Comparison of PD-L1 tumor cell expression with 22C3, 28-8, and SP142 IHC assays across multiple tumor types

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ABSTRACT

Background Multiple PD-L1 immunohistochemistry (IHC) assays, including DAKO 22C3, DAKO 28-8, and Ventana SP142 PD-L1 IHC assays, have been approved by the Food and Drug Administration as a companion diagnostic (CDx) for various antiprogrammed death-1 and antiprogrammed death ligand 1 (PD-L1) based cancer immunotherapies. Here we present 22C3, 28-8, and SP142 analysis of 418 tumor specimens encountered in routine clinical practice.

Methods All specimens were tested with 22C3, 28-8, and SP142 assays following the manufacturer's established staining protocols.

Results The same PD-L1 status (defined as tumor cell expression (TC) scores with all three assays ≥1% or all <1%) was observed in 60.0% (251/418) tumor specimens (45.9% (192/418) were triple negative and 14.1% (59/418) were triple positive). A total of 54.1% (226/418) tumor cases were positive with at least one IHC assay (94.2% (213/226), 77.0% (174/226), and 28.8% (65/226) of these were positive for 22C3, 28-8 and SP142, respectively). Among the 40.0% (167/418) tumor cases that showed a different PD-L1 status, 62.3% (104/167) were 22C3+/28-8+/SP142−, and 28.7% (48/167) were 22C3+/28-8−/SP142−. The same PD-L1 status with all three antibody clones was observed in 48.7% (97/199) of NSCLC cases, and among these, 54.6% (53/97) were triple negative and 45.4% (44/97) triple positive. A total of 73.4% (146/199) NSCLC cases were positive with at least one IHC assay (95.2% (n=139/146), 82.2% (n=120/146), and 32.2% (n=47/146) were positive for 22C3, 28-8, and SP142, respectively). Among the 51.3% (102/199) NSCLC cases that showed a different status among the three IHC assays, 67.6% (69/102) were 22C3+/28-8+/SP142−, and 23.5% (24/102) were 22C3+/28-8−/SP142−. A total of 81.1% (43/53) lung squamous cell carcinoma, 72.1% (88/122) of lung adenocarcinoma, 69.6% (16/23) of non-small cell lung cancer (NSCLC) not otherwise specified (NOS), and 50.0% (4/8) of small cell lung carcinoma cases were positive with at least one IHC assay.

Conclusions Our data suggest that 22C3 is the most sensitive PD-L1 IHC assay for tumor cell expression, followed by 28-8 and in turn by SP-142. These findings represent an additional factor for clinical teams to consider when deciding which PD-L1 IHC assay (and in turn which CDx-associated PD-L1 based immunotherapy) is most appropriate for each individual patient.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Prior studies have shown high concordance between 22C3 and 28-8 programmed death ligand 1 (PD-L1) immunohistochemistry (IHC) assays, with lower tumor cell (TC) expression with the SP142 assay in various controlled and clinical settings, which focused on either a limited number (<100) of tumor specimens, or a larger number (>100) of tumor specimens but of a single tumor type, or were unable to assess all three (22C3, 28-8, and SP142 assays) on each tumor specimen.

WHAT THIS STUDY ADDS

⇒ We present the PD-L1 TC expression scores of 22C3, 28-8, and SP142 IHC assays performed on 418 specimens of multiple tumor types encountered in routine clinical practice. We demonstrate that 22C3 is the most sensitive PD-L1 IHC assay, followed in turn by 28-8 and SP-142.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ The findings represent an additional factor for clinicians to consider when selecting which PD-L1 IHC assay is most appropriate for each individual patient.

INTRODUCTION

Immunotherapy has revolutionized cancer care over the past decade, and new treatment strategies continue to evolve. Programmed death-1/programmed death ligand 1 (PD-1/ PD-L1) inhibitors have been approved in the USA and around the world to treat and improve outcomes for patients with a range of tumor types, including non-small cell lung cancer (NSCLC),[1] melanoma,[2] triple-negative breast cancer,[3 4] and small cell lung cancer.[5 6]

PD-L1 is a transmembrane protein that binds to receptors, PD-1 or B7.1, to downregulate the immune response. PD-1 is an inhibitory receptor predominantly expressed on T cells following T cell activation in response to chronic inflammation, as may occur during infection or within the tumor

microenvironment.[7] Binding of PD-L1 to PD-1 inhibits T cell proliferation, cytokine production, and cytolytic activity, leading to functional inactivation of T cells. PD-L1 expression can occur on the surface of both tumor cells (TCs) and tumor-associated immune cells (ICs). Upregulated PD-L1 expression on TCs can therefore enable the tumor to evade the immune response.[7 8] Blockade of PD-L1/PD-1 ligation has become a strategy to restore tumor-specific T cell immunity, and positive expression of PD-L1 on the surface of TCs or ICs has been correlated with clinical benefit and successful treatment with PD-L1/PD-1 inhibitors across a range of cancer types.[3 8–14]

The PD-1/PD-L1 treatment landscape is continually adapting to new clinical trial and outcomes data and new Food and Drug Administration (FDA) approvals. Alongside the generation of new PD-1/PD-L1 inhibitors, various immunohistochemistry (IHC) assays have been developed to measure PD-L1 expression and have received approval from the FDA as either *companion* or *complementary* diagnostic assays.[15] A companion diagnostic assay (CDx) is defined by the FDA as a diagnostic test that provides required information that is 'essential for the safe and effective use of a corresponding drug or biological product'.[16] In the European Union, a CDx is defined as 'a device which is essential for the safe and effective use of a corresponding medicinal product to (a) identify, before and/or during treatment, patients who are most likely to benefit from the corresponding medicinal product; or (b) identify, before and/or during treatment, patients likely to be at increased risk of serious adverse reactions as a result of treatment with the corresponding medicinal product'.[17] To date, the FDA has approved four CDx PD-L1 IHC assays: Dako 22C3 (hereon referred to as 22C3; as a CDx for treatment with pembrolizumab in patients with a range of solid tumors, see table 1); Ventana SP142 (hereon referred to as SP142; as a CDx for treatment with atezolizumab in patients with urothelial carcinoma or NSCLC); Dako 28-8 (hereon referred to as 28-8; as a CDx for treatment with combination of ipilimumab and nivolumab in patients with NSCLC); and Ventana SP263 (hereon referred to as SP263; as a CDx for treatment with atezolizumab in patients with NSCLC).[15] It should be noted, however, that pembrolizumab is also FDA approved for the treatment of all solid tumors regardless of PD-L1 status if microsatellite instability - high (MSI-H)[18] or tumor mutational burden ≥10 mutations/megabase.[19]

Table 1 Summary of current FDA-approved companion and complementary PD-L1 diagnostic immunohistochemistry assays

US –FDA diagnostic name	Manufacturer	Tumor type	IHC scoring system	Cut-off score	Treatment	Diagnostic type	Year of FDA approval
PD-L1 IHC 22C3 pharmDx	Dako North America, Inc	Non-small cell lung cancer (NSCLC)	TPS	≥1 (≥50=high expression)	Pembrolizumab, cemiplimab-rwlc	Companion	2015
		Cervical cancer	CPS	≥1	Pembrolizumab	Companion	2018
		TNBC	CPS	≥10	Pembrolizumab	Companion	2020
		Head and neck squamous cell carcinoma (HNSCC)	CPS	≥1	Pembrolizumab	Companion	2019
		Esophageal squamous cell carcinoma	CPS	≥10	Pembrolizumab	Companion	2019
Ventana PD-L1 (SP142) Assay	Ventana Medical Systems, Inc.	Urothelial carcinoma	IC	IC ≥5	Atezolizumab	Companion	2018
		NSCLC	TC and IC	TC ≥50 or IC ≥10	Atezolizumab	Companion	2016
PD-L1 IHC 28-8 pharmDX	Dako North America, Inc	NSCLC	TC	≥ 1	Nivolumab in combination with ipilimumab	Companion	2016
		Non-squamous NSCLC	TC	≥1, ≥5, or ≥50	Nivolumab	Complementary	2015
		HNSCC	TC	≥1	Nivolumab	Complementary	2018
		Urothelial carcinoma	TC	≥1	Nivolumab	Complementary	2018
		Melanoma	TC	≥1	Nivolumab in combination with ipilimumab	Complementary	2016
Ventana PD-L1 (SP263) Assay	Ventana Medical Systems, Inc	NSCLC	TC	≥1	Atezolizumab	Companion	2021
		UC	TC and IC	TC ≥25; or ICP ≥1 and IC+ ≥25; or ICP=1 and IC+=100%	Durvalumab	Complementary	2017

Please check FDA website for most current indications.[15] ICP = per cent of tumor area occupied by any tumor-associated immune cells. ICP is used to calculate IC+, which is the per cent area of ICP exhibiting PD-L1 positive immune cell staining.
CPS, combined positive score; FDA, Food and Drug Administration; IC, immune cell; IHC, immunohistochemistry; NSCLC, non-small cell lung cancer; PD-L1, programmed death ligand 1; TC, tumor cell; TNBC, triple negative breast cancer; UC, urothelial carcinoma.

