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
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 SPI142 assay for ≥ 1% ICs.
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%.
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.

### Table 1. Clinicopathological characteristics of patients with 95 TNBC

| Characteristics                          | No. of patients |
|-----------------------------------------|-----------------|
| Age (yr)                                | 50 (30–83)      |
| Age (< 50)                              | 51 (53.7)       |
| Age (≥ 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.

**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...
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 SPI42 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 SPI42 assay in infiltrating ICs, and 50.5% and 52.6% of cases were positive for the 22C3 and SP263 assays in TCs, respectively. The
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 2. Expression of PD-L1 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 (16.6) | 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 | 3 (3.1) | 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) | 7 (7.1) |

Values are presented as number (%).

PD-L1 = programmed death-ligand 1; TNBC = triple-negative breast cancer.

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

| Assay   | 1% cutoff value (positive cases) | Fold-change | 5% cutoff value (positive cases) | Fold-change | 10% cutoff value (positive cases) | Fold-change |
|---------|----------------------------------|-------------|---------------------------------|-------------|----------------------------------|-------------|
| 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.
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

### 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 SPI42 and 22C3/SP263 assays according to cut-off values. At a 1% cut-off value, the OPA, PPA, and NPA between the SPI42 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 SPI42 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 SPI42 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 SPI42 assay. Forty-six (48.4%) of the fifty positive cases for the SPI42 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
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.
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 SPI42 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 SPI42 and SP263 assays at a 1% cut-off was high, while the concordance rate between the SPI42 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 SPI42 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 SPI42 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 SPI42 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 SPI42 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 SPI42 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.
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.

REFERENCES

1. Badve S, Dubbs DJ, Schnitt SJ, Baehner FL, Decker T, Eusebi V, et al. Basal-like and triple-negative breast cancers: a critical review with an emphasis on the implications for pathologists and oncologists. Mod Pathol 2011;24:157-67.
2. Sotiriou C, Pusztai L. Gene-expression signatures in breast cancer. N Engl J Med 2009;360:790-800.
3. Cetin I, Topcul M. Triple negative breast cancer. Asian Pac J Cancer Prev 2014;15:2427-31.
4. Eckstein M, Erben P, Kriegmair MC, Worst TS, Weiß CA, Wirtz RM, et al. Performance of the Food and Drug Administration/EMA-approved programmed cell death ligand-1 assays in urothelial carcinoma with emphasis on therapy stratification for first-line use of atezolizumab and pembrolizumab. Eur J Cancer 2019;106:234-43.
5. Mittendorf EA, Philips AV, Meric-Bernstam F, Qiao N, Wu Y, Harrington S, et al. PD-L1 expression in triple-negative breast cancer. Cancer Immunol Res 2014;2:361-70.
6. Tung N, Garber JE, Hacker MR, Torous V, Freeman GJ, Poles F, et al. Prevalence and predictors of androgen receptor and programmed death-ligand 1 in BRCA1-associated and sporadic triple-negative breast cancer. NPJ Breast Cancer 2016;2:16002.
7. Ali HR, Glont SE, Blows FM, Provenzano E, Dawson SJ, Liu B, et al. Prognostic value of PD-L1 in breast cancer: a meta-analysis. Breast J 2017;23:436-43.
8. Dill EA, Gru AA, Atkins KA, Friedman LA, Moore ME, Bullock TN, et al. PD-L1 expression and intratumoral heterogeneity across breast cancer subtypes and stages: an assessment of 245 primary and 40 metastatic tumors. Am J Surg Pathol 2017;41:334-42.
9. U.S. Food and Drug Administration. FDA approves atezolizumab for PD-L1 positive unresectable locally advanced or metastatic triple-negative breast cancer. https://www.fda.gov/drugs/drug-approvals-and-databases/fda-approves-atezolizumab-pd-l1-positive-unresectable-locally-advanced-or-metastatic-triple-negative. Accessed March 8th, 2019.
14. Schmid P, Adams S, Rugo HS, Schneeweiss A, Barrios CH, Iwata H, et al. Atezolizumab and nab-paclitaxel in advanced triple-negative breast cancer. N Engl J Med 2018;379:2108-21.

15. Nanda R, Chow LQ, Decs EC, Berger R, Gupta S, Geva R, et al. Pembrolizumab in patients with advanced triple-negative breast cancer: phase Ib KEYNOTE-012 study. J Clin Oncol 2016;34:2460-7.

16. Adams S, Ganti-Mays ME, Kalinsky K, Korde LA, Sharon E, Amiri-Kordestani L, et al. Current landscape of immunotherapy in breast cancer: a review. JAMA Oncol 2019;5:1205-14.

17. U.S. Food and Drug Administration. Dako PD-L1 IHC 22C3 pharmDx. http://www.accessdata.fda.gov/cdrh_docs/pdf15/P150013c.pdf. Accessed August 10th, 2016.

18. Bristol-Myers Squibb. Opdivo®. http://packageinserts.bms.com/pi/pi_opdivo.pdf. Accessed February 3rd, 2016.

19. Ventana Medical Systems. Ventana PD-L1 (SP142) assay. http://productlibrary.ventana.com/ventana_portal/executeSearch.do. Accessed October 27th, 2016.

20. Rebeletto MC, Midha A, Mistry A, Sabalos C, Schechter N, Li X, 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.

21. Gaule P, Smithy JW, Toki M, Rehman J, Patell-Socha F, Cougot D, et al. A quantitative comparison of antibodies to programmed cell death 1 ligand 1. JAMA Oncol 2017;3:256-9.

22. Scheel AH, Dietel M, Heukamp LC, Jöhrens K, Kirchner T, Reu S, et al. Harmonized PD-L1 immunohistochemistry for pulmonary squamous-cell and adenocarcinomas. Mod Pathol 2016;29:1165-72.

23. Ratcliffe MJ, Sharpe A, Midha A, Barker C, Scott M, Soper P, 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;23:3858-91.

24. Rimm DL, Han G, Taube JM, Yi ES, Bridge JA, Flieder DB, et al. A Prospective, multi-institutional, pathologist-based assessment of 4 immunohistochemistry assays for PD-L1 expression in non-small cell lung cancer. JAMA Oncol 2017;3:1051-8.

25. Beckers RK, Selinger CI, Vilain R, Madore J, Wilmott JS, Harvey K, et al. Programmed death ligand 1 expression in triple-negative breast cancer is associated with tumour-infiltrating lymphocytes and improved outcome. Histopathology 2016;69:25-34.

26. Borghaei H, Paz-Ares L, Horn L, Spigel DR, Steins M, Ready NE, et al. Nivolumab versus docetaxel in advanced nonsquamous non-small-cell lung cancer. N Engl J Med 2015;373:1627-39.

27. Garon EB, Rüzić NA, Hui R, Leight N, Balmanoukian AS, Eder JP, et al. Pembrolizumab for the treatment of non-small-cell lung cancer. N Engl J Med 2015;372:2018-28.

28. Fehrenbacher L, Spira A, Ballinger M, Kowanetz M, Vansteenkiste J, Mazieres J, 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;387:1897-46.

29. Brahmer J, Reckamp KL, Baas P, Crinò L, Eberhardt WE, Poddubskaya E, et al. Nivolumab versus docetaxel in advanced squamous-cell non-small-cell lung cancer. N Engl J Med 2015;373:123-35.

30. Sun WJ, Lee YK, Koo JS. Expression of PD-L1 in triple-negative breast cancer based on different immunohistochemical antibodies. J Transl Med 2016;14:173.