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Concordance of Programmed Death-Ligand 1 Expression between SP142 and 22C3/SP263 Assays in Triple-Negative Breast Cancer

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ABSTRACT

Purpose: Triple-negative breast cancer (TNBC) represents a major clinical challenge due to its aggressive and metastatic behavior and the lack of available targeted therapies. Therefore, therapeutic strategies are needed to improve TNBC patient management. Recently, atezolizumab and nab-paclitaxel chemotherapy has been approved by the Food and Drug Administration for the first-line treatment of patients with locally advanced and metastatic TNBC. The programmed death-ligand 1 (PD-L1) immunohistochemical SP142 assay was also approved as a companion diagnostic device for selecting TNBC patients for atezolizumab treatment. This study aimed to evaluate and compare the analytical performance of the PD-L1 22C3/SP263 assays in comparison with the SP142 assay for ≥ 1% immune cells (ICs).
Methods: Immunohistochemical expression for the PD-L1 22C3/SP263 assays, in comparison with the SP142 assay, was analyzed for the ≥ 1% ICs in 95 TNBCs.
Results: At the 1% cut-off value, the proportions of positive cases were 52.6% for the SP142 assay in infiltrating ICs and 50.5% and 52.6% for the 22C3 and SP263 assays in tumor cells, respectively. The PD-L1 SP263 assay had the highest while the PD-L1 22C3 assay had the lowest total positive expression rate at all cut-off values. The concordance rate between the assays was highest at a 1% cut-off value and decreased when the cut-off value increased. The concordance rate between the SP142 and SP263 assays at 1% cut-off was high, while in comparison, the concordance rate between the SP142 and 22C3 assays at 1% cut-off was relatively lower.
Conclusion: This study demonstrates that although the 22C3 assay at a 1% cut-off value compared with the PD-L1 SP142 assay at the clinically relevant cut-off shows comparable but not interchangeable analytical performance, the analytical performance of the SP263 assay at a 1% cut-off value shows interchangeable performance with the PD-L1 SP142 assay at the clinically relevant cut-off.

Keywords: B7-H1 antigen; Immunohistochemistry; Triple negative breast neoplasms

Conflict of Interest
The authors declare that they have no competing interests.

Author Contributions
Conceptualization: Lee SE; Data curation: Park HY, Lim SD, Han HS; Supervision: Kim WS; Validation: Yoo YB; Visualization: Park HY; Writing - original draft: Lee SE; Writing - review & editing: Lee SE.

INTRODUCTION

Triple-negative breast cancer (TNBC), defined by the lack of estrogen receptor and progesterone receptor expression and the absence of *HER2/neu* (*ERBB2*) gene amplification, accounts for approximately 10%–20% of invasive breast cancers [1-3]. TNBC represents a clinically and molecularly heterogeneous group. Currently, TNBC lacks clinically approved targeted therapies and is treated with traditional chemotherapy. TNBC represents a major clinical challenge due to its aggressive and metastatic behavior. Therefore, therapeutic strategies are needed to improve TNBC patient management.

Immunotherapy has changed the treatment landscape for melanoma and non-small cell lung cancer (NSCLC). Currently, nivolumab is used for NSCLC in the second-line treatment, pembrolizumab is used for NSCLC with high programmed death-ligand 1 (PD-L1) expression (≥ 50%) in the first-line treatment or in the second-line treatment for tumors with ≥ 1% PD-L1 expression, and atezolizumab is used for all subtypes of NSCLC in the second-line treatment [4,5].

Several studies have reported that 20%–58% of TNBCs express PD-L1. Higher expression of PD-L1 has been observed in TNBC than in non-TNBC [6-12]. Recently, the Food and Drug Administration (FDA) approved the use of atezolizumab and nab-paclitaxel chemotherapy for the first-line treatment of patients with locally advanced and metastatic TNBC [13]. Accelerated approval was granted based on Impassion130 (NCT02425891), a multicenter, international, double-blinded, placebo-controlled, randomized trial that included 902 patients with unresectable, locally advanced or metastatic TNBC [14]. Furthermore, several clinical trials are being conducted with PD-1 inhibitors, such as nivolumab and pembrolizumab, and PD-L1 inhibitors, such as atezolizumab and durvalumab, for TNBC treatment [15,16].