A complementary diagnostic test is broadly defined as 'a test that identifies a biomarker-defined subset of patients that respond particularly well to a drug and aid risk/benefit assessments for individual patients, but that are not prerequisites for receiving the drug'.[16] In the USA, the currently approved complementary diagnostic PD-L1 assays include the 28-8 assay for identifying patients with non-squamous NSCLC, head and neck squamous cell carcinoma (HNSCC) and urothelial carcinoma for treatment with nivolumab and the SP263 assay for identifying patients with urothelial carcinoma for treatment with durvalumab. Treatment with PD-1/PD-L1 inhibitors is indicated when the FDA-approved companion or complementary diagnostic assay demonstrates PD-L1 expression in the patient's tumor above a specified cut-off. Table 1 lists the cut-offs and various other features of the four main PD-L1 assays available in the USA.[15]

Prior studies have investigated the concordance between two or more PD-L1 IHC assays in controlled[20–27] and clinical settings.[28–32] The consensus of these reports is that there is high concordance in TC expression scores between 22C3 and 28-8 IHC assays[22–25 27 28 32] and much lower TC expression with the SP142 assay.[23 24 26 27 32] However, several of these studies focused on a limited number (<100) of tumor specimens,[21 22 24 31] while others that evaluated a larger number (>100) of specimens were unable to assess all three IHC assays (22C3, 28-8, and SP142)[25 26 28] or analyze more than a single tumor type.[23 27] In the current study, we analyzed 418 specimens from multiple tumor types (including 199 NSCLC specimens) with three PD-L1 IHC assays (22C3, 28-8, and SP142) during routine clinical practice. These specimens included a range of resection, core needle biopsy, and cytology patient samples. We present the PD-L1 TC expression scores for each specimen, as well as the histology and IHC staining patterns for select clinical cases to showcase important implications for patient treatment and clinical decision making.

MATERIALS AND METHODS
Patient cohort
The Foundation Medicine database (Morrisville, North Carolina, USA) was searched to collect advanced solid tumor specimens that were tested with all three PD-L1 IHC assays (DAKO 22C3, DAKO 28-8, and Ventana SP142) during the year starting in December 2020 and ending in December 2021. A total of 418 patient specimens (including 199 patient NSCLC specimens) were identified that fulfilled these criteria. Of these 418 specimens, 55.3% (231/418) were biopsies, 41.6% (174/418) were resection specimens, and 3.1% (13/418) were cytology specimens.

DAKO PD-L1 IHC 22C3 pharmDx assay
The DAKO PD-L1 IHC 22C3 pharmDx assay was performed per manufacturer's instructions in a Clinical Laboratory Improvement Amendments (CLIA)-certified and College of American Pathologists (CAP)-accredited reference laboratory (Foundation Medicine). The DAKO PD-L1 22C3 pharmDx assay is an immunohistochemical assay using mouse monoclonal anti-PD-L1 (22C3 clone) for use in the detection of PD-L1 protein in formalin-fixed, paraffin-embedded (FFPE) tissue using the EnVision FLEX visualization system on Autostainer Link 48 and associated staining protocol provided by the package insert and interpreted with the guidelines of the DAKO interpretation guide. All cases had accompanying controls, H&E-stained patient slide, negative reagent control stained patient slide, and a DAKO PD-L1 22C3-stained patient slide. PD-L1 stained slides were evaluated using a tumor proportion score (TPS) method, where TPS=(number of PD-L1-stained tumor cells/total number of viable tumor cells) × 100. A cut-off of TPS ≥1 was used to determine positivity for the 22C3 assay. It should be noted here that the terms TPS and TC expression (see below for 28-8) are often used interchangeably and refer to essentially the same scoring system. Of note, positivity in NSCLC was determined using a cut-off of TPS ≥1 per CDx for pembrolizumab.

DAKO PD-L1 28-8 pharmDx assays
The DAKO PD-L1 28-8 pharmDx assay was performed per manufacturer's instructions in a CLIA-certified and CAP-accredited reference laboratory (Foundation Medicine). In brief, the DAKO PD-L1 28-8 pharmDx assay is an immunohistochemical assay using rabbit monoclonal anti-PD-L1, for use in the detection of PD-L1 protein in FFPE tissue with the EnVision FLEX visualization system on Autostainer Link 48, and associated staining protocol provided by the package insert and interpreted with the guidelines of the DAKO interpretation guide. All cases had accompanying controls, H&E-stained patient slide, negative reagent control-stained patient slide, and a DAKO PD-L1 28-8-stained patient slide. PD-L1 stained slides were evaluated using a tumor cell expression (TC) scoring method, where TC=(number of PD-L1-stained tumor cells/total number of viable tumor cells) × 100. A cut-off of TC ≥1% was used to determine positivity for the DAKO PD-L1 28-8 pharmDx assay. Of note, positivity in NSCLC was determined using a cut-off per CDx for nivolumab in combination with ipilimumab using a TC cut-off score of 1.

Ventana PD-L1 SP142 CDx assay
PD-L1 SP142 testing was performed using the Ventana SP142 CDx assay per manufacturer's instructions in a CLIA-certified and CAP-accredited reference laboratory (Foundation Medicine). In brief, the Ventana SP142 CDx assay consists of the rabbit monoclonal anti-PD-L1 SP142 clone, the Opti-View DAB IHC detection kit, and the Opti-View Amplification Kit stained on the Ventana BenchMark ULTRA instrument using the staining protocol provided by the package insert and interpreted with the guidelines of the Ventana interpretation guide. All cases had an accompanying H&E-stained patient

Table 2 Summary of patient cohort demographics

Tumor type	Number of cases (n)	Mean age (years)	Median age (years)	Female patients (%)	Specimen site (% samples in each tumor type)		
					Primary site	Metastatic	Ambiguous or unknown
NSCLC	199	69.7	69.0	41.7 (n=83)	52.8 (n=105)	30.7 (n=61)	16.6 (n=33)
Gastrointestinal	70	65.7	65.5	55.7 (n=39)	28.6 (n=20)	54.3 (n=38)	17.1 (n=12)
Gynecological	37	64.1	67.0	100 (n=37)	27.0 (n=11)	32.4 (n=12)	37.8 (n=14)
Genitourinary	19	72.3	72.0	0 (n=0)	52.7 (n=10)	26.3 (n=5)	21.1 (n=4)
Neuroendocrine tumor	17	68.7	70.0	41.2 (n=7)	17.6 (n=3)	58.8 (n=12)	11.8 (n=2)
Head and neck tumor	7	57.6	62.0	71.4 (n=5)	0 (n=0)	57.1 (n=4)	42.9 (n=3)
Melanoma	16	70.6	73.0	31.3 (n=5)	50 (n=8)	37.5 (n=6)	12.5 (n=2)
Soft tissue	15	59.4	63.0	46.7 (n=7)	60.0 (n=9)	13.3 (n=2)	26.7 (n=4)
Central nervous system	10	51.3	53.5	50.0 (n=5)	90.0 (n=9)	0 (n=0)	10.0 (n=1)
Unknown primary malignant neoplasm	28	63.2	69.0	46.4 (n=13)	N/A	N/A	N/A
Total	418	67.3	68.5	48.1 (n=201)	39.0 (n=163)	32.8 (n=137)	28.2 (n=118)

NSCLC, non-small cell lung cancer.

slide, negative regent control stained patient slide with an on-slide tonsil control and a Ventana PD-L1 SP142 stained patient slide with an on-slide tonsil control. TC levels were determined similarly to the TPS method. A cut-off level of ≥1% staining TCs was used to determine tumor cell expression. (As part of routine clinical practice, the tumor infiltrating ICs scoring method was also used to generate an IC score, which is defined as proportion of tumor area that is occupied by PD-L1 staining IC of any intensity and is not reported in this study).

Data analysis

Comparison of all continuous variables were performed using Kruskal-Wallis test using the R software V.4.0.3.

RESULTS
Patient cohort demographics

A detailed summary of patient cohort demographics is provided in table 2. The mean and median ages of the *entire* patient cohort were 67.3 and 68.5 years old, respectively. A total of 48.1% (201/418 cases) of the patients were female. A total of 39.0% (163/418) specimens received were from a primary tumor site, 32.8% (137/418) specimens from metastatic site, and in the remaining 28.2% (118/418) of specimens the tumor site was ambiguous or unknown.

