	—	—	—	—	Correlation coefficient = 0.66	

Staining reviewed by Merck-, Dako-, or Bristol Myers Squibb–trained pathologists[i]

Pathologist trained in a CLIA program-certified laboratory

Scoring performed by pathologist experienced with histology and cytology specimens from malignant pulmonary lesions

NR

(Continued on following page)

TABLE 3. Assay Concordance and Agreement for Assessment of PD-L1 Expression on TCs (All Available Cutoffs) (Continued)

Study (No.)	Assay Comparison[a]	Cutoff (Reference[b])	OPA (%)	PPA (%)	NPA (%)	Statistical Test Result	Regression Analysis	Pathologist Training
Krigsfeld et al[22] (1,506)	28-8 *v* 22C3	Continuous	—	—	—	—	$\rho = 0.96$	
		≥ 1% (28-8)	96.2	96.7	95.3	—	—	NR
		≥ 1% (22C3)	96.2	96.9	95.0	—	—	
Lisberg et al[35] (28)	22C3 *v* SP142	Continuous	—	—	—	—	$r^2 = 0.58$	NR
Motoi et al[20] (486)	28-8 *v* 22C3	< 1%, 1%-49%, ≥ 50%	—	—	—	Cohen's k = 0.896	—	
	22C3 *v* SP263	< 1%, 1%-49%, ≥ 50%	—	—	—	Cohen's k = 0.729	—	NR
	22C3 *v* SP142	< 1%, 1%-49%, ≥ 50%	—	—	—	Cohen's k = 0.159	—	
Quinn et al[28] (100)	22C3 *v* SP263	Continuous	—	—	—	—	$r^2 = 0.9025$	
			—	—	—	—		NR
			—	—	—	—		
Saito et al[19] (147)	28-8 *v* 22C3	Continuous	—	—	—	—	$\rho = 0.851$	NR
Scott et al[37] (493)	SP263 *v* SP142	≥ 1%	63.5	—	—	—	—	
		≥ 5%	68.0	—	—	—	—	
		≥ 10%	68.5	—	—	—	—	NR
		≥ 25%[g]	68.5	—	—	—	—	
		≥ 50%[g]	80.5	—	—	—	—	
Wilberger et al[29] (23)	22C3 *v* SP263	—	91.3	—	—	—	—	Some cases scored by a Ventana pathologist[l]
Xu et al[33] (49)	22C3 *v* SP142	≥ 1%[d]	—	—	—	Cohen's k = 0.608	—	NR
		≥ 50%[g]	—	—	—	Cohen's k = 0.545	—	
Zhang et al[24] (45)	28-8 *v* SP263	Continuous	—	—	—	—	$r^2 = 0.91$	
		≥ 1%[d]	—	—	—	—	$r^2 = 0.73$	NR
		28-8: ≥ 1%[d]; SP263: ≥ 25%[g]	—	—	—	—	$r^2 = 0.58$	
		≥ 25%[g]	—	—	—	—	$r^2 = 0.95$	
B- or T-cell lymphoma								
Published article								
Vranic et al[46] (78)	SP263 *v* SP142	Continuous	—	—	—	—	$\rho = 0.919$	NR
Breast cancer								
Published article								
Karnik et al[41] (136)	22C3 *v* SP263	≥ 1%	—	—	—	Cohen's k = 0.902	—	NR
Malignant pleural mesothelioma								
Published article								
Watanabe et al[43] (32)	28-8 *v* 22C3	1%	84.4	—	—	—	—	
	28-8 *v* SP263	28-8: 1%; SP263: 25%	75.0	—	—	—	—	NR
	22C3 *v* SP263	22C3: 1%; SP263: 25%	71.9	—	—	—	—	

(Continued on following page)

TABLE 3. Assay Concordance and Agreement for Assessment of PD-L1 Expression on TCs (All Available Cutoffs) (Continued)