Therefore, the identification of patients who may benefit from immune checkpoint inhibition in TNBC is a critical issue. PD-L1 expression is an important biomarker for the prediction of the response to anti-PD1 and anti-PD-L1 immunotherapy. Currently, there are multiple PD-L1 immunohistochemistry (IHC) assays to determine the expression of PD-L1 in tumor cells (TCs). Each assay is linked to a specific therapeutic agent. In NSCLC, the Dako PD-L1 IHC 22C3 assay was approved for use with pembrolizumab [17,18], and the Ventana PD-L1 IHC SP142 assay was approved for use with atezolizumab [19]. The Ventana PD-L1 IHC SP263 assay is in development for use with durvalumab [20].

In TNBC, the FDA also recently approved the Ventana PD-L1 IHC (SP142) assay as a companion diagnostic device for selecting TNBC patients for atezolizumab treatment [14]. However, each PD-L1 IHC assay was found to have many unresolved issues such as the use of different antibody clones, different IHC protocols and platforms, different cut-off values for PD-L1 positivity, and different types of cells in which PD-L1 is assessed (TCs vs. immune cells [ICs]). It is not clear whether the cut-off points for PD-L1 positivity are valuable in predicting the response to immunotherapy in TNBC. Furthermore, there is a question of whether PD-L1 FDA-approved assays have comparable performance. In this study, the analytical performance of the PD-L1 22C3/SP263 assay was evaluated and compared with the SP142 assay for ≥ 1% ICs.

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METHODS

Patients

Ninety-five patients with TNBC were analyzed who underwent surgical resection for primary breast cancer at the Konkuk University Medical Center (KUMC) between January 2012 and December 2016. The archival data were fully anonymized prior to the study, the requirement for informed consent was waived by the Institutional Review Board of KUMC, Seoul, Korea (KUMC 2019-06-031). Clinicopathological information was obtained by reviewing medical records and hematoxylin and eosin stain (H&E)-stained sections. The following histopathological variables of the invasive carcinomas were determined: histologic subtype, T stage, N stage, AJCC stage, and Bloom-Richardson histologic grade.

Tissue microarray construction

All 95 H&E-stained slides were reviewed, and the most representative area was selected from each case. Two 3-mm tissue cores derived from the representative tumors in formalin-fixed paraffin-embedded tissue blocks were taken. On-slide control tissues (tonsil) were used.

Programmed death-ligand 1 immunohistochemistry

From each tissue microarray (TMA) block, 4-μm sections were cut and stained with 3 validated PD-L1 assays according to the manufacturer's instructions: staining for SP142 (Ventana Medical Systems Inc., Tucson, USA), SP263 (Ventana Medical Systems Inc.), and 22C3 (Agilent Technologies/Dako, Carpinteria, USA) were performed on the Ventana Benchmark Ultra platform using an OptiView DAB IHC detection kit.

TCs (invasive component) and tumor-infiltrating inflammatory cells, composed of mononuclear cells including lymphocytes, macrophages, and plasma cells, were scored separately. PD-L1 expression was scored on the basis of the percentage of TCs/ICs showing membranous positivity, irrespective of the staining intensities. Placenta was used as an external control and macrophages were used as internal controls in order to validate the adequacy of the PD-L1 staining reaction. PD-L1 evaluation was performed blindly by 2 trained pathologists (SEL and WSK), who routinely use SP263, 22C3, and SP142 assays in clinical practice.

Statistical analysis

To compare the agreement of different assays at different cut-off values, the overall percentage agreement (OPA), positive percentage agreement (PPA), negative percentage agreement (NPA), and Cohen κ statistic were calculated at different cut-offs. Venn diagrams were constructed to illustrate the discordance/concordance of different assays and cut-off systems. A *p*-value of less than 0.05 was considered to indicate a statistically significant difference. All analyses were carried out using SPSS version 22 (IBM Corp., Armonk, USA).