The mean and median ages of the *NSCLC* patient cohort were 69.7 and 69.0 years old, respectively. A total of 41.7% (83/199 cases) of the patients were female. A total of 52.8% (105/199) NSCLC specimens were from the primary tumor site (lung), 30.7% (61/199) specimens from a metastatic site, and 16.6% (33/199) from a specimen site that was either ambiguous or unknown.

In addition, NSCLC specimens were received from patients with stage IV (61.3%, 122/199), stage III (16.6%, 33/199), stage II (5.0%, 10/199), and stage I disease (8.0%, 16/199); the remaining specimens were received

from patients with unknown stage of disease (9.0%, 18/199). The disease diagnoses for NSCLC specimens included lung adenocarcinoma (61.3%, 122/199 cases), lung squamous cell carcinoma (26.6%, 53/199 cases), NSCLC (not otherwise specified) (11.6%, 23/199 cases), and adenosquamous carcinoma (0.5%, 1/199 cases). In addition, the total tumor cohort included eight small cell lung cancer (SCLC) specimens (8/418 total tumor cases, or 1.9% of all tumor cases).

Table 3 PD-L1 tumor cell expression status with 22C3, 28-8, and SP142 IHC assays in different types of tumor specimen

Category	Resection specimens	Biopsy specimens	Cytology specimens
22C3+/28-8+/SP142+	12.1% (21/174)	15.6% (36/231)	15.4% (2/13)
22C3-/28-8-/SP142-	53.4% (93/174)	39.8% (92/231)	53.8% (7/13)
22C3+/28-8+/SP142-	24.1% (42/174)	26.4% (61/231)	7.7% (1/13)
22C3+/28-8-/SP142-	6.9% (12/174)	14.7% (34/231)	15.4% (2/13)
22C3-/28-8+/SP142-	1.7% (3/174)	2.2% (5/231)	7.7% (1/13)
22C3-/28-8+/SP142+	0.6% (1/174)	0.4% (1/231)	0.0% (0/13)
22C3-/28-8-/SP142+	0.6% (1/174)	0.4% (1/231)	0.0% (0/13)
22C3+/28-8-/SP142+	0.6% (1/174)	0.4% (1/231)	0.0% (0/13)

Positive status of each PD-L1 IHC test is here defined as tumor cell expression (TC) ≥1%; negative status TC <1%.
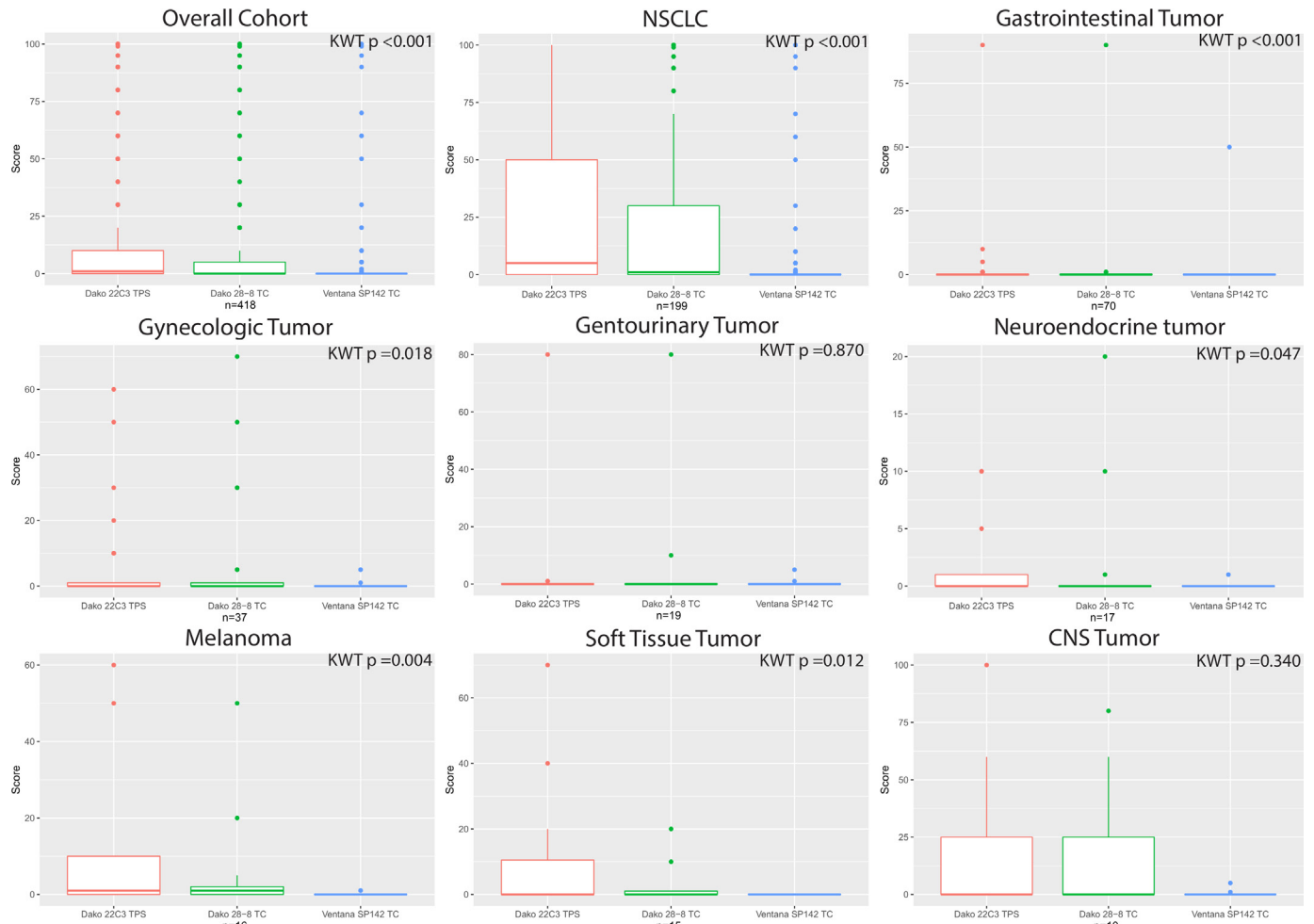IHC, immunohistochemistry; PD-L1, programmed death ligand 1.

Figure 1 Tumor cell PD-L1 expression detected with 22C3, 28-8, and SP142 IHC assays across all tumor types. Higher positive tumor cell staining was observed with 22C3 compared with 28-8 and SP142 in NSCLC, neuroendocrine tumor, melanoma, soft tissue, and overall tumor specimens. There were no clear differences in positive tumor cell staining between the three different antibody clones for GYN, genitourinary, GI, or CNS tumor specimens. IHC, immunohistochemistry; NSCLC, non-small cell lung cancer; PD-L1, programmed death ligand 1.

Overall PD-L1 tumor cell expression patterns

Table 3 shows the PD-L1 status with all three antibody clones in resection, biopsy and cytology specimens. No appreciable difference in tumor cell expression was observed between specimen types. A higher percentage of triple negative (22C3−/28-8−/SP142−) cases are observed in cytology specimens, but this corresponds to only 7 out of 13 total cytology specimens. The proportion of resection, biopsy and cytology specimens with an IC score of ≥1 were 62.6% (65/174), 46.3% (107/231), and 30.8% (4/13), respectively, and the proportion of resection, biopsy and cytology specimens with an IC score <1 were 37.4% (65/174), 53.2% (123/231), and 69.2% (9/13), respectively.

Tumor cell PD-L1 expression as detected with 22C3, 28-8 and SP142 IHC assays across all tumor types are shown in figure 1. Higher positive tumor cell staining was observed with 22C3 compared with 28-8 and SP142 in NSCLC, neuroendocrine tumor, melanoma, soft tissue, and overall tumor specimens. There were no clear or significant differences in PD-L1 tumor cell expression

status between the three different antibody clones for GYN, genitourinary, GI, or CNS tumor specimens.

Figure 2A,B provides an analytical comparison of tumor cell expression staining, by case, for 22C3 (dark blue triangles), 28-8 (light green circles), and SP142 (yellow squares) PD-L1 IHC assays across all tumor cases (n=418; figure 2A) and all NSCLC cases (n=199; figure 2B). The 'best fit' curves show that for all tumor cases (and NSCLC cases) that were positive with all three IHC assays, the average 22C3 TC score was typically 10–20 percentage points higher than 28-8 scores, which were in turn approximately 40–60 percentage points higher than SP142 scores.

