Abstract

Background

Triple-negative breast cancer (TNBC) is the most aggressive type of breast cancer. A recent study demonstrated the efficacy of anti-PD-L1 (anti-programmed death ligand-1) immunotherapy in patients with TNBC. However, the identification of TNBC patients who may benefit from immunotherapy is a critical issue. Several assays have been used to evaluate PD-L1 expression, and a few studies comparing PD-L1 expression using various primary antibodies in TNBC tissues have been reported. However, the expression profiles of the PD-L1 using the 73–10 assay have not yet been analyzed in TNBC tissues.

Methods

We analyzed the PD-L1 immunohistochemical profiles of 62 women with TNBC using the 73–10, SP142 (companion diagnostic for atezolizumab), and E1L3N assays. PD-L1 expression on immune cells (ICs) and tumor cells (TCs) was also evaluated, and PD-L1 positivity was defined as a PD-L1-expressing ICs or TCs \( \geq 1\% \).

Results

The expression rates of PD-L1 were 79.0%, 67.7%, and 46.8% on ICs, and 17.7%, 6.5%, and 12.9% on TCs using the 73–10, SP142, and E1L3N assays, respectively. The concordance rates between the 73–10 and SP142 assays were 85.5% (on ICs) and 88.7% (on TCs), respectively, and substantial agreement on ICs (coefficient 0.634) and moderate agreement (coefficient 0.485) on TCs were noted. Sample age and tumor diameter did not influence the ratio of PD-L1 expression among the assays.

Conclusions

The positive rate on ICs and TCs of the 73–10 assay was higher than that of the SP 142 and E1L3N assays. Although substantial agreement on ICs and moderate agreement on TCs between the 73–10 and SP142 assays was noted in the present cohort, further studies are necessary to clarify the clinical usefulness of the 73–10 assay for TNBC patients.
needed to clarify the PD-L1 expression status using various primary antibodies in a larger patient population. This would lead to the establishment of an effective evaluation method to assess the predictive value of anti-PD-L1 immunotherapy.

**Introduction**

Triple-negative breast cancer (TNBC), characterized by the absence of estrogen and progesterone receptors and human epidermal growth factor receptor 2 (HER2), accounts for 12%–17% of breast cancers [1–3]. It is well known that the rates of recurrence, distant metastasis, and mortality rate are significantly higher in TNBC than in other breast cancer subtypes [1, 2]. One of the reasons for the high mortality rate is the limited therapeutic options. However, immune checkpoint inhibitors, such as anti-programmed death ligand 1 (PD-L1) and anti-programmed death protein 1 (PD-1), have been breakthroughs in the treatment of patients with TNBC. Some studies have reported that 20%–58% of TNBC patients express PD-L1, and higher expression of PD-L1 was observed in TNBC patients than in non-TNBC individuals [4–10]. Moreover, several studies have demonstrated the effectiveness of immune checkpoint inhibitors in patients with TNBC. For example, the IMpassion130 trial (NCT02425891) showed that as the first-line treatment, anti-PD-L1 agent (atezolizumab) plus nab-paclitaxel was superior to placebo plus nab-paclitaxel for advanced or metastatic TNBC patients showing \( \geq 1\% \) PD-L1 expression on immune cells (ICs) [11]. Therefore, the identification of TNBC patients who may benefit from immune checkpoint inhibitors is a critical issue.

Immunohistochemical assays are used to evaluate PD-L1 expression. Currently, several primary antibodies for PD-L1 and immunohistochemical protocols and platforms are available for commercial use [12]. Each assay is linked to a specific therapeutic agent. For example, in non-small cell lung cancer, the 22C3 assay has been approved as a companion diagnostic for pembrolizumab [13, 14] and the SP263 assay for durvalumab [15]. In TNBC, the SP142 assay is the companion diagnostic for atezolizumab [11, 12], the 73–10 assay is the companion diagnostic for avelumab (JAVELIN Solid Tumor study; NCT01772004l) [16], and the E1L3N assay is used as a laboratory-developed test [17]; these assays have different cut-off values for PD-L1 immunoreactivity and use different types of positive cells (tumor cells (TCs) vs. ICs). Moreover, the differences in positive immunoreactivity among primary PD-L1 antibodies are well known [12]. In lung cancer, some studies, including the Blueprint PD-L1 immunohistochemical assay comparison study, evaluated the differences in the properties of PD-L1 primary antibodies [18–20]. Although a few studies have analyzed PD-L1 immunoreactivity using the 22–8, 22C3, SP142, SP263, and E1L3N assays in TNBC patients [21–25], the immunoreactivity of PD-L1 using the 73–10 assay has not been compared with that of the SP142 assay. Thus, we aimed to evaluate PD-L1 immunoreactivity using the SP142, 73–10, and E1L3N assays in TNBC tissues.