RESULTS

Clinicopathological characteristics of 95 triple-negative breast cancer patients

A total of 95 TNBCs were analyzed in this study. The clinicopathological characteristics are presented in **Table 1**. The median age at diagnosis was 50 years (range, 30–80 years). Of them, 58 patients (61.1%) were pre-menopausal and 37 patients (38.9%) were post-menopausal. The major histologic type was invasive ductal carcinoma (92.2%). About 90% of the cases were histologic grade III, and the majority of cases had a high Ki-67 expression of ≥ 20%. Of

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Table 1. Clinicopathological characteristics of patients with 95 TNBC

Characteristics	No. of patients
Age (yr)	50 (30–83)
Age (yr)	
< 50	51 (53.7)
≥ 50	44 (46.3)
Menopause	
Pre	58 (61.1)
Post	37 (38.9)
Histologic type	
IDC	87 (91.6)
Carcinoma with medullary feature	5 (5.3)
Pleomorphic carcinoma	3 (3.2)
T-stage	
1	38 (40.0)
2	49 (51.6)
3	8 (8.4)
N-stage	
0	53 (55.8)
1	27 (28.4)
2	7 (7.4)
3	4 (4.2)
Stage	
I	8 (8.4)
II	69 (72.6)
III	14 (14.7)
IV	0
Histologic grade	
1	0
2	10 (10.5)
3	85 (89.5)
Ki-67 proliferation index	
High (≥ 20%)	87 (91.6)
Low (< 20%)	8 (8.4)
Neoadjuvant CTx	
Yes	6 (6.3)
No	89 (93.7)
CTx	
Yes	88 (92.6)
No	7 (7.4)
RTx	
Yes	78 (82.1)
No	17 (17.9)
Recur	
Yes	21 (22.1)
No	74 (77.9)

Values are presented as median (interquartile range) or number (%).
TNBC = triple-negative breast cancer; IDC = invasive ductal carcinoma; CTx = chemotherapy; RTx = radiation therapy.

these 95 patients, 6 (6.3%) received neoadjuvant chemotherapy, 88 (92.6%) received adjuvant chemotherapy, and 78 (82.1%) received radiation therapy. Among the 95 patients, 21 (22.1%) showed recurrence. The median time until recurrence after curative resection was 12 months.

Correlation of programmed death-ligand 1 expression between the SP142 assay and SP263/22C3 assay

The staining intensity of the Ventana 22C3 assay was weaker compared with those of the SP263 and SP142 assays. A representative IHC image of 3 PD-L1 assays is depicted in **Figure 1**. The correlations between SP142 and SP263/22C3 are shown in **Figure 2**. The Spearman correlation

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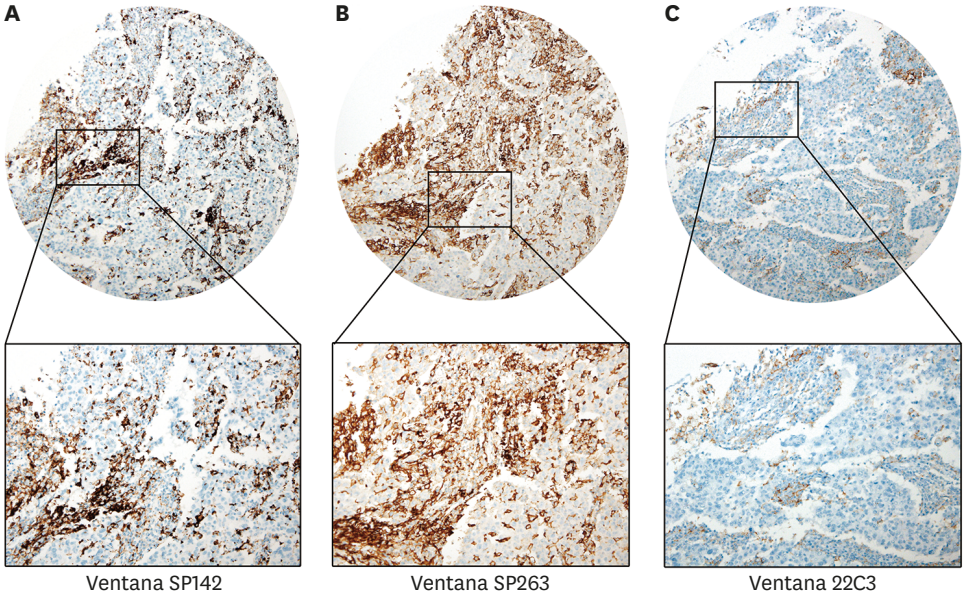