The analytical concordance between the three antibody clones across all tumor specimens (n=418) is shown in figure 3A. The same PDL-1 status with all three antibody clones (either TC ≥1% with all three assays or TC <1% with all three assays) was observed in 60.0% (251/418) of all tumor specimens: 45.9% (192/418) of all tumor cases were triple negative and 14.1% (59/418) of all tumor cases were triple positive. A different PD-L1 status
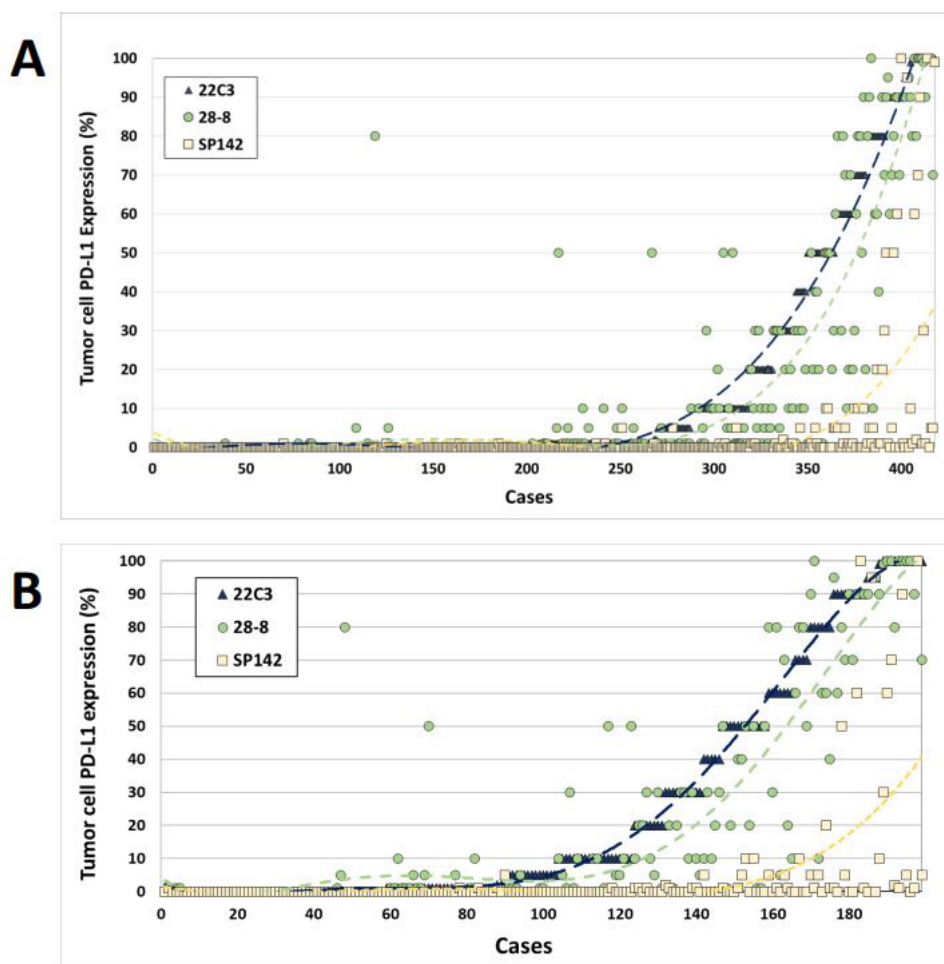
Figure 2 (A) PD-L1 tumor cell expression scores with 22C3, 28-8, and SP142 PD-L1 IHC assays in all tumor specimens (n=418). (B) PD-L1 tumor cell expression scores with 22C3, 28-8, and SP142 PD-L1 IHC assays in all NSCLC specimens (n=199). Analytical comparison of percentage tumor cell expression staining, by case, for 22C3 (dark blue triangles), 28-8 (light green circles), and SP142 (yellow squares) PD-L1 IHC assays. Superimposed points indicate identical values. 'Best fit' colored curves allow comparison of score range between the three assays. IHC, immunohistochemistry; NSCLC, non-small cell lung cancer; PD-L1, programmed death ligand 1.

was observed with the three antibody clones in 40.0% (167/418) of all tumor specimens; and among these cases, 62.3% (104/167) were 22C3+/28-8+/SP142−, and 28.7% (48/167) were 22C3+/28-8−/SP142−. A total of 54.1% (226/418) of all tumor cases were positive with at least 1 IHC assay (22C3, 28-8 or SP142) and among these cases, 94.2% (213/226) were positive for 22C3, 77.0% (174/226) were positive for 28-8, and 28.8% (65/226) were positive for SP142.

Figure 3B shows the positive and negative PD-L1 status with all three antibody clones for NSCLC specimens (n=199). The same PD-L1 status with all three antibody clones (either all assays demonstrating TC ≥1% or all demonstrating TC<1%) was observed in 48.7% (97/199) of all NSCLC specimens. Of these NSCLC specimens showing the same PD-L1 status with all three antibody clones, 54.6% (53/97) were triple negative, and 45.4% (44/97) were triple positive. A different PD-L1 status was observed among all three antibody clones in 51.3% (102/199) of all NSCLC specimens. Among these NSCLC cases showing a different status, 67.6% (69/102) of cases

were 22C3+/28-8+/SP142−, and 23.5% (24/102) were 22C3+/28-8−/SP142−. A total of 73.4% (146/199) of all NSCLC cases were positive with at least one IHC assay (22C3, 28-8, or SP142), compared with 54.1% (226/418) of tumor specimens overall. Among these cases that were positive with at least one IHC assay, 22C3 was positive in 95.2% (139/146) of cases, 82.2% (120/146) were positive for 28-8, and 32.2% (47/146) were positive for SP142.

Table 4 shows the positive and negative PD-L1 status with all three antibody clones for different types of lung cancer specimens. The PD-L1 status was the same (≥ 1% TC expression with all three clones, or <1% TC expression with all three clones) with all three antibody clones in 50.8% (62/122) of all lung adenocarcinoma specimens, 32.1% (17/53) of all lung squamous cell carcinoma specimens, 69.6% (16/23) of all NSCLC (NOS) specimens, and 62.5% (5/8) of all small cell lung carcinoma specimens. A total of 72.1% (88/122) of adenocarcinoma cases were positive with at least one antibody clone, compared with 81.1% (43/53) of all squamous cell carcinoma specimens, 69.6% (16/23) of all NSCLC (NOS) specimens,
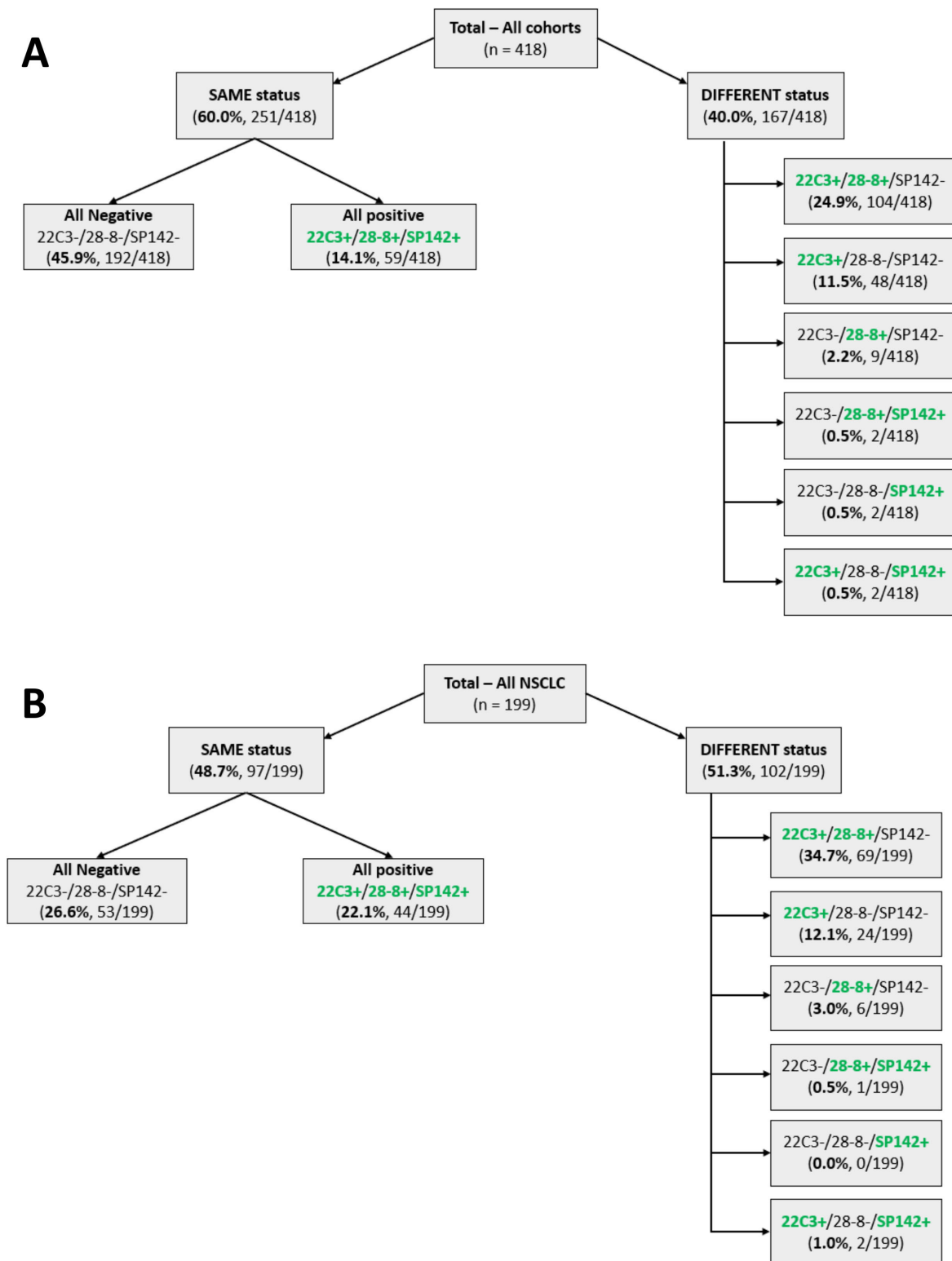
Figure 3 (A) Analytical concordance in status of PD-L1 result with 22C3, 28-8, and SP142 IHC assays, in all tumor specimens (n=418). Most cases (60.0% of all tumor specimens, n=251) showed the same PDL-1 status, and the remainder (40.0% of all tumor specimens, n=167) showed different status between the three antibody clones. Among cases that showed the same status, most were negative for all antibody clones (45.0%, n=192), and the remainder were all positive (14.1%, n=59). Among cases that showed a different status, most were either 22C3+/28-8+/SP142– (24.9%, n=104) or 22C3+/28-8–/SP142– (11.5%, n=48). (B) Analytical concordance in status of PD-L1 result with 22C3, 28-8, and SP142 IHC assays, in NSCLC tumor specimens. A percentage of 51.3 (102/199) of all NSCLC specimens showed a different PDL1 status between the three antibody clones, while 48.7% (97/199) showed the same status. Among NSCLC cases that showed a different status, most were either 22C3+/28-8+/SP142– (34.7%, n=69) or 22C3+/28-8–/SP142– (12.1%, n=24). IHC, immunohistochemistry; NSCLC, non-small cell lung cancer; PD-L1, programmed death ligand 1.