Study (No.)	Assay Comparison[a]	Cutoff (Reference[b])	OPA (%)	PPA (%)	NPA (%)	Statistical Test Result	Regression Analysis	Pathologist Training
Melanoma								
Congress abstract								
Krigsfeld et al[42] (202)	28-8 *v* 22C3	≥ 1%	93.1	82.1	97.3	—	—	NR
SCCHN								
Published article								
De Meulenaere et al[13] (99)	22C3 *v* SP142	≥ 1%	75	—	—	Cohen's k = 0.511	—	NR
		≥ 5%	81.9	—	—	—		
		≥ 10%	83.3	—	—	—		
Congress abstract								
Scott et al[14] (486)	28-8 *v* SP263	≥ 1%[d]	84	77	95	—	—	NR
		≥ 25%[g]	93	62	100	—	—	
	22C3 *v* SP263	≥ 1%[d]	79	68	95	—	—	
		≥ 25%[g]	91	56	99	—	—	
	SP142 *v* SP263	≥ 1%	59	31	100	—	—	
		≥ 25%	85	15	100	—	—	
Thymic carcinoma								
Published article								
Sakane et al[44] (53)	28-8 *v* 22C3	Continuous	—	—	—	—	ρ = 0.9561	NR
	28-8 *v* SP263	Continuous	—	—	—	—	ρ = 0.9234	
	28-8 *v* SP142	Continuous	—	—	—	—	ρ = 0.9197	
	22C3 *v* SP263	Continuous	—	—	—	—	ρ = 0.9114	
	22C3 *v* SP142	Continuous	—	—	—	—	ρ = 0.9122	
	SP263 *v* SP142	Continuous	—	—	—	—	ρ = 0.9192	
UC								
Congress abstracts								
Krigsfeld et al[45] (13)	28-8 *v* 22C3	Continuous	—	—	—	—	ρ = 0.94	NR
Multiple tumor types								
Published articles								
Abdul Karim et al[48] (> 175)	22C3 *v* SP142	NR	95-100	—	—	—	—	NR
Batenchuk et al[17] (1,930)	28-8 *v* 22C3	≥ 1%[d] (28-8)	97	97	97	Cohen's k = 0.94	—	Pathologists trained and certified by Dako[e]
		≥ 5% (28-8)	97	97	97	Cohen's k = 0.93		
		≥ 10% (28-8)	98	98	98	Cohen's k = 0.95		
		≥ 25% (28-8)	98	98	97	Cohen's k = 0.95		
		≥ 50% (28-8)	97	99	96	Cohen's k = 0.92		

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TABLE 3. Assay Concordance and Agreement for Assessment of PD-L1 Expression on TCs (All Available Cutoffs) (Continued)

Study (No.)	Assay Comparison[a]	Cutoff (Reference[b])	OPA (%)	PPA (%)	NPA (%)	Statistical Test Result	Regression Analysis	Pathologist Training
Congress abstract								
Krigsfeld et al[47] (3,113)	28-8 *v* 22C3	≥ 1% (28-8)	96.2	96.8	95.4	—	—	NR
		≥ 1% (22C3)	96.2	96.4	96.0	—	—	
		Continuous	—	—	—	—	ρ = 0.96	

NOTE. Data are sorted by tumor type and alphabetical order by first author, with studies evaluating lung cancers shown at the top and multiple tumor types shown at the bottom. Additional tumor types are shown in alphabetical order.

Abbreviations: ρ, Spearman correlation coefficient; CLIA, Clinical Laboratory Improvement Amendments; IHC, immunohistochemistry; NPA, negative percentage agreement; NR, not reported; OPA, overall percentage agreement; PD-L1, programmed death ligand 1; PPA, positive percentage agreement; r^2, Pearson correlation coefficient; SCCHN, squamous cell carcinoma of the head and neck; TPS, tumor proportion score; UC, urothelial carcinoma.

[a]Assays were based on antibodies from US Food and Drug Administration–approved diagnostics.

[b]Reference test reported in the table if indicated in the publication. A reference test is defined as a standard test used for comparison with a novel test to determine PPA and NPA.

[c]Most studies reported concordance results in non–small-cell lung cancer only, but a number of studies reported results across heterogeneous types of lung tumors, including small-cell lung cancer and non–small-cell lung cancer, or did not specify the types of lung tumors included.

[d]US Food and Drug Administration–approved cutoff for the Dako PD-L1 IHC 28-8 or 22C3 pharmDx Assays.

[e]Dako, an Agilent Technologies Inc company.

[f]US Food and Drug Administration–approved cutoff for the Ventana PD-L1 (SP142) Assay.

[g]US Food and Drug Administration–approved cutoff for the Ventana PD-L1 (SP263) Assay.

[h]Scoring scale 0-3: 0 (< 1%), 1 (≥ 1% to < 5%), 2 (≥ 5% to < 50%), 3 (≥ 50%).

[i]22C3 staining reviewed by Merck- and/or Dako-trained pathologists, and 28-8 staining reviewed by Bristol Myers Squibb– and/or Dako-trained pathologists.

[j]The value reported is the average positive agreement.

[k]The value reported is the average negative agreement.

[l]A total of 17 cases were scored before and after interpretation training by a Ventana pathologist, and six cases were scored after training.