**Materials and methods**

**Patient selection**

We selected 165 consecutive patients with TNBC who underwent surgical resection at the Department of Surgery of the Kansai Medical University Hospital between January 2006 and December 2018. Patients who received neoadjuvant chemotherapy were excluded from the study because neoadjuvant chemotherapy may influence PD-L1 expression. Patients who were
diagnosed with invasive breast carcinoma of no special type according to the recent World
Health Organization Classification of Breast Tumors [26] were selected. Patients with a special
type of invasive carcinoma were excluded from the study because each special type of carci-
noma has unique clinicopathological features. In total, 62 patients with TNBC were included
in the study cohort. This study cohort was fundamentally the same as that used in our previous
studies [27–29]. In a previous study, we analyzed the relationship between adipophilin expres-
sion, a lipid droplet-associated protein, and the clinicopathological features of patients with
TNBC [27]. In our previous studies, we examined the significance of PD-L1 expression in can-
cer-associated fibroblasts [28], and the relationship between CD155, an immune checkpoint
protein, and PD-L1 expression [29] in TNBC tissues. Thus, the contents of the present study
do not overlap with those of our previous studies [27–29].

This retrospective single-institution study was conducted in accordance with the principles
of the Declaration of Helsinki, and the study protocol was approved by the Institutional
Review Board of the Kansai Medical University Hospital (Approval #2019041). All data were
fully anonymized. Institutional Review Board waived the requirement for informed consent,
because of the retrospective design of the study; medical records and archival samples were
used with no risk to the participants. Moreover, the present study did not include minors.
Information regarding this study, such as the inclusion criteria and opportunity to opt-out,
was provided through the institutional website.

Histopathological analysis

Surgically resected specimens were fixed with formalin, sectioned, and stained with hematoxy-
lin and eosin. All histopathological diagnoses were independently evaluated by more than two
experienced diagnostic pathologists. We used the TNM Classification of Malignant Tumors,
Eighth Edition. Histopathological grading was based on the Nottingham histological grade
[30]. According to a meta-analysis of patients with TNBC, the Ki-67 labeling index (LI) ≥ 40%
was considered high in operative specimens [31]. Stromal tumor-infiltrating lymphocytes
(TILs) were identified using hematoxylin and eosin staining and were considered lymphocyte-
predominant breast cancer (LPBC) at ≥ 60% and non-LPBC at < 59%, according to the TIL
Working Group recommendation [32, 33].

Tissue microarray

Hematoxylin and eosin-stained slides were used to select the regions that were morphologi-
cally most representative of carcinoma, and three tissue cores with a diameter of 2 mm were
punched out from the paraffin-embedded blocks for each patient. The tissue cores were
arrayed in the recipient paraffin blocks.

Immunohistochemistry

Immunohistochemical analyses were performed using an autostainer (the SP142 and E1L3N
assays on Discovery ULTRA System; Roche Diagnostics, Basel, Switzerland; and the 73–10
assay on Leica Bond-III; Leica Biosystems, Bannockburn, IL, USA) according to the manufac-
turer’s instructions. Three different primary monoclonal antibodies were used to detect
PD-L1: SP142 (Roche Diagnostics, Basel, Switzerland), E1L3N (Cell Signaling Technology,
Danvers, MA, USA), and 73–10 (Leica Biosystems, Newcastle, UK). A minimum of two
researchers independently evaluated the immunohistochemical staining results. PD-L1 expres-
sion on the ICs (lymphocytes, macrophages, dendritic cells, and granulocytes) of all samples
was evaluated. PD-L1 expression on ICs was assessed as the proportion of tumor area occupied
by PD-L1-positive ICs of any intensity using the same method as previously reported [11, 34,
Tumor area was defined as the area containing viable tumor cells, associated intratumoral stroma, and contiguous peritumoral stroma. PD-L1-positivity was assessed by the percentage of PD-L1-positive ICs related to the total number of ICs and defined as positive when PD-L1-expressing ICs were $\geq 1\%$ in the tumor area [11, 34, 35]. PD-L1 expression on TCs was assessed as the proportion of viable invasive carcinoma cells showing membranous staining of any intensity divided by the total number of viable invasive carcinoma cells [22, 34]. PD-L1 expression on $\geq 1\%$ TCs was defined as positive [22, 34].

Statistical analyses

All analyses were performed using Statistical Package for the Social Sciences (SPSS) Statistics software (version 27.0, IBM, Armonk, NY, USA). The differences in the PD-L1 expression levels of identical specimens detected by the SP142, 73–10, and E1L3N assays were analyzed using the Wilcoxon matched-pairs signed-rank test. Correlations between two groups were determined using Fisher’s exact test for categorical variables. Agreement between two groups was analyzed using the kappa test. Statistical significance was set at $p < 0.05$.

Results

Patients’ characteristics

This study included 62 female patients, and Table 1 summarizes their clinical and pathological characteristics. The median age at the time of initial diagnosis was 68 years (range, 31–93 years). Based on the biopsy results, all patients had TNBC (invasive carcinomas of no special type).