Table 4 PD-L1 tumor cell expression status with 22C3, 28-8, and SP142 IHC assays in different types of lung cancer

Category	Adenocarcinoma (n=122)	Squamous cell carcinoma (n=53)	NSCLC (NOS) (n=23)	Small cell carcinoma (n=8)
22C3+/28-8+/SP142+	23.0% (28/122)	13.2% (7/53)	39.1% (9/23)	12.5% (1/8)
22C3−/28-8−/SP142−	38.5% (34/122)	18.9% (10/53)	30.4% (7/23)	50.0% (4/8)
22C3+/28-8+/SP142−	33.6% (41/122)	45.3% (24/53)	13.0% (3/23)	25.0% (2/8)
22C3+/28-8−/SP142−	12.3% (15/122)	15.1% (8/53)	13.0% (3/23)	12.5% (1/8)
22C3−/28-8+/SP142−	2.5% (3/122)	5.7% (3/53)	0.0% (0/23)	0.0% (0/8)
22C3−/28-8+/SP142+	0.8% (1/122)	0% (0/53)	0.0% (0/23)	0.0% (0/8)
22C3−/28-8−/SP142+	0.0% (0/122)	0% (0/53)	0.0% (0/23)	0.0% (0/8)
22C3+/28-8−/SP142+	0.0% (0/122)	1.9% (1/53)	4.3% (1/23)	0.0% (0/8)

Positive status of each PD-L1 IHC test is here defined as tumor cell expression (TC) ≥1%; negative status TC <1%.
IHC, immunohistochemistry; NSCLC, non-small cell lung cancer; PD-L1, programmed death ligand 1.

Table 5 Comparison of PD-L1 tumor cell expression status in primary versus metastatic non-small cell lung cancer

Category	Primary site (n=106)	Metastatic site (n=69)	Undetermined site (n=24)
22C3+/28-8+/SP142+	20.8% (22/106)	20.3% (14/69)	33.3% (8/24)
22C3−/28-8−/SP142−	21.7% (23/106)	34.8% (24/69)	16.7% (4/24)
22C3+/28-8+/SP142−	37.7% (40/106)	27.5% (19/69)	41.7% (10/24)
22C3+/28-8−/SP142−	17.0% (18/106)	11.6% (8/69)	0.0% (0/24)
22C3−/28-8+/SP142−	1.9% (2/106)	2.9% (2/69)	8.3% (2/24)
22C3−/28-8+/SP142+	0.0% (0/106)	1.4% (1/69)	0.0% (0/24)
22C3−/28-8−/SP142+	0.0% (0/106)	0.0% (0/69)	0.0% (0/24)
22C3+/28-8−/SP142+	0.0% (0/106)	1.4% (1/69)	0.0% (0/24)

Positive status of each PD-L1 IHC test is here defined as tumor cell expression (TC) ≥1%; negative status TC <1%.
IHC, immunohistochemistry; PD-L1, programmed death ligand 1.

and 50.0% (4/8) of all small cell lung carcinoma specimens. Among lung adenocarcinoma specimens that were positive with at least one antibody clone: 95.5% (84/88) were positive for 22C3, 83.0% (73/88) were positive for 28-8, and 23.8% (29/122) were positive for SP142. Among squamous cell carcinoma specimens that were positive with at least one antibody clone: 93.0% (40/43) were positive for 22C3, 79.1% (34/43) were positive for 28-8, and 16.3% (7/43) were positive for SP142. Among NSCLC (NOS) specimens that were positive with at least one antibody clone: 100.0% (16/16) were positive for 22C3, 75.0% (12/16) were positive for 28-8, and 62.5% (10/23) were positive for SP142. Finally, among small cell lung carcinoma specimens that were positive with at least one antibody clone: 100.0% (4/4) were positive for 22C3, 75.0% (3/4) were positive for 28-8, and 25.0% (1/4) were positive for SP142.

Table 5 shows the positive and negative PD-L1 status with all three antibody clones for primary versus metastatic NSCLC specimens. The PD-L1 status was the same (either ≥1% TC expression with all three clones, or <1% TC expression with all three clones) with all three antibody clones in 42.5% (45/106) of all primary NSCLC specimens, 50.7% (35/69) of all metastatic NSCLC specimens, and 50.0% (12/24) of all NSCLC specimens with undetermined specimen location. It was observed that 78.3% (83/106) primary NSCLC specimens were positive with at least one antibody clone, compared with 65.2% (45/69) of metastatic NSCLC specimens, and 83.3% (20/24) of all NSCLC specimens with undetermined specimen location. Among primary NSCLC specimens that were positive with at least one antibody clone: 97.6%

(81/83) were positive for 22C3, 78.3% (65/83) were positive for 28-8, and 27.7% (23/83) were positive for SP142. Among metastatic NSCLC specimens that were positive with at least one antibody clone: 93.3% (42/45) were positive for 22C3, 80.0% (36/45) were positive for 28-8, and 31.1% (14/45) were positive for SP142. Among NSCLC cases with undetermined specimen location that were positive with at least one antibody clone: 91.7% (22/24) were positive for 22C3, 100.0% (24/24) were positive for 28-8, and 50.0% (12/24) were positive for SP142.

Clinical implication with clinical vignettes

The histology and PD-L1 tumor cell expression pattern with each of the three antibody clones (22C3, 28-8 and SP142) for three separate NSCLC patient specimens are shown in figure 4. These three cases are representative of most tumor specimens observed with a positive PD-L1 status, in that TC score is greatest with 22C3, followed by 28-8, followed by SP142 having the lowest TC score. A score of TC ≥1 was reported with 22C3 for all three specimens, qualifying all three patients for treatment with pembrolizumab or cemiplimab-rwlc. Furthermore, a score of TC ≥1 with 28-8 was reported for all three specimens, qualifying all three patients for treatment with nivolumab or nivolumab in combination with ipilimumab. While specimens from patient 1 and patient 3 both exhibited SP142 scores of TC ≥1 (and are therefore considered having a positive PD-L1 status for purposes of this study), the scores of TC 5 for each of these specimens did not reach the NSCLC SP142 CDx threshold of TC ≥50 to qualify for treatment with atezolizumab. However, in should be noted that patient 3 also had an IC ≥10 (not reported here), which reached the NSCLC SP142 CDx threshold of IC ≥10 for treatment with atezolizumab.