Figure 1. Representative IHC image of the same TMA core stained with 3 PD-L1 assays. (A) An SP142 assay on the Ventana platform showed prominent granular staining in infiltrating immune cells (IHC staining, 20× magnification). (B) An SP263 assay on the Ventana platform showed membranous staining in TCs (IHC staining, 20× magnification). (C) A 22C3 assay on the Ventana platform showed membranous staining in TCs (IHC staining, 20× magnification).
IHC = immunohistochemistry; TMA = tissue microarray; PD-L1 = programmed death-ligand 1; TC = tumor cell.

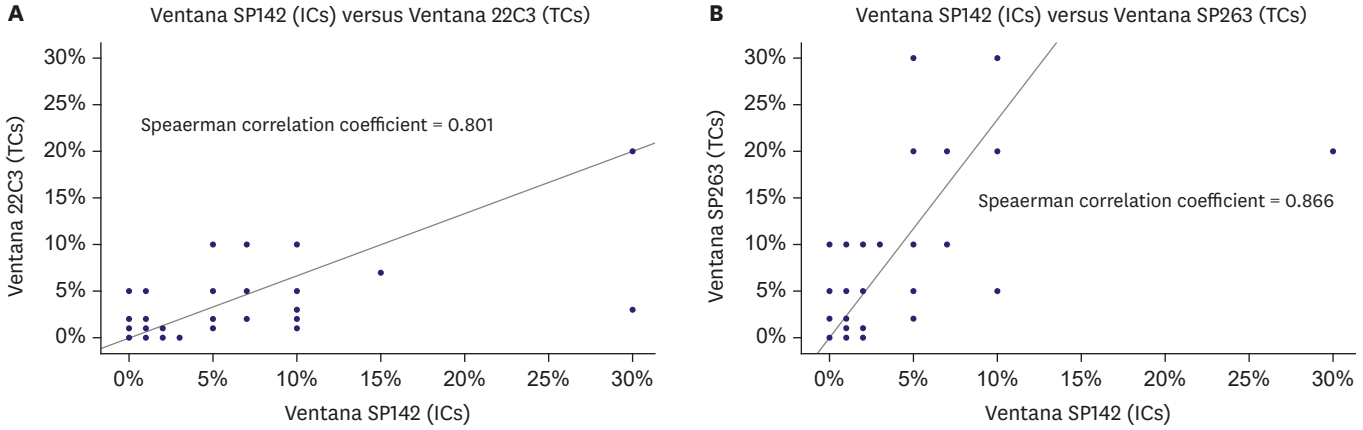

Figure 2. Scatter plots showing pairwise comparisons of the percentages of TC staining between the 22C3 and SP142 immunohistochemical assays (A) /SP263 and SP142 immunohistochemical assays (B).
IC = immune cell; TC = tumor cell.

coefficients were 0.866 (SP142 vs. SP263) and 0.801 (SP142 vs. 22C3), indicating strong associations between the other assays and the SP142 assay.

Overall positivity according to the anti-programmed death-ligand 1 assay and cut-off value

PD-L1 SP142 assay and SP263/22C3 assay staining in TNBC TCs and ICs is summarized in **Table 2**. To evaluate the analytical performance of the PD-L1 22C3/SP263 assays in comparison with the SP142 assay for ≥1% ICs, analyses were conducted on the TCs in the 22C3 and SP263 assays and the infiltrating ICs in the SP142 assay (**Table 3**). At the 1% cut-off value, 52.6% of cases were positive for the SP142 assay in infiltrating ICs, and 50.5% and 52.6% of cases were positive for the 22C3 and SP263 assays in TCs, respectively. The

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Table 2. Expression of PD-L1 assay in TNBC