PD-L1 expression status using different assays

The prevalence of PD-L1 expression on ICs was 79.0% (49 patients), 67.7% (42 patients), and 46.8% (29 patients) as determined using the 73–10, SP142, and E1L3N assays, respectively (Table 2), while the prevalence of PD-L1 expression on TCs was 17.7% (11 patients), 6.5% (4 patients), and 12.9% (8 patients) using the 73–10, SP142, and E1L3N assays, respectively (Table 3). Representative expression patterns of PD-L1 on ICs and TCs were shown by each assay (Figs 1–3).

Comparison of PD-L1 expression analysis on ICs among the 73–10, SP 142, and E1L3N assays

The expression levels of PD-L1 on ICs analyzed by the 73–10, SP142, and E1L3N assays are illustrated in Fig 4. Higher PD-L1 expression was noted using the 73–10 assay compared to using the SP142 assay (median [range], 8% [0–80%] (73–10 assay) vs. 1% [0–50%] (SP142 assay), $p < 0.001$). Fifty patients (80.6%) were positive for PD-L1 expression on their ICs using either the 73–10 or the SP142 assay, and the remaining 12 patients (19.4%) tested negative for PD-L1 based on the results of both primary assays (Table 2A). The concordance rate between the 73–10 and SP142 assays was 85.5%, and Cohen’s kappa coefficient was 0.634 (substantial agreement, $p < 0.001$). Higher PD-L1 expression was also noted using the 73–10 assay than the E1L3N assay (median [range], 8% [0%–80%] (73–10 assay) vs. 0% [0%–40%] (E1L3N assay), $p < 0.001$). Forty-eight patients (79.0%) tested positive for PD-L1 expression on their ICs as determined using either the 73–10 or the E1L3N assay, and the remaining 13 (21.0%) patients tested negative according to the results of both the assays (Table 2B); the concordance rate was 67.7%, and Cohen’s kappa coefficient was 0.378 (fair agreement, $p < 0.001$). Higher PD-L1 expression was also noted using the SP142 assay compared to the E1L3N assay (median [range], 1% [0%–50%] (SP142 assay) vs. 0% (0%) (E1L3N assay), $p = 0.002$). Forty-two patients
Table 1. Clinical characteristics of patients with triple-negative breast cancer.

| Factors                          | n  | %    |
|----------------------------------|----|------|
| Total                            | 62 |      |
| Age (years old)                  |    |      |
| Median (range) 68 (31–93)        |    |      |
| Menopausal status                |    |      |
| Premenopausal                     | 9  | 14.5 |
| Postmenopausal                    | 52 | 83.9 |
| Unknown                          | 1  | 1.6  |
| Tumour size (mm)                 |    |      |
| ≤ 10                             | 8  | 12.9 |
| 10 < and ≤ 20                    | 23 | 37.1 |
| 20 < and ≤ 50                    | 27 | 43.5 |
| 50 <                             | 4  | 6.5  |
| Pathological stage               |    |      |
| I                                | 26 | 41.9 |
| IIA                              | 23 | 37.1 |
| IIB                              | 5  | 8.1  |
| IIIA                             | 4  | 6.5  |
| IIIB                             | 3  | 4.8  |
| IIIC                             | 1  | 1.6  |
| Lymph node status                |    |      |
| positive                         | 14 | 22.6 |
| negative                         | 33 | 53.2 |
| not tested                       | 15 | 24.2 |
| Lymphatic invasion               |    |      |
| positive                         | 53 | 85.5 |
| negative                         | 9  | 14.5 |
| Venous invasion                  |    |      |
| positive                         | 37 | 59.7 |
| negative                         | 25 | 40.3 |
| Nottingham histological grade    |    |      |
| 1                                | 2  | 3.2  |
| 2                                | 28 | 45.2 |
| 3                                | 32 | 51.6 |
| Ki-67 labeling index (LI)        |    |      |
| high                             | 37 | 59.7 |
| low                              | 21 | 33.9 |
| not tested                       | 4  | 6.5  |
| Stromal TILs                     |    |      |
| LPBC                             | 19 | 30.6 |
| non-LPBC                         | 43 | 69.4 |
| sample age (years)               |    |      |
| < 5                              | 19 | 30.6 |
| 5 ≤ and < 10                     | 24 | 38.7 |
| 10 ≤                             | 19 | 30.6 |

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(67.7%) were positive for PD-L1 expression on their ICs using either the SP142 or the E1L3N assay, and the remaining 20 (32.3%) patients were negative according to both the assays (Table 2C); the concordance rate was 79.0%, and Cohen’s kappa coefficient was 0.590 (moderate agreement, $p < 0.001$).