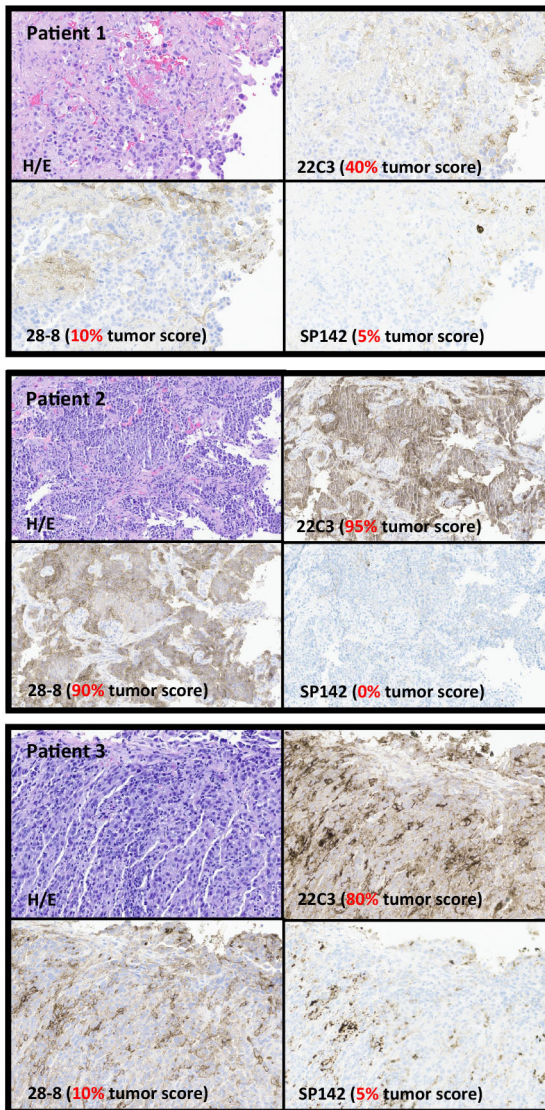**Figure 4** PD-L1 IHC (22C3, 28-8 and SP142) in NSCLC patient specimens. The H&E histology and PD-L1 IHC (22C3, 28-8 and SP142) is shown for three separate NSCLC specimens (patients 1–3). All three specimens received a score of PD-L1 tumor expression score (TC) of TC >1 with 22C3 and 28-8, qualifying each patient for treatment with pembolizumab (or cemiplimab-rwlc) and nivolumub (or nivolumbab in combination with ipilimumab), respectively. The SP142 score of TC 5 for specimens from patients 1 and 3 was a positive PD-L1 status for purposes of this study but was <50 and therefore did not qualify for treatment with atezolizumab based on TC alone. It should be noted that patient 3 also received an SP142 immune cell (IC) score of 10 (not shown), qualifying them for CDx-associated treatment atezolizumab. IHC, immunohistochemistry; NSCLC, non-small cell lung cancer; PD-L1, PD-L1; TC, tumor cell.

DISCUSSION

This report represents one of the largest studies to date of tumor specimens analyzed with multiple PD-L1 IHC assays in routine clinical practice. The result of each FDA-approved PD-L1 IHC CDx assay is an indication for a specific cancer treatment (table 1). Some tumor types, such as NSCLC, have multiple associated PD-L1 IHC CDx assays, each specifically linked to a different cancer treatment. For example, there are four PD-L1 IHC CDx assays for NSCLC, including 22C3 (linked to pembrolizumab or cemiplimab-rwlc treatment), 28-8 (linked to nivolumab in combination with ipilimumab), SP142 and SP263 (both linked to atezolizumab treatment). Several factors might be considered when selecting a PD-L1 IHC assay for each individual patient, including which IHC assay has a CDx indication for the patient's specific tumor type, which CDx-associated cancer treatment is preferred by the clinician, which immunotherapy has the least side effects, as well as the current clinical status of the patient. The findings presented here, including the sensitivity of various PD-L1 IHC assays across a range of tumor types, represent an additional factor to consider when selecting which PD-L1 IHC assay is most appropriate for each individual patient. It should also be noted that, while PD-L1 assays can help to establish the eligibility of patients for immune checkpoint inhibitors, additional biomarkers such as tumor mutational burden (TMB) can be useful to anticipate the efficacy and durability of benefit of these drugs, in an additive and independent manner across many tumor types.[33–35]

Selecting the most appropriate PD-L1 assay to run for a particular patient, especially in cases of tissue specimen scarcity, is important. For example, an NSCLC case with a positive 22C3 result (tumor cell expression, or TC ≥1) and negative 28-8 result (TC <1), would lead to an FDA-approved indication for treatment with pembrolizumab or cemiplimab-rwlc, but not with nivolumab in combination with ipilimumab (the CDx-associated treatment for 28-8). In this scenario, if the clinician had ordered a 28-8 assay only, without 22C3 (eg, due to limited tissue), a single negative 28-8 PD-L1 score would likely be reported, and the patient would not be eligible for any specific PD-L1 immunotherapy. In this study of 418 tumor specimens tested with 22C3, 28-8 and SP142 assays, the same PD-L1 status (either all three assays scored with TC <1, or all scored with TC ≥1) was observed in 60.0% (251/418) of all tumor specimens, and 48.7% (97/199) of all NSCLC specimens. In these cases that received the same PD-L1 status with all three IHC assays, if one IHC assay was ordered instead of all three, the final 'positive' PD-L1 status reported for that patient would not have been altered (although the patient would only be eligible for the CDx indicated treatment associated with the single IHC assay that was ordered). However, in a significant proportion of cases (ie, 40.0% (167/418) of all tumor specimens, and 51.3% (102/199) of all NSCLC specimens), the PD-L1 status was different among the three assays. In these cases that received a different PD-L1 status, if one IHC assay was ordered instead of all three, the final PD-L1 status reported for that patient may have been altered if only one IHC assay was ordered instead of all three assays. This raises the question, which PD-L1 IHC assay should the patient's clinical team choose, especially if they have no CDx treatment preference, or if the sample size is not sufficient for multiple PD-L1 IHC assays?

In this study, our data suggest that 22C3 is the most sensitive IHC CDx assay for tumor cell PD-L1 expression and identifies a positive PD-L1 status more frequently than 28-8, which in turn is positive more often than SP142. These findings are consistent with previous reports, such as Blueprint phase 1 and phase 2 studies,[21 31] as well as analyses of PD-L1 IHC assays in cell lines and lung cancer specimens,[20 31 36] all of which reported greater sensitivity with 22C3 and 28-8 assays and consistently lower TC staining with the Ventana SP142 assay. In this study, the 22C3, 28-8 and SP142 assays were positive (TC ≥1) in 94.2% (213/226), 77.0% (174/226), and 28.8% (65/226), respectively, of all tumor cases that were positive with at least one IHC assay (ie, 54.1% (226/418) of all tumor cases). Similarly, among the 73.4% (146/199) NSCLC specimens that were positive with at least one IHC assay, 22C3 was positive in 95.2% (139/146), 28-8 was positive in 82.2% (120/146), and SP142 was positive in 32.2% (47/146) of cases. While the Blueprint phase 2 study (which evaluated 39 NSCLC tumor cases), concluded that 22C3 and 28-8 assays were highly comparable,[21] the current study suggests that the 22C3 assay returns a positive PD-L1 status to a slightly greater extent than 28-8 in routine clinical practice. Furthermore, as shown in figure 2A,B, in tumor cases that are positive with all three assays, the average 22C3 score is consistently 10–20 percentage points higher than 28-8. This conclusion is further supported by our findings that among tumor specimens that showed a different PD-L1 status between the three IHC assays, 22C3 was positive in most cases: among all tumor specimens with a different PD-L1 status, 62.3% (104/167) were 22C3+/28-8+/SP142−, and 28.7% (48/167) were 22C3+/28-8−/SP142−, with only 7.8% (13/167) reported as negative for 22C3. Similarly, among all NSCLC cases with a different PD-L1 status, 67.6% (69/102 cases) were 22C3+/28-8+/SP142−, 23.5% (24/102 cases) were 22C3+/28-8−/SP142−, with only 6.9% (7/102) reported as negative for 22C3. While 22C3 may not be the optimal PD-L1 IHC choice for every tumor type or every clinical scenario (eg, some clinicians may prefer to treat with atezolizumab in certain situations, for which 22C3 is not a CDx assay), our data indicate that a positive tumor cell PD-L1 status is more often reported with the 22C3 assay compared with other IHC assays. In reference to the current study, it is important to point out that the Ventana SP142 assay scoring system also includes an IC score, which is not included in the 22C3 and 28-8 assays (see table 1). For example, an SP142 IC score of ≥5 and ≥10 are indications for treatment of urothelial carcinoma and NSCLC, respectively (both with atezolizumab), regardless of SP142 TC score. Therefore, while an SP142 case would be regarded as negative for the purposes of our study if TC <1, there remains a possibility that the IC score may have been at or above the CDx threshold to be regarded as having a 'positive PD-L1 status'.