PD-L1 assay	SP142	SP263	22C3
Tumor cell component			
1% cut-off value			
Negative	79 (83.2)	45 (47.4)	47 (49.5)
Positive	16 (16.8)	50 (52.6)	48 (50.5)
5% cut-off value			
Negative	89 (93.7)	52 (54.7)	78 (82.1)
Positive	6 (6.3)	43 (45.3)	17 (17.9)
10% cut-off value			
Negative	93 (97.9)	65 (68.4)	89 (93.7)
Positive	2 (2.1)	30 (31.6)	6 (6.3)
Immune cell component			
1% cut-off value			
Negative	45 (47.4)	27 (28.4)	46 (48.4)
Positive	50 (52.6)	68 (71.6)	49 (51.6)
5% cut-off value			
Negative	68 (71.6)	38 (40.0)	83 (87.4)
Positive	27 (28.4)	57 (60.0)	12 (12.6)
10% cut-off value			
Negative	80 (84.2)	55 (57.9)	93 (97.9)
Positive	15 (15.8)	40 (42.1)	2 (2.1)

Values are presented as number (%).
PD-L1 = programmed death-ligand 1; TNBC = triple-negative breast cancer.

Ventena 22C3 assay identified fewer tumors as positive (0.96-fold) than did the reference assay (SP142), while the SP263 assay showed similar results as the reference assay (1-fold). At the 5% cut-off value, 28.4% of cases were positive for the SP142 assay in infiltrating ICs, and 17.9% and 45.3% were positive for the 22C3 and SP263 assays in TCs, respectively. The Ventena 22C3 assay identified significantly fewer tumors as positive (0.63-fold) than did the reference assay (SP142), while the SP263 assay identified significantly more tumors as positive (1.59-fold) than did the reference assay. At the 10% cut-off value, 15.8% of cases were positive for the SP142 assay in infiltrating ICs, and 6.3% and 31.6% were positive for the 22C3 and SP263 assays in TCs, respectively. The Ventena 22C3 assay identified significantly fewer tumors as positive (0.4-fold) than did the reference assay (SP142), while the SP263 assay showed significantly more tumors as positive (2.0-fold) than did the reference assay. At the 50% cut-off value, only 12.2% of cases were positive for the SP263 assay. Of 95 cases, 39 (41.1%) showed no expression of any of the 3 PD-L1 antibodies.

Concordance rate comparison between SP142 and 22C3/SP263 assays according to cut-off values

Regarding the current FDA-approved assay (≥ 1% in SP142), the concordance rate between SP142 and 22C3/SP263 assays was highest when a 1% cut-off value was used, and the kappa value between the SP142 and SP263 assays was highest when a 1% cut-off value was used (κ = 0.831) (**Table 4**). On the other hand, the kappa value between the SP142 and 22C3 assays was the lowest at the 10% cut-off value (κ = 0.114).

Table 3. Overall PD-L1 positivity according to assays and cutoff value

Assay	1% cutoff value	Fold-change (positive cases)	5% cutoff value	Fold-change (positive cases)	10% cutoff value	Fold-change (positive cases)
SP142 (ICs)	50 (52.6)	Reference	27 (28.4)	Reference	15 (15.8)	Reference
22C3 (TCs)	48 (50.5)	0.96	17 (17.9)	0.63	6 (6.3)	0.4
SP263 (TCs)	50 (52.6)	1	43 (45.3)	1.59	30 (31.6)	2

Values are presented as number (%).
PD-L1 = programmed death-ligand 1; IC = immune cell; TC = tumor cell.

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Table 4. Kappa value for inter-PD-L1 assay concordance according to cutoff value

Assay	1% cutoff value	5% cutoff value	10% cutoff value
SP142 (ICs) vs 22C3 (TCs)	0.747	0.592	0.215
SP142 (ICs) vs SP263 (TCs)	0.831	0.605	0.465
22C3 (TCs) vs SP263 (TCs)	0.705	0.372	0.255

PD-L1 = programmed death-ligand 1; IC = immune cell; TC = tumor cell.

Table 5. PPA, NPA, and OPA between SP142 assay and 22C3/SP263 assay at multiple PD-L1 expression cutoff values

Assay	Cutoff value	vs SP142 (IC ≥ 1%)		
		PPA	NPA	OPA
22C3 (TCs)	1%	86.0	88.9	87.4
	5%	55.6	97.1	85.3
	10%	20.0	96.3	84.2
SP263 (TCs)	1%	92.0	91.1	91.2
	5%	96.3	75.0	81.1
	10%	86.7	78.8	80.0

PPA = positive percent agreement; NPA = negative percent agreement; OPA = overall percent agreement; PD-L1 = programmed death-ligand 1; IC = immune cell; TC = tumor cell.