TABLE 4. Assay Concordance and Agreement for Assessment of PD-L1 Expression on ICs or Combined ICs and TCs

Study (No.)	Assay Comparison[a]	Cells Scored (Algorithm)	Cutoff (Reference[b])	OPA (%)	PPA (%)	NPA (%)	Statistical Test Result	Regression Analysis	Pathologist Training
Lung cancer[c]									
Published articles									
Conde et al[38] (69)	SP263 *v* SP142	% IC	Continuous	—	—	—	—	ρ = 0.68 (validation cohort); 0.74 (discovery cohort)	NR
Ilie et al[25] (56)	28-8 *v* SP263	% IC	Scoring scale 0-3[d]	—	—	—	Cohen's k = 0.721	—	NR
			Continuous	—	—	—	—	ρ = 0.880	
	28-8 *v* SP142	% IC	Scoring scale 0-3[d]	—	—	—	Cohen's k = 0.134	—	
			Continuous	—	—	—	—	ρ = 0.590	
	SP263 *v* SP142	% IC	Scoring scale 0-3[d]	—	—	—	Cohen's k = 0.018	—	
			Continuous	—	—	—	—	ρ = 0.568	
Kim et al[27] (97)	22C3 *v* SP142	TC (22C3); TC plus IC (SP142)	$\geq$ 1%	—	—	—	Cohen's k = 0.468	—	NR
			$\geq$ 5%	—	—	—	Cohen's k = 0.214	—	
			$\geq$ 10%	—	—	—	Cohen's k = 0.160	—	
			$\geq$ 25%	—	—	—	Cohen's k = 0.108	—	
	SP263 *v* SP142	TC (SP263); TC plus IC (SP142)	$\geq$ 1%	—	—	—	Cohen's k = 0.501	—	
			$\geq$ 5%	—	—	—	Cohen's k = 0.236	—	
			$\geq$ 10%	—	—	—	Cohen's k = 0.232	—	
			$\geq$ 25%	—	—	—	Cohen's k = 0.151	—	
Xu et al[34] (135)	22C3 *v* SP142	IC (% tumor area) and TC	Scoring scale TC0 $<$ 1%, TC1 $\geq$ 1% to $<$ 5%, TC2 $\geq$ 5% to $<$ 50%, TC3 $\geq$ 50%, IC0 $<$ 1%, IC1 $\geq$ 1% to $<$ 5%, IC2 $\geq$ 5% to $<$ 10%, IC3 $\geq$ 10% (SP142)	—	—	—	Weighted k = 0.324	—	NR
Congress abstracts									
Motoi et al[20] (486)	28-8 *v* SP142	TC (28-8)	$<$ 1%, $\geq$ 1% to $<$ 50%, $\geq$ 50%	—	—	—	k = 0.241	—	NR
		TC plus IC (SP142)	$<$ 1%, $\geq$ 1% to $<$ 50%, $\geq$ 50%						
	22C3 *v* SP142	TC (22C3)	$<$ 1%, $\geq$ 1% to $<$ 50%, $\geq$ 50%	—	—	—	k = 0.213	—	
		TC plus IC (SP142)	$<$ 1%, $\geq$ 1% to $<$ 50%, $\geq$ 50%						
	SP263 *v* SP142	TC (SP263)	$<$ 1%, $\geq$ 1% to $<$ 50%, $\geq$ 50%	—	—	—	k = 0.291	—	
		TC plus IC (SP142)	$<$ 1%, $\geq$ 1% to $<$ 50%, $\geq$ 50%						

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TABLE 4. Assay Concordance and Agreement for Assessment of PD-L1 Expression on ICs or Combined ICs and TCs (Continued)

Study (No.)	Assay Comparison[a]	Cells Scored (Algorithm)	Cutoff (Reference[b])	OPA (%)	PPA (%)	NPA (%)	Statistical Test Result	Regression Analysis	Pathologist Training
Scott et al[37] (493)	28-8 *v* SP263	% IC	≥ 1%	93.1	—	—	—	—	
			≥ 5%	93.1					
			≥ 10%	91.9					
			≥ 25%	86.8					
			≥ 50%	97.0					
	22C3 *v* SP263	% IC	≥ 1%	89.9	—	—	—	—	
			≥ 5%	89.9					
			≥ 10%	89.7					NR
			≥ 25%	87.8					
			≥ 50%	96.1					
	SP263 *v* SP142	% IC	≥ 1%	64.5	—	—	—	—	
			≥ 5%	63.5					
			≥ 10%[e]	60.0					
			≥ 25%	96.0					
			≥ 50%	98.0					
Malignant pleural mesothelioma									
Published article									
Watanabe et al[43] (32)	28-8 *v* SP142	28-8: % TC	≥ 1%	81.3	—	—	—	—	
		SP142: % TC or IC (% tumor area)	≥ 1%						
	22C3 *v* SP142	22C3: % TC	≥ 1%	84.4	—	—	—	—	
		SP142: % TC or IC (% tumor area)	≥ 1%						NR
	SP263 *v* SP142	SP263: % TC	≥ 25%	75.0	—	—	—	—	
		SP142: % TC or IC (% tumor area)	≥ 1%						
RCC									
Congress abstract									
Zhu et al[60] (32)	28-8 *v* SP142	IC or TC	NR	91	—	—	—	—	NR