### Comparison of PD-L1 expression levels on TCs using the 73–10, SP 142, and E1L3N assays

Higher PD-L1 expression was noted using the 73–10 assay compared to the SP142 assay. Eleven patients (17.7%) tested positive for PD-L1 expression on their TCs using either the 73–10 or the SP142 assay, and the remaining 51 patients (82.3%) tested negative for PD-L1 according to both the assays (Table 3A). The concordance rate between the 73–10 and SP142 assays was 88.7%, and Cohen’s kappa coefficient was 0.485 (moderate agreement, $p < 0.001$). Higher PD-L1 expression was noted using the 73–10 assay than the E1L3N assay. Eleven patients (17.7%) tested positive for PD-L1 expression on their TCs using either the 73–10 or the E1L3N

| Table 2. Comparison of PD-L1 expression on ICs by 73–10, SP142, and E1L3N assays. |
|---------------------------------|---------------------------------|---------------------------------|
| (A)                             | 73–10                           |                                |
| SP142                           | PD-L1 ≥ 1%                      | PD-L1 < 1%                      |
| PD-L1 ≥ 1%                      | 41 (66.1%)                      | 1 (1.6%)                        |
| PD-L1 < 1%                      | 8 (12.9%)                       | 12 (19.4%)                      |
|                                | concordance rate                | kappa coefficient               |
|                                | 85.5%                           | 0.634                           |
| (B)                             | 73–10                           |                                |
| E1L3N                           | PD-L1 ≥ 1%                      | PD-L1 < 1%                      |
| PD-L1 ≥ 1%                      | 29 (46.8%)                      | 0                               |
| PD-L1 < 1%                      | 20 (32.2%)                      | 13 (21.0%)                      |
|                                | concordance rate                | kappa coefficient               |
|                                | 67.7%                           | 0.345                           |
| (C)                             | E1L3N                           |                                |
| SP142                           | PD-L1 ≥ 1%                      | PD-L1 < 1%                      |
| PD-L1 ≥ 1%                      | 29 (46.8%)                      | 13 (21.0%)                      |
| PD-L1 < 1%                      | 0                               | 20 (32.2%)                      |
|                                | concordance rate                | kappa coefficient               |
|                                | 95.2%                           | 0.814                           |

| Table 3. Comparison of PD-L1 expression on TCs by 73–10, SP 142, and E1L3N assays. |
|---------------------------------|---------------------------------|---------------------------------|
| (A)                             | 73–10                           |                                |
| SP142                           | PD-L1 ≥ 1%                      | PD-L1 < 1%                      |
| PD-L1 ≥ 1%                      | 4 (6.5%)                        | 0                               |
| PD-L1 < 1%                      | 7 (11.3%)                       | 51 (82.3%)                      |
|                                | concordance rate                | kappa coefficient               |
|                                | 88.7%                           | 0.485                           |
| (B)                             | 73–10                           |                                |
| E1L3N                           | PD-L1 ≥ 1%                      | PD-L1 < 1%                      |
| PD-L1 ≥ 1%                      | 8 (12.9%)                       | 0                               |
| PD-L1 < 1%                      | 3 (4.8%)                        | 51 (82.3%)                      |
|                                | concordance rate                | kappa coefficient               |
|                                | 95.2%                           | 0.814                           |
| (C)                             | E1L3N                           |                                |
| SP142                           | PD-L1 ≥ 1%                      | PD-L1 < 1%                      |
| PD-L1 ≥ 1%                      | 4 (6.5%)                        | 0                               |
| PD-L1 < 1%                      | 4 (6.5%)                        | 54 (87.1%)                      |
|                                | concordance rate                | kappa coefficient               |
|                                | 93.5%                           | 0.635                           |

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assay, and the remaining 51 (82.3%) patients tested negative according to both the assays (Table 3B); the concordance rate was 95.2%, and Cohen’s kappa coefficient was 0.814 (almost perfect agreement, \( p < 0.001 \)). Higher PD-L1 expression was also noted using the E1L3N assay compared to the SP142 assay. Eight patients (12.9%) tested positive for PD-L1 expression on their TCs using either the E1L3N or the SP142 assay, and the remaining 54 (87.1%) patients tested negative according to both the assays (Table 3C); the concordance rate was 93.5%, and Cohen’s kappa coefficient was 0.635 (substantial agreement, \( p < 0.001 \)).

PD-L1 expression status on ICs based on sample age using the 73–10, SP142, and E1L3N assays

The rates of PD-L1 expression in samples of different ages as determining using the 73–10, SP142, and E1L3N assays are illustrated in Fig 5A. The positivity rates of PD-L1 expression...
using the 73–10, SP142, and E1L3N assays were 84.2%, 84.2%, and 52.6% in the samples aged < 5 years; 79.2%, 58.3%, and 45.8% in the samples aged 5 years ≤ and < 10 years, and 73.7%, 63.2%, and 42.1% in the samples aged > 10 years, respectively. The concordance rates of the SP 142 and E1L3N assays with the 73–10 assay in the samples aged < 5 years were 89.5% and 68.4%, and the Cohen’s kappa coefficients were 0.604 (substantial agreement, p = 0.008) and 0.345 (fair agreement, p = 0.047), respectively (Table 4A and 4B). The concordance rate between the SP142 and E1L3N assays in the samples aged < 5 years was 68.4%, and the Cohen’s kappa coefficient was 0.345 (fair agreement, p = 0.047) (Table 4C).