Additionally, the OPA, PPA, and NPA were compared pairwise between the SP142 and 22C3/SP263 assays according to cut-off values. At a 1% cut-off value, the OPA, PPA, and NPA between the SP142 and 22C3 assays were 87.4%, 86.0%, and 88.9%, respectively. At cut-off values of 5% and 10%, the OPA and PPA decreased, while the NPA increased. At a 1% cut-off, the OPA, PPA, and NPA between the SP142 and SP263 assays were 91.2%, 92.0%, and 91.1%, respectively. At cut-off values of 5% and 10%, the PPA increased, while the OPA and NPA decreased (**Table 5**).

To analyze the concordance or discordance between the SP142 assay (≥ 1% of ICs) and the 22C3/SP263 assays in detail, a Venn diagram was constructed (**Figure 3**). At the 1% cut-off value for the SP263 and 22C3 assays (**Figure 3A**), the total number of positive cases in either assay were 56 (58.9%). Thirty-nine cases (41.1%) were identified as positive for all 3 PD-L1 assays. Fifty cases (52.6%) were positive (≥ 1% ICs) in the SP142 assay. Forty-six (48.4%) of the fifty positive cases for the SP142 assay were concordantly positive with the SP263 assay, while 4 cases (4.2%) were exclusively positive for the SP263 assay. Forty-three (45.3%) of the

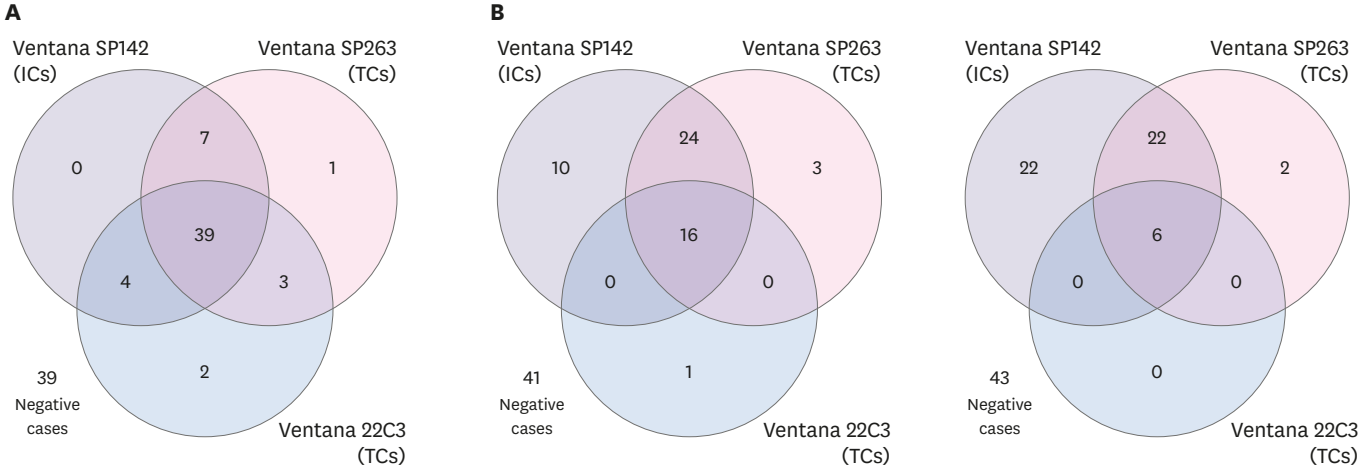

Figure 3. A Venn diagram representing the concordance or discordance between the SP142 assay (≥ 1% of immune cells) and the 22C3/SP263 assays. (A) 22C3/SP263 assays at a 1% cut-off value, (B) 22C3/SP263 assays at a 5% cut-off value, (C) 22C3/SP263 assays at a 10% cut-off value.
IC = immune cell; TC = tumor cell.