(Continued on following page)

TABLE 4. Assay Concordance and Agreement for Assessment of PD-L1 Expression on ICs or Combined ICs and TCs (Continued)

Study (No.)	Assay Comparison[a]	Cells Scored (Algorithm)	Cutoff (Reference[b])	OPA (%)	PPA (%)	NPA (%)	Statistical Test Result	Regression Analysis	Pathologist Training
SCCHN									
Congress abstract									
Scott et al[14] (486)	28-8 *v* SP263	% IC	≥ 25%	81	41	96	—	—	NR
		CPS[f]	≥ 1	83	78	96			
			≥ 10	84	64	99			
		% TC or % IC	≥ 25%	80	53	96			
	22C3 *v* SP263	% IC	≥ 25%	82	41	97	—	—	
		CPS[f]	≥ 1[g]	75	68	93			
			≥ 10	79	55	98			
		% TC or % IC	≥ 25%	79	48	97			
	SP142 *v* SP263	% IC	≥ 25%	74	6	99	—	—	
		CPS[f]	≥ 1	69	57	99			
			≥ 10	68	26	100			
		% TC or % IC	≥ 25%	75	37	97			
Thymic carcinoma									
Published article									
Sakane et al[44] (53)	28-8 *v* 22C3	% IC	Continuous	—	—	—	—	ρ = 0.8732	NR
	28-8 *v* SP263	% IC	Continuous	—	—	—	—	ρ = 0.6192	
	28-8 *v* SP142	% IC	Continuous	—	—	—	—	ρ = 0.5553	
	22C3 *v* SP263	% IC	Continuous	—	—	—	—	ρ = 0.5994	
	22C3 *v* SP142	% IC	Continuous	—	—	—	—	ρ = 0.5005	
	SP263 *v* SP142	% IC	Continuous	—	—	—	—	ρ = 0.4787	
UC									
Published article									
Zavalishina et al[49] (100)	22C3 *v* SP263	% TC or % IC	≥ 25%[h] (22C3)	—	50	100	—	r^2 = 0.99 (TC); r^2 = 0.69 (IC)	Pathologists certified by Ventana/ Roche and Agilent/ Dako
		% TC plus % IC or % TC	≥ 10% (SP263)	—	100	92	—		
	22C3 *v* SP142	% IC	≥ 5%[e] (22C3)	—	43	97	—	r^2 = 0.93 (TC); r^2 = 0.50 (IC)	
		% TC plus % IC or % TC	≥ 10% (SP142)	—	67	91	—		
	SP263 *v* SP142	% TC or % IC	≥ 25%[h] (SP142)	—	56	98	—	r^2 = 0.91 (TC); r^2 = 0.85 (IC)	
		% IC	≥ 5%[e] (SP263)	—	71	96	—		
Congress abstracts									
Walker et al[61] (335)	22C3 *v* SP263	CPS[f]	≥ 1 (SP263)	77.0	90.7	69.6	—	—	NR
		CPS[f]	≥ 10[g] (SP263)	81.5	62.7	91.7	—	—	
	SP263 *v* SP142	IC (% tumor area)	≥ 5%[h] (SP263)	69.9	15.3	99.5	—	—	
	28-8 *v* SP263	TC	≥ 1% (SP263)	75.5	66.9	80.2	—	—	
Zhu et al[60] (18)	22C3 *v* SP263	NR	NR	94	—	—	—	—	NR

(Continued on following page)

TABLE 4. Assay Concordance and Agreement for Assessment of PD-L1 Expression on ICs or Combined ICs and TCs (Continued)

Study (No.)	Assay Comparison[a]	Cells Scored (Algorithm)	Cutoff (Reference[b])	OPA (%)	PPA (%)	NPA (%)	Statistical Test Result	Regression Analysis	Pathologist Training
Multiple tumor types									
Published article									
Abdul Karim et al[48] (> 175)	22C3 *v* SP142	TC or IC	90-94	—	—	—	—	—	NR
Congress abstract									
Nakasaki et al[50] (87)	SP263 *v* SP142	TC or IC ≥ 25% (SP263); TC or IC scoring scale (SP142)[i]	—	78	—	—	k = 0.262	—	NR

NOTE. Data are sorted by tumor type and alphabetical order by first author, with studies evaluating lung cancers shown at the top and multiple tumor types shown at the bottom. All other tumor types are shown in alphabetical order.