Many explanations have been postulated to explain different staining characteristics among the various

PD-L1 IHC assays available today. Some reports suggest that differences in the number, size, and accessibility of specific epitopes recognized by the various PD-L1 antibodies (22C3, 28-8 and SP142) could be a factor.[37–40] PD-L1 is a 290-amino acid transmembrane glycoprotein, with two extracellular immunoglobulin (Ig) domains and a 31-amino acid cytoplasmic domain.[41 42] The greater sensitivity of 22C3 and 28-8 IHC assays (compared with SP142) might be explained by the findings that DAKO 22C3 and DAKO 28-8 antibodies each bind *multiple* epitopes in the extracellular domain of PD-L1 (although at different sites),[43] while the Ventana SP142 antibody binds to a *single* 7-amino acid stretch ($_{284}$DTHLEET$_{290}$) epitope in the cytoplasmic domain[44–46] and that therefore the 22C3 and 28-8 antibodies have more binding 'targets' per PD-L1 molecule with which to bind. Furthermore, the 22C3 and 28-8 antibodies recognize *larger* epitopes that are possibly *more accessible* to antibodies during the IHC assay. The epitope recognized by 22C3 spans 31 amino acids and lies predominantly in extracellular residues 166–190, while the main epitopes recognized by 28-8 lie within extracellular residues 86–93, 125–145, and 205–223.[43] However, it is also important to point out that other factors could contribute to the different sensitivities and staining characteristics among the three assays. Some studies indicate that variation in TC PD-L1 staining between 22C3, 28-8 and SP142 assays is independent of antibody used (and epitope recognized), and more likely due to differences in tumor heterogeneity, as well as platform and assay variables.[36 43] Other reports indicate that variation in patient treatment (eg, with chemotherapy regimens) at the time of biopsy may alter tumor cell expression of PD-L1.[35] Finally, others have proposed that initial negative PD-L1 test results could be followed by repeat PD-L1 testing at a later point with alternative specimens, or with a different clone if necessary.[47]

Our data show that 81.1% (43/53) of lung squamous cell carcinoma cases were positive with at least one IHC assay, compared with 72.1% (88/122) cases of lung adenocarcinoma. This finding is consistent with several recent studies that have shown greater tumor cell PD-L1 expression in squamous cell carcinoma compared with lung adenocarcinoma.[48–50] While it is a limited number of samples, our data also show that an even lower proportion (50.0%, 4/8 cases) of SCLC cases demonstrate positive PD-L1 status with at least one IHC assay, a finding consistent with previous reports of lower, although variable, PD-L1 tumor cell expression in SCLC ranging from 0–3%[51 52] to 71%.[53]

Finally, we show that a greater proportion (34.8%, 24/69 cases) of metastatic NSCLC specimens were triple negative, compared with only 21.7% (23/106) of primary NSCLC cases. While some reports have demonstrated similarly lower PD-L1 tumor cell expression in metastatic versus primary sites,[54 55] others have reported higher PD-L1 tumor cell expression in metastatic specimens.[50 56] This variation in reported TC expression in metastatic versus primary sites might be due to differences in the

specific tumor type studied, differences in the local tumor microenvironment such as the local cytokine milieu,[57] or the specific metastatic site in question. For example, in metastatic triple negative breast cancer, TC expression of PD-L1 is reported to be substantially lower in liver, skin and bone sites, compared with those in lung, soft tissue, and lymph nodes.[55]

One limitation of this study is that all the specimens were stained using the manufacturer's staining protocol for their assay, and our results are therefore not generalizable if a lab uses a different staining protocol. A further limitation is that each of the IHC assays were performed on different tissue sections from the same block for each specimen, rather than an IHC multiplex assay on the same tissue section. As such, not all tissue sections through a tumor specimen are identical, due to variations in tissue size and shape, as well as tumor heterogeneity, a factor that likely caused approximately 5–6 'outlier' specimens to show variant 28-8 TC expression, compared with the best fit 28-8 curve. The 28-8 IHC assay was disproportionately affected by these factors in our study, because tissue sections for 22C3 and SP142 IHC assays were often cut first, followed by tissue sections cut for next-generation sequencing testing, and only finally followed by tissue sections cut for 28-8 IHC. Therefore, the tissue profile and amount of tumor present on the 28-8 tissue section could on occasion vary significantly from that seen in the 22C3 and SP142 tissue sections.

Conclusion

In conclusion, we show that 22C3 is the most sensitive PD-L1 IHC assay for tumor cell expression and returns a positive PD-L1 status more frequently than 28-8, which in turn is positive more often than SP-142. Multiple factors should be considered in the selection of a PD-L1 IHC assay for a particular patient, including which IHC assay is a CDx for the patient's specific tumor type, which CDx-associated therapy is most clinically appropriate, the side effect profile of that therapy, as well as the current clinical status of the patient. The findings presented here represent an additional resource to help guide clinicians select which PD-L1 IHC CDx assay is most suitable for their patient.

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REFERENCES

1 Pai-Scherf L, Blumenthal GM, Li H, et al. FDA approval summary: pembrolizumab for treatment of metastatic non-small cell lung cancer: first-line therapy and beyond. *Oncologist* 2017;22:1392–9.
2 Schachter J, Ribas A, Long GV, et al. Pembrolizumab versus ipilimumab for advanced melanoma: final overall survival results of a multicentre, randomised, open-label phase 3 study (KEYNOTE-006). *Lancet* 2017;390:1853–62.
3 Schmid P, Adams S, Rugo HS, et al. Atezolizumab and nab-paclitaxel in advanced triple-negative breast cancer. *N Engl J Med* 2018;379:2108–21.
4 Schmid P, Rugo HS, Adams S, et al. Atezolizumab plus nab-paclitaxel as first-line treatment for unresectable, locally advanced or metastatic triple-negative breast cancer (IMpassion130): updated efficacy results from a randomised, double-blind, placebo-controlled, phase 3 trial. *Lancet Oncol* 2020;21:44–59.
5 Horn L, Mansfield AS, Szczęsna A, et al. First-line atezolizumab plus chemotherapy in extensive-stage small-cell lung cancer. *N Engl J Med* 2018;379:2220–9.
6 Pacheco J, Bunn PA. Advancements in small-cell lung cancer: the changing landscape following IMpower-133. *Clin Lung Cancer* 2019;20:148–60.
7 Blank C, Mackensen A. Contribution of the PD-L1/PD-1 pathway to T-cell exhaustion: an update on implications for chronic infections and tumor evasion. *Cancer Immunol Immunother* 2007;56:739–45.
8 Herbst RS, Soria J-C, Kowanetz M, et al. Predictive correlates of response to the anti-PD-L1 antibody MPDL3280A in cancer patients. *Nature* 2014;515:563–7.
9 Bellmunt J, Mullane SA, Werner L, et al. Association of PD-L1 expression on tumor-infiltrating mononuclear cells and overall survival in patients with urothelial carcinoma. *Ann Oncol* 2015;26:812–7.
10 Brahmer JR, Drake CG, Wollner I, et al. Phase I study of single-agent anti-programmed death-1 (MDX-1106) in refractory solid tumors: safety, clinical activity, pharmacodynamics, and immunologic correlates. *J Clin Oncol* 2010;28:3167–75.
11 Fehrenbacher L, Spira A, Ballinger M, et al. Atezolizumab versus docetaxel for patients with previously treated non-small-cell lung cancer (POPLAR): a multicentre, open-label, phase 2 randomised controlled trial. *Lancet* 2016;387:1837–46.
12 Powles T, Eder JP, Fine GD, et al. MPDL3280A (anti-PD-L1) treatment leads to clinical activity in metastatic bladder cancer. *Nature* 2014;515:558–62.
13 Rosenberg JE, Hoffman-Censits J, Powles T, et al. Atezolizumab in patients with locally advanced and metastatic urothelial carcinoma who have progressed following treatment with platinum-based chemotherapy: a single-arm, multicentre, phase 2 trial. *Lancet* 2016;387:1909–20.
14 Topalian SL, Hodi FS, Brahmer JR, et al. Safety, activity, and immune correlates of anti-PD-1 antibody in cancer. *N Engl J Med* 2012;366:2443–54.
15 FDA. List of cleared or approved companion diagnostic devices (in vitro and imaging tools), 2022. Available: https://www.fda.gov/medical-devices/in-vitro-diagnostics/list-cleared-or-approved-companion-diagnostic-devices-in-vitro-and-imaging-tools
16 Scheerens H, Malong A, Bassett K, et al. Current status of companion and complementary diagnostics: strategic considerations for development and launch. *Clin Transl Sci* 2017;10:84–92.
17 Ritzhaupt A, Hayes I, Ehmann F. Implementing the EU in vitro diagnostic regulation - a European regulatory perspective on companion diagnostics. *Expert Rev Mol Diagn* 2020;20:565–7.
18 FDA. *FDA grants accelerated approval to pembrolizumab for first tissue/site agnostic indication*, 2017.