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fifty positive cases for the SP142 assay were concordantly positive with the 22C3 assay, while 5 cases (5.3%) were exclusively positive for the 22C3 assay. At the 5% cut-off value for the SP263 and 22C3 assays (**Figure 3B**), the total number of positive cases for either assay were 53 (55.8%). Sixteen cases (16.8%) were identified as positive for all 3 PD-L1 assays. Forty (42.1%) of the fifty positive cases for the SP142 assay were concordantly positive with the SP263 assay, while 3 cases (3.2%) were exclusively positive for the SP263 assay. Sixteen (16.8%) of the fifty positive cases for the SP142 assay were concordantly positive with the 22C3 assay, while one case (1.1%) was exclusively positive for the 22C3 assay. At the 10% cut-off value for the SP263 and 22C3 assays (**Figure 3C**), the total number of positive cases for either assay were 32 (58.9%). Twenty-eight cases (29.5%) were identified as positive for all 3 PD-L1 assays. Twenty-eight (29.5%) of the fifty positive cases for the SP142 assay were concordantly positive with the SP263 assay, while 2 cases (2.1%) were exclusively positive for the SP263 assay. Six (6.3%) of the fifty positive cases for the SP142 assay were concordantly positive with the 22C3 assay, while no cases were exclusively positive for the 22C3 assay.

DISCUSSION

Immune checkpoint inhibitors are emerging as therapeutic options for TNBC [14,15]. Recently, the FDA approved the combination of atezolizumab and nab-paclitaxel chemotherapy for the first-line treatment of patients with locally advanced or metastatic TNBC with PD-L1 positivity [14]. The Impassion130 trial showed that atezolizumab plus nab-paclitaxel chemotherapy prolonged progression-free survival among patients with metastatic TNBC in the PD-L1-positive group. In PD-L1-positive patients, the response rates were 58.9% with atezolizumab–nab-paclitaxel and 42.6% with placebo–nab-paclitaxel. In this trial, PD-L1 positivity was defined as ≥ 1% of infiltrating ICs in the PD-L1 SP142 assay. Of the 902 patients, 369 (41%) were classified as the PD-L1-positive group, 243 (27%) had low levels at 1% to < 5%, and 126 (14%) had levels in their ICs at ≥ 5%. In contrast, only 81 (9%) of the patients in the study exhibited PD-L1 expression on TCs [14]. In the present study, 52.6% of patients showed PD-L1 expression at a 1% cut-off value, 28.4% at a 5% cut-off value, and 15.8% at a 10% cut-off value using the SP142 assay for infiltrating ICs. On the contrary, only 16.8% of the patients showed PD-L1 expression at a 1% cut-off value in TCs. These findings are in agreement with the expression rate of PD-L1 in the Impassion130 trial. The Ventana SP142 assay consistently showed fewer positive rates in TCs compared with the other assays in NSCLC and urothelial carcinoma [4,21-24]. Furthermore, the concordance rate between the TCs and ICs was lower in the Ventana SP142 assay compared with the other assays. Unlike NSCLC and urothelial carcinoma, the high PD-L1 expression rate of ICs found with the SP142 assay may be related to high levels of tumor-infiltrating lymphocytes (TIL) in TNBC. PD-L1 expression in ICs may reflect an association with a TIL-mediated antitumor inflammatory response [25].

Nanda and colleagues [15] reported the results of the first single-agent anti-PD-1 therapy (pembrolizumab) in the metastatic TNBC cohort within KEYNOTE-012. This trial screened 111 metastatic TNBC patients for PD-L1 positivity. PD-L1 positivity was defined as positive staining in ≥ 1% of TCs, which was immunohistochemically assessed using the PD-L1 22C3 assay. Of the 111 patients, 58.6% had PD-L1 positive tumors in this trial [15]. In the present study, 50.5% of patients showed PD-L1 expression at a 1% cut-off value, 17.9% at a 5% cut-off value, and 6.3% at a 10% cut-off value in the 22C3 assay. The PD-L1 expression rate sharply decreased as the cut-off value increased. The PD-L1 22C3 assay had the lowest total positive expression rate at all cut-off values in the present study.