The concordance rates of the SP 142 and E1L3N assays with the 73–10 assay in samples aged 5 years ≤ and < 10 years were 79.2% and 66.7%, and the Cohen’s kappa coefficients were 0.538 (moderate agreement, p = 0.003) and 0.364 (fair agreement, p = 0.021), respectively (Table 4D and 4E). The concordance rate between the SP142 and E1L3N assays in samples

**Fig 2. Immunohistochemical staining for PD-L1 using the SP142 assay.** (A) PD-L1 is expressed in immune cells (×400). (B) No PD-L1 expression is noted in immune cells (×400). (C) PD-L1 is expressed in carcinoma cells (×400). (D) PD-L1 is not expressed in carcinoma cells (×400).

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aged 5 years ≤ and < 10 years was 87.5%, and the Cohen’s kappa coefficient was 0.753 (substantial agreement, \( p < 0.001 \)) (Table 4F).

The concordance rates of the SP 142 and E1L3N assays with the 73–10 assay in the samples aged > 10 years were 89.5% and 68.4%, and the Cohen’s kappa coefficients were 0.759 (substantial agreement, \( p = 0.001 \)) and 0.412 (moderate agreement, \( p = 0.026 \)), respectively (Table 4G and 4H). The concordance rate between the SP142 and E1L3N assays in the samples aged > 10 years was 78.9%, and the Cohen’s kappa coefficient was 0.596 (moderate agreement, \( p = 0.005 \)) (Table 4I).

**PD-L1 expression status on TCs based on sample age using the 73–10, SP142, and E1L3N assays**

PD-L1 expression rates on TCs based on different sample ages using the 73–10, SP142, and E1L3N assays are illustrated in Fig 5B. The positivity rates of PD-L1 expression using the 73–
Fig 4. Comparison of PD-L1 expression level on immune cells using the 73–10, SP142, and E1L3N assays.
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Fig 5. Comparison of PD-L1-positive ratio on immune cells (A) and tumor cells (B) based on sample age.
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10, SP142, and E1L3N assays were 5.3%, 0%, and 5.3% in the samples aged < 5 years; 20.8%, 8.3%, and 12.5% in the samples aged 5 years ≤ and < 10 years, and 26.3%, 10.5%, and 20.1% in the samples aged > 10 years, respectively. The concordance rates of the SP 142 and E1L3N assays with the 73–10 assay in the samples aged < 5 years were 94.7% and 100.0%, and the Cohen’s kappa coefficients were noncalculable and 1.000 (perfect agreement, \( p < 0.001 \)), respectively (Table 5A and 5B). The concordance rate between the SP142 and E1L3N assays in the samples aged < 5 years was 94.7%, and the Cohen’s kappa coefficient was noncalculable (Table 5C).

The concordance rates of the SP 142 and E1L3N assays with the 73–10 assay in samples aged 5 years ≤ and < 10 years were 87.5% and 91.7%, and the Cohen’s kappa coefficients were 0.514 (moderate agreement, \( p = 0.012 \)) and 0.855 (perfect agreement, \( p < 0.001 \)), respectively (Table 5D and 5E). The concordance rate between the SP142 and E1L3N assays in the samples aged 5 years ≤ and < 10 years was 95.8%, and the Cohen’s kappa coefficient was 0.778 (substantial agreement, \( p < 0.001 \)) (Table 5F).

The concordance rates of the SP 142 and E1L3N assays with the 73–10 assay in the samples aged > 10 years were 84.2% and 94.7%, and the Cohen’s kappa coefficients were 0.496 (moderate agreement, \( p = 0.012 \)) and 0.855 (perfect agreement, \( p < 0.001 \)), respectively (Table 5G and 5H). The concordance rate between the SP142 and E1L3N assays in the samples aged > 10 years was 89.5%, and the Cohen’s kappa coefficient was 0.612 (substantial agreement, \( p = 0.004 \)) (Table 5I).

PD-L1 expression status on ICs according to tumor diameter using the 73–10, SP142, and E1L3N assays

Positivity rates of PD-L1 expression for different tumor diameters according to the 73–10, SP142, and E1L3N assays are illustrated in Fig 6A. According to tumor diameter, the positivity rates of PD-L1 expression using the 73–10, SP142, and E1L3N assays were 87.1%, 77.4%, and 54.8% for tumor diameter ≤ 20 mm, and 71.0%, 58.1%, and 38.7% for tumor diameter > 20 mm, respectively. The concordance rates of the SP 142 and E1L3N assays with the 73–10 assay for tumors with a diameter ≤ 20 mm were 90.3% and 67.7%, and the Cohen’s kappa coefficients were 0.674 (substantial agreement, \( p < 0.001 \)) and 0.305 (fair agreement, \( p = 0.018 \)), respectively (Table 6A and 6B). The concordance rate between the SP142 and E1L3N assays for tumors with a diameter ≤ 20 mm was 77.4%, and the Cohen’s kappa coefficient was 0.523 (moderate agreement, \( p = 0.001 \)) (Table 6C). The concordance rates of the SP 142 and E1L3N assays with the 73–10 assay for tumors with a diameter > 20 mm were 80.6% and 67.7%, and the Cohen’s kappa coefficients were 0.585 (moderate agreement, \( p = 0.001 \)) and 0.411 (moderate agreement, \( p = 0.005 \)), respectively (Table 6D and 6E). The concordance rate between the SP 142 and E1L3N assays for tumors with a diameter > 20 mm was 80.6%, and the Cohen’s kappa coefficient was 0.627 (substantial agreement, \( p < 0.001 \)) (Table 6F).