Abbreviations: ρ, Spearman correlation coefficient; CPS, combined positive score; IC, immune cell; IHC, immunohistochemistry; NPA, negative percentage agreement; NR, not reported; OPA, overall percentage agreement; PD-L1, programmed death ligand 1; PPA, positive percentage agreement; r^2, Pearson correlation coefficient; RCC, renal cell carcinoma; SCCHN, squamous cell carcinoma of the head and neck; TC, tumor cell; UC, urothelial carcinoma.

[a]Assays were based on antibodies from US Food and Drug Administration–approved diagnostics.

[b]Reference test reported in the table if indicated in the corresponding publication. A reference test is defined as a standard test used for comparison with a novel test to determine PPA and NPA.

[c]Most studies reported concordance results in non–small-cell lung cancer only, but a number of studies reported results across heterogeneous types of lung tumors, including small-cell lung cancer and non–small-cell lung cancer, or did not specify the types of lung tumors included.

[d]Scoring scale 0-3: 0 (< 1%), 1 (≥ 1% to < 5%), 2 (≥ 5% to < 10%), 3 (≥ 10%).

[e]US Food and Drug Administration–approved cutoff for the Ventana PD-L1 (SP142) Assay.

[f]CPS is the number of PD-L1–staining cells (TCs, lymphocytes, and macrophages) divided by the total number of viable TCs, multiplied by 100.

[g]US Food and Drug Administration–approved cutoff for the Dako PD-L1 IHC 22C3 pharmDx Assay.

[h]US Food and Drug Administration–approved cutoff for the Ventana PD-L1 (SP263) Assay.

[i]Scoring scale TC0 or IC0 < 1%, TC1 or IC1 ≥ 1% to < 5%, TC2 ≥ 5% to < 50%, IC2 ≥ 5% to < 10%, TC3 ≥ 50%, IC3 ≥ 10%.

assays in SCCHN and strong concordance in B- or T-cell lymphoma and thymic carcinoma.[13,14,44,46]

Multiple tumor types. Strong concordance between 28-8- and 22C3-based assays was observed in two real-world studies evaluating concordance for TC scoring across samples from multiple tumor types.[17,47] In a third study, strong agreement was also seen between 22C3- and SP142-based assays in samples from multiple tumor types, with OPAs of 95%-100%; however, this study was published as a research letter, and information on the assay methodology used was limited.[48]

Part III: Analytical Concordance in Studies Assessing PD-L1 Expression on ICs or Combined ICs and TCs

Assay concordance in studies where evaluation of PD-L1 expression with 28-8-, 22C3-, SP263-, and SP142-based assays included ICs is shown, with algorithm definitions, in Table 4. Only one study reported on the training status of pathologists.

Analytical concordance for assessment of PD-L1 expression on ICs was variable and generally lower than for PD-L1 expression on TCs.

Lung cancer. Most studies reported concordance results in NSCLC only, but a number of studies reported results across heterogeneous types of lung tumors, including small-cell lung cancer and NSCLC, or did not specify the types of lung tumors included. Agreement and concordance for IC scoring was generally high between 28-8- and SP263-based assays[25,37] and between 22C3- and SP263-based assays,[37] although the number of studies where these assays and algorithms were compared was small. There were no studies directly comparing the 28-8- and 22C3-based assays using IC scoring. Generally poor concordance between 22C3- and SP142-based assays for scoring of ICs or combined ICs and TCs was seen in three studies in lung cancer.[20,27,34] In separate studies, analytical concordance between SP142- and SP263-based assays for IC scoring was poor to fair,[25,38] and no studies compared 28-8- and SP142-based assays using IC scoring, aside from the Blueprint studies.

In the Blueprint studies, IC staining was generally comparable between 28-8-, 22C3-, and SP263-based assays.[39,40] Staining with an SP142-based assay was less sensitive than with 28-8-, 22C3-, or SP263-based assays.[39,40] As with concordance analyses in TCs, formal statistics for comparisons between assays were not presented in the publications from the Blueprint studies.