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For early-stage TNBC, several studies are testing durvalumab in combination with other agents (NCT02826434; NCT02489448; NCT02685059). PD-L1 expression was assessed on TCs using the PD-L1 SP263 assay. In the present study, 52.6% of patients showed PD-L1 SP263 expression at a 1% cut-off value, 45.3% at a 5% cut-off value, and 31.6% at a 10% cut-off value in the SP263 assay. The PD-L1 SP263 assay had the highest total positive expression rate at all cut-off values in present study.

Identifying which patients will respond to immune checkpoint inhibitors is a significant challenge. This identification has been mainly based on IHC evaluation of PD-L1 expression. However, variations in PD-L1 expression rates have been attributed to different antibody clones, staining protocols and platforms, cut-off values, and scoring algorithms [26-28]. Different PD-L1 IHC assays have been developed and approved in parallel with different therapeutic agents, with different cut-offs determined according to clinical response. The PD-L1 expression rates of TCs and ICs were diverse in previous studies on TNBC as well [6,14,25]. The PD-L1 expression of TCs was 19% at a 5% cut-off with clone 5H1 [29] and 64%–80% at a 1% cut-off with clone E1L3N [6]. Sun et al. [30] reported that PD-L1 expression was significantly higher in ICs with all 3 PD-L1 assays including 28-8, E1L3N, and SP142 and varied at different cut-off values. In the present study, the PD-L1 expression rate ranged from 49.0%–51.0% with a 1% cut-off value, 17.3%–43.9% with a 5% cut-off value, and 6.1%–30.6% with a 10% cut-off value.

The analytical performance of inter-PD-L1 assays should be comparable to allow for the appropriate interpretation of the use of PD-L1 diagnostic assays in selecting immune checkpoint inhibitors. Therefore, the SP263 and 22C3 assays were evaluated for performance in comparison with the PD-L1 SP142 assay, the currently FDA-approved assay. The concordance rate between assays was highest at a 1% cut-off value and decreased when the cut-off value increased. This may be due to the pathologist's tendency to interpret a result as positive at a 1% cut-off, even with low-level intensity and slight expression (1+), and their tendency to make more subjective interpretations as cut-off values increase. This finding was similar to those previously reported [30]. The concordance rate between the SP142 and SP263 assays at a 1% cut-off was high, while the concordance rate between the SP142 and 22C3 assays at a 1% cut-off was relatively lower. Relatively high degrees of agreement (OPA: 85.3% at a 5% cut-off value, 84.2% at a 10% cut-off value) were observed between the 22C3 and SP142 assays at cut-off values of 5% and 10%, likely due to the high NPAs (NPA: 97.1% at a 5% cut-off value, 96.3% at a 10% cut-off value). The concordance and discordance between the SP142 assay (≥ 1% of ICs) and the 22C3/SP263 assays were analyzed in detail. At a 1% cut-off value for the SP263 and 22C3 assays, few cases were exclusively positive by a specific assay, but relevant amounts of cases were exclusively classified as positive by the SP142 assay at a 5% cut-off value and the SP263 and 22C3 assays at a 10% cut-off value. Considered together, these data indicate that the 22C3 assay at a 1% cut-off value exhibits comparability with the SP142 assay for the clinically relevant cut-off, but it could not be used interchangeably, while the SP263 assay at a 1% cut-off value is interchangeable with the SP142 assay at the clinically relevant cut-off.

A limitation of this study was the potential difference in the PD-L1 expression between the TMAs and the whole cancer tissue sections, although the TMAs were constructed by collecting 2 cores for each case to limit the impact of the heterogenous expression of PD-L1. Furthermore, an analysis comparing the therapeutic responses to the immune checkpoint inhibitors could not be performed. Therefore, further studies on TNBC are needed to define the clinically relevant cut-off value based on therapeutic response.

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This is the first study to compare the analytical performance of PD-L1 22C3/SP263 diagnostic assays in comparison with the recently FDA-approved SP142 assay for ≥ 1% ICs in TNBC.

In conclusion, this study demonstrates that although the analytical performance of the 22C3 assay at a 1% cut-off value is comparable to but not interchangeable with the PD-L1 SP142 assay at the clinically relevant cut-off, the analytical performance of the SP263 assay at a 1% cut-off value shows interchangeability with the PD-L1 SP142 assay at the clinically relevant cut-off.

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