PD-L1 expression status on TCs based on tumor diameter using the 73–10, SP142, and E1L3N assays

Positivity rates of PD-L1 expression on TCs with different tumor diameters according to the 73–10, SP142, and E1L3N assays are illustrated in Fig 6B. According to tumor diameter, the positivity rates of PD-L1 expression using the 73–10, SP142, and E1L3N assays were 16.1%, 3.2%, and 9.7% at tumor diameter ≤ 20 mm, and 19.4%, 9.7%, and 16.1% at tumor diameter > 20 mm, respectively. The concordance rates of the SP 142 and E1L3N assays with the 73–10 assay for tumors with a diameter ≤ 20 mm were 87.1% and 93.5%, and the Cohen’s kappa coefficients were 0.674 (substantial agreement, \( p < 0.001 \)) and 0.305 (fair agreement, \( p = 0.018 \)), respectively (Table 6A and 6B). The concordance rate between the SP142 and E1L3N assays for tumors with a diameter ≤ 20 mm was 77.4%, and the Cohen’s kappa coefficient was 0.523 (moderate agreement, \( p = 0.001 \)) (Table 6C). The concordance rates of the SP 142 and E1L3N assays with the 73–10 assay for tumors with a diameter > 20 mm were 80.6% and 67.7%, and the Cohen’s kappa coefficients were 0.585 (moderate agreement, \( p = 0.001 \)) and 0.411 (moderate agreement, \( p = 0.005 \)), respectively (Table 6D and 6E). The concordance rate between the SP 142 and E1L3N assays for tumors with a diameter > 20 mm was 80.6%, and the Cohen’s kappa coefficient was 0.627 (substantial agreement, \( p < 0.001 \)) (Table 6F).
| Sample Age | Assay       | PD-L1 ≥ 1% | PD-L1 < 1% | Concordance Rate | Kappa Coefficient |
|------------|-------------|------------|------------|------------------|-------------------|
| ≥ 5 years  | 73–10       |            |            |                  |                   |
|            | SP142 PD-L1 | 15 (78.9%) | 1 (5.3%)   |                  |                   |
|            | E1L3N PD-L1 | 6 (31.6%)  | 3 (15.8%)  |                  |                   |
|            |            |            |            |                  |                   |
|            | E1L3N      |            |            |                  |                   |
|            | SP142 PD-L1 | 10 (52.6%) | 0          |                  |                   |
|            | E1L3N PD-L1 | 6 (31.6%)  | 3 (15.8%)  |                  |                   |
| < 10 years | 73–10      |            |            |                  |                   |
|            | SP142 PD-L1 | 14 (58.3%) | 0          |                  |                   |
|            | E1L3N PD-L1 | 5 (20.8%)  | 5 (20.8%)  |                  |                   |
| < 10 years | E1L3N      |            |            |                  |                   |
|            | SP142 PD-L1 | 11 (45.8%) | 0          |                  |                   |
|            | E1L3N PD-L1 | 8 (33.3%)  | 5 (20.8%)  |                  |                   |
| ≤ 10 years | 73–10      |            |            |                  |                   |
|            | SP142 PD-L1 | 12 (63.2%) | 0          |                  |                   |
|            | E1L3N PD-L1 | 2 (10.5%)  | 5 (26.3%)  |                  |                   |

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### Table 5. Comparison of PD-L1 expression on TCs by 73–10, SP142, and E1L3N assays in sample ages.

#### 5 years

| Assay   | PD-L1 ≥ 1% | PD-L1 < 1% | Concordance Rate | Kappa Coefficient |
|---------|------------|------------|------------------|-------------------|
| (A) 73–10 |            |            |                  |                   |
| SP142   |            |            |                  |                   |
| PD-L1 ≥ 1% | 0          | 0          |                  |                   |
| PD-L1 < 1% | 1 (5.3%)  | 18 (94.7%) |                  |                   |
| E1L3N   |            |            |                  |                   |
| PD-L1 ≥ 1% | 1 (5.3%)  | 0          |                  |                   |
| PD-L1 < 1% | 0          | 18 (94.7%) |                  |                   |