Nonlung tumor types. As was the case for analytical concordance for TC scoring in nonlung tumor types, data were limited for IC scoring in nonlung tumor types. Single studies were identified in most tumor types, with the exception of three studies in UC. Among nonlung tumor studies, only one study in thymic carcinoma and one study in UC reported formal concordance statistics. In thymic carcinoma, strong concordance for IC scoring was seen between 28-8- and 22C3-based assays, whereas poor-to-fair analytical concordance for IC scoring was seen between all other possible combinations of 28-8-, 22C3-, SP263-, and SP142-based assays.[44] In UC, concordance for IC scoring between SP142- and SP263-, 22C3- and SP263-, and 22C3- and SP142-based assays was generally poor to fair, and higher concordance between assays was reported with TC scoring than with IC scoring.[49]

Multiple tumor types. Concordance between SP263- and SP142-based assays for TC or IC scoring was poor in a cohort of patients with various tumor types.[50]

DISCUSSION

This systematic review identified 42 studies that assessed concordance between assays utilizing antibodies from FDA-approved diagnostics across a range of tumor types. Concordance between PD-L1 assays was most frequently evaluated in lung cancer, particularly NSCLC, reflecting the approval of multiple PD-(L)1 inhibitors and associated companion or complementary PD-L1 diagnostic assays across multiple treatment lines for the treatment of advanced lung cancers,[51-53] the relatively early approval of PD-L1 assays in NSCLC compared with other tumor types,[54] and the high incidence of lung cancers compared with other cancers in which PD-(L)1 inhibitors and PD-L1 assays have been approved.[55] The combination of these factors would be expected to lead to greater interest in the analytical concordance between assays in lung cancer, as well as make it the tumor type of choice for concordance studies because of the higher absolute number of cases and widespread use of PD-L1 testing.

The current review was designed to focus on interassay concordance data, but a number of studies identified by the literature search also evaluated interobserver variability and concordance between sample types (eg, resections, core needle or bronchial biopsy samples, tumor-positive lymph node excision biopsy or resection samples, and cytology specimens).[39,40] These studies used a variety of designs and assessment measures to investigate the contribution of these factors to PD-L1 test variability. Concordance between pathologists, centers, and sample types has been examined extensively in previous reviews of the literature.[10,11,56] Interobserver reproducibility is generally good for assessment of PD-L1 expression on TCs but is variable for assessment of PD-L1 expression on ICs because of a range of factors, including assessment of both cytoplasmic and cell membrane staining of ICs and scoring of percentage area staining rather than the percentage of PD-L1–positive cells.[10,11,32] Sample types may also play an important role in concordance between tests, with limited available data suggesting generally good concordance between cytology specimens and tumor tissue, as well as between core biopsy samples and surgical specimens.[10] Studies evaluating concordance between original

diagnostic material and newly acquired tissue and evaluating the effects of intertumoral and intratumoral heterogeneity also suggest that these factors can affect reproducibility of PD-L1 assessment.[10,57] A possible limitation of this review is the absence of the assessment of the impact that these factors may have on concordance results. Variation in assay methodology across the included studies may represent another possible limitation, despite efforts to exclude studies that did not use assay manufacturers' specified materials and methods.

Concordance between 28-8-, 22C3-, and SP263-based assays was generally high for assessment of PD-L1 expression on TCs in tumor types for which one or more assays have been approved. Of note, there is a sizable body of evidence for generally high concordance between assays in lung cancers, reflecting pathologists' level of experience and supporting the potential interchangeability of approved assays in this tumor type. High concordance was also seen for TC and IC scoring in SCCHN and for TC scoring in UC, although more data are needed to allow comprehensive evaluation of analytical concordance in these tumor types. Data from studies in which PD-L1 expression agreement was assessed across multiple cutoffs suggested a trend for higher agreement with increasing cutoff, possibly because of variability in pathologist assessment at low expression levels.[13,15,58] Adequately powered studies are required to confirm this observation. It is important to note that analytical concordance alone is insufficient to guide decisions around assay choice. Clinicians and pathologists should place these results in context with relevant data on assay predictive performance, sensitivity, and specificity, as well as performance around relevant clinical cutoffs, when making decisions for their laboratory and clinical practices and selecting treatment.

The causative factors for the low staining intensity obtained with SP142-based assays compared with other assays remain unclear but are suggested to be the result of differences in assay methodology rather than variation in the epitope binding site targeted by each antibody clone.[59] Agreement between SP142-based assays and other assays was higher for TC scoring in lymphomas and thymic carcinoma and IC scoring in renal cell carcinoma than in other tumor types. However, the study comparing SP142- and SP263-based assays in B- and T-cell lymphomas included only 78 samples,[46] whereas the study in thymic carcinoma included only 53 samples.[44] Similarly, the study with renal cell carcinoma specimens had a small sample size (n = 32), and the reported results were limited to overall agreement at an unknown PD-L1 expression cutoff.[60] The results of these studies should be confirmed in larger studies of concordance between the SP142 assay and other assays.