19 FDA. FDA approves pembrolizumab for adults and children with TMB-H solid tumors, 2020. Available: https://www.fda.gov/drugs/drug-approvals-and-databases/fda-approves-pembrolizumab-adults-and-children-tmb-h-solid-tumors

20 Büttner R, Gosney JR, Skov BG, et al. Programmed death-ligand 1 immunohistochemistry testing: a review of analytical assays and clinical implementation in non-small-cell lung cancer. J Clin Oncol 2017;35:3867–76.

21 Hirsch FR, McElhinny A, Stanforth D, et al. PD-L1 immunohistochemistry assays for lung cancer: results from phase 1 of the blueprint PD-L1 IHC assay comparison project. J Thorac Oncol 2017;12:208–22.

22 Ahn S, Kim K-M. PD-L1 expression in gastric cancer: interchangeability of 22C3 and 28-8 pharmDx assays for responses to immunotherapy. Mod Pathol 2021;34:1719–27.

23 Hendry S, Byrne DJ, Wright GM, et al. Comparison of four PD-L1 immunohistochemical assays in lung cancer. J Thorac Oncol 2018;13:367–76.

24 Huang X, Ding Q, Guo H, et al. Comparison of three FDA-approved diagnostic immunohistochemistry assays of PD-L1 in triple-negative breast carcinoma. Hum Pathol 2021;108:42–50.

25 Narita Y, Sasaki E, Masuishi T, et al. PD-L1 immunohistochemistry comparison of 22C3 and 28-8 assays for gastric cancer. J Gastrointest Oncol 2021;12:2696–705.

26 Venina AR, Ivantsov AO, Iyevleva AG, et al. PCR-based analysis of PD-L1 RNA expression in lung cancer: comparison with commonly used immunohistochemical assays. Ann Diagn Pathol 2022;59:151968.

27 Widmaier M, Wiestler T, Walker J, et al. Comparison of continuous measures across diagnostic PD-L1 assays in non-small cell lung cancer using automated image analysis. Mod Pathol 2020;33:380–90.

28 Batenchuk C, Albitar M, Zerba K, et al. A real-world, comparative study of FDA-approved diagnostic assays PD-L1 IHC 28-8 and 22C3 in lung cancer and other malignancies. J Clin Pathol 2018;71:1078–83.

29 Velcheti V, Patwardhan PD, Liu FX, et al. Real-world PD-L1 testing and distribution of PD-L1 tumor expression by immunohistochemistry assay type among patients with metastatic non-small cell lung cancer in the United States. PLoS One 2018;13:e0206370.

30 Krigsfeld GS, Prince EA, Pratt J, et al. Analysis of real-world PD-L1 IHC 28-8 and 22C3 pharmDx assay utilisation, turnaround times and analytical concordance across multiple tumour types. J Clin Pathol 2020;73:656–64.

31 Tsao MS, Kerr KM, Kockx M, et al. PD-L1 immunohistochemistry comparability study in real-life clinical samples: results of blueprint phase 2 project. J Thorac Oncol 2018;13:1302–11.

32 Prince EA, Sanzari JK, Pandya D, et al. Analytical Concordance of PD-L1 Assays Utilizing Antibodies From FDA-Approved Diagnostics in Advanced Cancers: A Systematic Literature Review. JCO Precis Oncol 2021;5:953–73.

33 Borcoman E, Le Tourneau C. Keynote-158 study, FDA granted accelerated approval of pembrolizumab for the treatment of patients with advanced PD-L1-positive cervical cancer. Ann Transl Med 2020;8:1611.

34 Chung HC, Ros W, Delord J-P, et al. Efficacy and safety of pembrolizumab in previously treated advanced cervical cancer: results from the phase II KEYNOTE-158 study. J Clin Oncol 2019;37:1470–8.

35 Piha-Paul SA, Oh D-Y, Ueno M, et al. Efficacy and safety of pembrolizumab for the treatment of advanced biliary cancer: results from the KEYNOTE-158 and KEYNOTE-028 studies. Int J Cancer 2020;147:2190–8.

36 Gaule P, Smithy JW, Toki M, et al. A quantitative comparison of antibodies to programmed cell death 1 ligand 1. JAMA Oncol 2017;3:256–9.

37 Callea M, Pedica F, Doglioni C. Programmed death 1 (PD-1) and its ligand (PD-L1) as a new frontier in cancer immunotherapy and challenges for the pathologist: state of the art. Pathologica 2016;108:48–58.

38 Ratcliffe MJ, Sharpe A, Midha A, et al. Agreement between programmed cell death ligand-1 diagnostic assays across multiple protein expression cutoffs in non-small cell lung cancer. Clin Cancer Res 2017;23:3585–91.

39 Rebelatto MC, Midha A, Mistry A, et al. Development of a programmed cell death ligand-1 immunohistochemical assay validated for analysis of non-small cell lung cancer and head and neck squamous cell carcinoma. Diagn Pathol 2016;11:95.

40 Xu H, Lin G, Huang C, et al. Assessment of concordance between 22C3 and SP142 immunohistochemistry assays regarding PD-L1 expression in non-small cell lung cancer. Sci Rep 2017;7:16956.

41 Dong H, Zhu G, Tamada K, et al. B7-H1, a third member of the B7 family, co-stimulates T-cell proliferation and interleukin-10 secretion. Nat Med 1999;5:1365–9.

42 Zak KM, Grudnik P, Magiera K, et al. Structural biology of the immune checkpoint receptor PD-1 and its ligands PD-L1/PD-L2. Structure 2017;25:1163–74.

43 Lawson NL, Dix CI, Scorer PW, et al. Mapping the binding sites of antibodies utilized in programmed cell death ligand-1 predictive immunohistochemical assays for use with immuno-oncology therapies. Mod Pathol 2020;33:518–30.

44 do Couto F, Liao Z, Zhu Y. ANTI-PD-L1 antibodies and diagnostic uses thereof., a. Spring Bioscience Corporation, Editor.

45 Vennapusa B, Liao Z, Kowanetz M, et al. Roche, PC anti-PD-L1 antibodies and diagnostic uses thereof. I.S.B.C. Genentech, assignee., Editor

46 Systems VM, I. VENTANA PD-L1 (SP142) assay. summary of safety and effectiveness data 2016.

47 Shen X, Wang Y, Jin Y, et al. PD-L1 expression in non-small cell lung cancer: heterogeneity by pathologic types, tissue sampling and metastasis. J Thorac Dis 2021;13:4360–70.

48 Mansour MSI, Malmros K, Mager U, et al. PD-L1 expression in non-small cell lung cancer specimens: association with clinicopathological factors and molecular alterations. Int J Mol Sci 2022;23:23094517. doi:10.3390/ijms23094517

49 Saez de Gordoa K, Lopez I, Marginet M, et al. PD-L1 expression in non-small cell lung cancer: data from a referral center in Spain. Diagnostics 2021;11:11081452 doi:10.3390/diagnostics11081452

50 Hwang DM, Albaqer T, Santiago RC, et al. Prevalence and heterogeneity of PD-L1 expression by 22C3 assay in routine population-based and reflexive clinical testing in lung cancer. J Thorac Oncol 2021;16:1490–500.

51 Schultheis AM, Scheel AH, Ozretić L, et al. PD-L1 expression in small cell neuroendocrine carcinomas. Eur J Cancer 2015;51:421–6.

52 Guleria P, Kumar S, Malik PS, et al. PD-L1 expression in small cell and large cell neuroendocrine carcinomas of lung: an immunohistochemical study with review of literature. Pathol Oncol Res 2020;26:2363–70.

53 Ishii H, Azuma K, Kawahara A, et al. Significance of programmed cell death-ligand 1 expression and its association with survival in patients with small cell lung cancer. J Thorac Oncol 2015;10:426–30.

54 Szekely B, Bossuyt V, Li X, et al. Immunological differences between primary and metastatic breast cancer. Ann Oncol 2018;29:2232–9.

55 Rozenblit M, Huang R, Danziger N, et al. Comparison of PD-L1 protein expression between primary tumors and metastatic lesions in triple negative breast cancers. J Immunother Cancer 2020;8.

56 Schoenfeld AJ, Rizvi H, Bandlamudi C, et al. Clinical and molecular correlates of PD-L1 expression in patients with lung adenocarcinomas. Ann Oncol 2020;31:599–608.

57 Chen S, Crabill GA, Pritchard TS, et al. Mechanisms regulating PD-L1 expression on tumor and immune cells. J Immunother Cancer 2019;7:305.