#### 7–10 years

| Assay   | PD-L1 ≥ 1% | PD-L1 < 1% | Concordance Rate | Kappa Coefficient |
|---------|------------|------------|------------------|-------------------|
| (B) 73–10 |            |            |                  |                   |
| SP142   |            |            |                  |                   |
| PD-L1 ≥ 1% | 2 (8.3%)  | 0          |                  |                   |
| PD-L1 < 1% | 3 (12.5%) | 19 (79.2%) |                  |                   |
| E1L3N   |            |            |                  |                   |
| PD-L1 ≥ 1% | 3 (12.5%) | 0          |                  |                   |
| PD-L1 < 1% | 2 (8.3%)  | 19 (79.2%) |                  |                   |

#### 5 years and < 10 years

| Assay   | PD-L1 ≥ 1% | PD-L1 < 1% | Concordance Rate | Kappa Coefficient |
|---------|------------|------------|------------------|-------------------|
| (C) 73–10 |            |            |                  |                   |
| SP142   |            |            |                  |                   |
| PD-L1 ≥ 1% | 1 (5.3%)  | 18 (94.7%) |                  |                   |
| PD-L1 < 1% | 0          | 18 (94.7%) |                  |                   |
| E1L3N   |            |            |                  |                   |
| PD-L1 ≥ 1% | 1 (5.3%)  | 0          |                  |                   |
| PD-L1 < 1% | 0          | 18 (94.7%) |                  |                   |

#### 73–10 years

| Assay   | PD-L1 ≥ 1% | PD-L1 < 1% | Concordance Rate | Kappa Coefficient |
|---------|------------|------------|------------------|-------------------|
| (D) 73–10 |            |            |                  |                   |
| SP142   |            |            |                  |                   |
| PD-L1 ≥ 1% | 2 (8.3%)  | 0          |                  |                   |
| PD-L1 < 1% | 3 (12.5%) | 19 (79.2%) |                  |                   |
| E1L3N   |            |            |                  |                   |
| PD-L1 ≥ 1% | 3 (12.5%) | 0          |                  |                   |
| PD-L1 < 1% | 2 (8.3%)  | 19 (79.2%) |                  |                   |

#### ≤ 10 years

| Assay   | PD-L1 ≥ 1% | PD-L1 < 1% | Concordance Rate | Kappa Coefficient |
|---------|------------|------------|------------------|-------------------|
| (E) 73–10 |            |            |                  |                   |
| SP142   |            |            |                  |                   |
| PD-L1 ≥ 1% | 2 (8.3%)  | 1 (4.2%)  |                  |                   |
| PD-L1 < 1% | 0          | 21 (87.5%) |                  |                   |
| E1L3N   |            |            |                  |                   |
| PD-L1 ≥ 1% | 0          | 18 (94.7%) |                  |                   |
| PD-L1 < 1% | 4 (21.1%) | 14 (73.7%) |                  |                   |

#### ≤ 10 years

| Assay   | PD-L1 ≥ 1% | PD-L1 < 1% | Concordance Rate | Kappa Coefficient |
|---------|------------|------------|------------------|-------------------|
| (F) 73–10 |            |            |                  |                   |
| SP142   |            |            |                  |                   |
| PD-L1 ≥ 1% | 2 (10.5%) | 0          |                  |                   |
| PD-L1 < 1% | 3 (15.8%) | 14 (73.7%) |                  |                   |
| E1L3N   |            |            |                  |                   |
| PD-L1 ≥ 1% | 2 (10.5%) | 0          |                  |                   |
| PD-L1 < 1% | 4 (21.1%) | 15 (78.9%) |                  |                   |

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The concordance rate between the SP 142 and E1L3N assays for tumors with diameter \( \leq 20 \text{ mm} \) was 93.5%, and the Cohen’s kappa coefficient was 0.475 (moderate agreement, \( p = 0.002 \)) (Table 7C). The concordance rates of the SP 142 and E1L3N assays with the 73–10 assay for tumors with diameter > 20 mm were 90.3% and 96.8%, and the Cohen’s kappa coefficients were 0.617 (substantial agreement, \( p < 0.001 \)) and 0.890, respectively (perfect agreement, \( p < 0.001 \)) (Table 7D and 7E). The concordance rate between the SP 142 and E1L3N assays for tumors with diameter > 20 mm was 93.5%, and the Cohen’s kappa coefficient was 0.716 (substantial agreement, \( p < 0.001 \)) (Table 7F).

**Discussion**

In the present study, for the first time, we analyzed the PD-L1 expression status on ICs and TCs in TNBC tissues using the 73–10 assay and compared it with the expression status according to the SP142 and E1L3N assays. The highest positivity rate on ICs was observed using the 73–10 assay, followed by the SP142 and E1L3N assays, and the highest positivity rate on TCs was observed using the 73–10 assay followed by the E1L3N and SP142 assays. For ICs, a substantial agreement was observed in the concordance rate between the 73–10 and SP142 assays, and fair agreement in the concordance rate between and the 73–10 and E1L3N assays. For TCs, the concordance rate between the 73-10 and SP142 assays was in moderate agreement, and the rate between the 73-10 and E1L3N assays was in almost perfect agreement.