Assessment of PD-L1 expression on ICs generally showed lower concordance than TC scoring across all assays and tumor types evaluated. Reduced concordance for IC scoring may be related to greater subjectivity when interpreting IC staining compared with TC staining, due to the small size of ICs, ultimately reflected in the high interobserver variability reported.[32,39,40] A relative lack of pathologist experience with IC scoring compared with TC scoring and less methodological standardization of IC scoring may also contribute to reduced concordance.[32,39,40]

Pembrolizumab has been approved for the treatment of PD-L1–expressing gastric cancer, cervical cancer, UC, esophageal squamous cell carcinoma, triple-negative breast cancer, and SCCHN based on assessment with the PD-L1 combined positive score (CPS) algorithm.[6] One study in UC and one study in SCCHN identified in this systematic review assessed interassay concordance using the PD-L1 CPS algorithm, both of which found generally similar concordance to that seen with % TC scoring and/or % IC scoring.[14,61] In those two studies, the training status of the pathologist and, hence, any potential impact on concordance were not reported. Reproducibility of scoring between pathologists appeared to be higher with the CPS algorithm than with the mononuclear IC density score using a 22C3-based assay in UC specimens.[62] Future studies are needed to assess analytical concordance using the CPS algorithm across multiple tumor types and assays.

Although most of the studies included here did not report whether pathologists received specific training, it is reasonable to assume that pathologists participating in the cited studies completed training, since assay-specific training is frequently provided for pathologists.[10] Effective training is an important part of efforts to improve scoring accuracy, which may be reflected in higher levels of concordance seen in lung tumors and for TC-based scoring methods. At present, it is unclear if pathologist training improves interassay concordance, but several studies have found strong concordance between observers in studies where practical training was required.[10,32,63-67] Given the comparatively low concordance for IC scoring seen in this review and the likely introduction of scoring systems that incorporate ICs, such as CPS, in a wider range of tumor types in the future, it is important that effective training is put in place to aid in consistent and accurate interpretation. As well as supporting standardized assay interpretation, training should educate pathologists on how to approach tumor-specific challenges, such as scoring of PD-L1 staining in tumors with heterogeneous morphology.[10] Provision of tumor type–specific training is another important consideration, as pathologists' familiarity with the tissue structures and cell types present in a sample is important for assessment of PD-L1 expression.[10]

Greater uptake of digital pathology might also promote a shift toward centralization of test interpretation by pathologists with subspecialty expertise.[68] Adoption of artificial intelligence–based assessment may also improve the reproducibility of test results by supporting process

harmonization, reducing interobserver and intraobserver variability, and assisting with interpretation and standardization of scoring.[63,68-71] Of note, the uPath PD-L1 (SP263) image analysis algorithm suite received CE-IVD status in Europe in June 2020 for evaluation of PD-L1 expression in NSCLC samples,[72] highlighting the importance of evaluating the potential benefits of these technologies for assisting in interpretation of PD-L1 immunostaining as they enter clinical practice. In line with these points, a possible limitation of this study is the exclusion of studies using digital images. Although a number of studies investigating these technologies were identified during the course of this systematic review, the inclusion criteria restricted the studies evaluated to those investigating "glass slide" pathology only, so as to reflect PD-L1 diagnostic assay approvals at the time the literature search was performed.

In summary, 28-8-, 22C3-, and SP263-based assays show strong analytical concordance for the assessment of PD-L1 expression on TCs in lung cancers and UC. The body of evidence in other tumor types was limited, preventing a conclusion on assay concordance. When placed in context with data for predictive performance, sensitivity, and specificity, the large body of evidence for analytical concordance in lung cancer supports the potential interchangeability of these assays in clinical practice. Care must be taken in tumor types where data for predictive value and/or analytical concordance are limited. As the body of evidence for PD-L1 as a predictor of response to PD-(L)1 inhibitor therapy expands, further studies assessing the comparability and interchangeability of PD-L1 assays with scoring algorithms such as CPS are necessary in additional tumor types.

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AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

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APPENDIX

TABLE A1. Studies Assessing Analytical Concordance of Two or More Assays Utilizing Antibodies From US Food and Drug Administration–Approved PD-L1 Diagnostics

Study	Location	Tumor Type	No.	PD-L1 Assays	Scoring Algorithm
Published articles					
Abdul Karim et al[48]	United States	Multiple	> 175	22C3, SP142	TC or TC/IC
Batenchuk et al[17]	United States	Multiple[a]	1,930	28-8	TC
				22C3	TPS
Conde et al[38]	Spain	Squamous cell lung carcinoma	69	SP142, SP263	TC or IC
De Meulenaere et al[13]	Belgium	SCCHN	99	22C3, SP142	TC
Fujimoto et al[15]	Japan	NSCLC	40	28-8, 22C3, SP142, SP263	TPS
Fujimoto et al[16]	Japan	NSCLC	99	22C3, SP263	TPS
Hirsch et al[39]	Multinational	NSCLC	39	28-8, 22C3, SP263	TPS
				SP142	TC or IC
Ilie et al[25]	France	Lung squamous cell carcinoma	56	28-8, SP142, SP263	TC or IC
Karnik et al[41]	United States	Breast cancer	136	22C3, SP263	TC
Kim et al[27]	South Korea	NSCLC	97	22C3, SP263	TPS
				SP142	TPS with IC[b]
Nakamura et al[21]	Japan	NSCLC	137	28-8, 22C3	TC
Pang et al[36]	China	NSCLC	84	SP142, SP263	TC
Ratcliffe et al[18]	United Kingdom and United States	NSCLC	500	28-8, 22C3, SP263	TC
Saito et al[23]	Japan	NSCLC	420	28-8, 22C3	TC
Sakane et al[44]	Japan	Thymic carcinoma or NET	53	28-8, 22C3, SP142, SP263	TC or IC
Scheel et al[32]	Germany	Lung cancer[c]	30	28-8, 22C3, SP142, SP263	TC
Skov and Skov[74]	Denmark	Lung cancer[c]	86	28-8, 22C3	TPS
Tsao et al[40]	Multinational	Lung cancer[c]	81	28-8, 22C3, SP142, SP263	TPS or IC
Velcheti et al[75]	United States	NSCLC	6,024	28-8, 22C3, SP142	TC
Villaruz et al[31]	United States	NSCLC	302	22C3, SP263	TC
Vranic et al[46]	Bosnia and Herzegovina and United States	B- or T-cell lymphoma	78	SP142, SP263	TC
Watanabe et al[43]	Japan	Pleural mesothelioma	32	28-8, 22C3, SP263	TC
				SP142	TC or IC
Xu et al[34]	China	NSCLC	135	22C3	TPS
				SP142	TC or IC
Zavalishina et al[49]	Russia	UC	100	22C3	TC plus IC or TC
				SP142	IC
				SP263	TC or IC
Congress abstracts					
Beck et al[26]	South Korea	NSCLC	80	22C3, SP263	TC
Cho et al[30]	South Korea	Lung cancer[c]	109	22C3, SP263	TC
Krigsfeld et al[42]	United States	Melanoma	202	28-8, 22C3	TC
Krigsfeld et al[22,45,47]	United States	Multiple[d]	3,113[e]	28-8, 22C3	TC
Lisberg et al[35]	United States	NSCLC	28	22C3, SP142	TC
Motoi et al[20]	Japan	Lung cancer[c]	486	28-8, 22C3, SP263	TC
				SP142	TC or TC plus IC
Nakasaki et al[50]	United States	Multiple	87	SP142, SP263	TC or IC
Quinn et al[28]	United Kingdom	NSCLC	100	22C3, SP263	TPS

(Continued on following page)

TABLE A1. Studies Assessing Analytical Concordance of Two or More Assays Utilizing Antibodies From US Food and Drug Administration–Approved PD-L1 Diagnostics (Continued)

Study	Location	Tumor Type	No.	PD-L1 Assays	Scoring Algorithm
Saito et al[19]	Japan	NSCLC	147	28-8, 22C3	TC
Scott et al[37]	United Kingdom and United States	NSCLC	493	28-8, 22C3	IC
				SP142, SP263	TC or IC
Scott et al[14]	United Kingdom and United States	SCCHN	486	28-8, 22C3, SP142, SP263	TC, IC, CPS, TC or IC
Walker et al[61]	United Kingdom and United States	UC	335	28-8	TC
				22C3	CPS
				SP142	IC
				SP263	TC or IC
Wilberger et al[29]	United States	NSCLC	23	22C3, SP263	TC
Xu et al[33]	China	NSCLC	49	22C3, SP142	TC
Zhang et al[24]	Multinational	NSCLC	45	28-8, SP263	TC
Zhu et al[60]	United States	RCC	32	28-8, SP142	TC or IC
		UC	18	22C3, SP263	TC or IC

Abbreviations: CPS, combined positive score; IC, immune cell; NET, neuroendocrine tumor; NSCLC, non–small-cell lung cancer; PD-L1, programmed death ligand 1; RCC, renal cell carcinoma; SCCHN, squamous cell carcinoma of the head and neck; TC, tumor cell; TPS, tumor proportion score; UC, urothelial carcinoma.

[a]Includes evaluation of lung subgroup.

[b]For SP142-based assays, the area of PD-L1–positive ICs was integrated into the scoring system.

[c]When lung cancer is indicated, studies performed concordance analyses across several types of lung tumors or did not specify the types of lung tumors.

[d]Includes evaluation of lung cancer and UC subgroups.

[e]A total of 1,506 samples from lung cancer and 13 samples from UC were analyzed.[22,45]