The SP142 assay is used for companion diagnostics for atezolizumab in patients with TNBC. The IMPassion130 trial clearly demonstrated that atezolizumab plus nab-paclitaxel significantly prolonged progression-free survival in advanced or metastatic PD-L1-positive TNBC patients [11]; the study defined PD-L1-positivity as PD-L1-expressing ICs \( \geq 1\% \) in the tumor area [11], which is the same definition used in this study. However, various immunohistochemical platforms have been developed to evaluate PD-L1 expression; thus, few studies have compared the differences between all the PD-L1 immunohistochemical assays in TNBC tissues [21–25]. For example, the 73–10 assay, which is the companion diagnostic tests for ave-lumab [16, 36], has not yet been analyzed in TNBC tissues.

Table 8 summarizes the comparisons of PD-L1 expression on ICs using different antibodies according to the results of previous studies and the present one. The rates of PD-L1 expression were relatively different among these studies [21–25]. The PD-L1 positivity rates using the
SP142 assay ranged from 19.3% to 67.7% and those using the 22C3 assay ranged from 32.6% to 81% (the 22C3 assay was analyzed by a combined positive score). Although PD-L1-positivity rate on ICs was defined as more than one PD-L1-positive IC in one study [23], the remaining studies, including the present one, used the same definition (PD-L1-expressing IC ≥ 1%). Our cohort showed the highest positivity rate using the SP142 assay (67.7%).

Table 9 summarizes the comparisons of PD-L1 expression status on TCs using different antibodies according to the results of previous studies and the present one. In contrast to the results obtained for ICs, the positivity rates of PD-L1 expression on TCs were relatively consistent among all previous studies [21–23]. The PD-L1 positivity rates based on the SP142 assay ranged from 5.1% to 16.8%. All studies, including the present one, defined PD-L1-expressing TC ≥ 1% as positive [21–23].

The sample size, population, and interobserver variation may have influenced these results [22, 37]. Except for the post-hoc immunohistochemical analysis of the IMpassion130 trial [24], and another study [25], four studies, including the present one, used the tissue microarray (TMA) technique to evaluate PD-L1 expression. Selection bias of the tumor sample may...
influence the positivity rate of PD-L1 expression because PD-L1 expression can show heterogeneity within the same tumor tissue [22]. Moreover, the patient population may also influence the difference in PD-L1 expression. The patients in the IMpassion130 trials had metastatic or unresectable advanced TNBC [24]. In contrast, most of our patients had no

Table 7. Comparison of PD-L1 expression on TCs by 73–10, SP142, and E1L3N assays in tumor diameters.

| Tumor Diameter | 73–10 | 20mm < Tumor Diameter |
|----------------|-------|-----------------------|
|                |       |                       |
| (A)            |       |                       |
| SP142          |       |                       |
| PD-L1 ≥ 1%     | 73–10 | 3 (9.7%)              |
| PD-L1 < 1%     | 73–10 | 2 (6.5%)              |

| (B)            |       |                       |
| E1L3N          |       |                       |
| PD-L1 ≥ 1%     | 73–10 | 3 (9.7%)              |
| PD-L1 < 1%     | 73–10 | 2 (6.5%)              |

| (C)            |       |                       |
| E1L3N          |       |                       |
| PD-L1 ≥ 1%     | 73–10 | 3 (9.7%)              |
| PD-L1 < 1%     | 73–10 | 2 (6.5%)              |

| (D)            |       |                       |
| SP142          |       |                       |
| PD-L1 ≥ 1%     | 73–10 | 3 (9.7%)              |
| PD-L1 < 1%     | 73–10 | 2 (6.5%)              |

| (E)            |       |                       |
| E1L3N          |       |                       |
| PD-L1 ≥ 1%     | 73–10 | 5 (16.1%)             |
| PD-L1 < 1%     | 73–10 | 1 (3.2%)              |

| (F)            |       |                       |
| SP142          |       |                       |
| PD-L1 ≥ 1%     | 20mm  | 3 (9.7%)              |
| PD-L1 < 1%     | 20mm  | 2 (6.5%)              |

Table 8. Comparison of PD-L1 expression among different primary antibodies.

| Reference | 28–8 | 22C3 | SP142 | SP263 | E1L3N | 73–10 | No. of patients | Specimens |
|-----------|------|------|-------|-------|-------|-------|----------------|-----------|
| 21        | ND   | 51.6%| 52.6% | 71.6% | ND    | ND    | 95             | TMA       |
| 22        | 35.8%| 32.6%| 28.4% | ND    | ND    | ND    | 95             | TMA       |
| 23        | 36.7%| ND   | 19.3% | ND    | 37.6% | ND    | 218            | TMA       |
| 24        | ND   | 80.9%| 46.4% | 74.9% | ND    | ND    | 614            | Whole     |
| 25        | 63.3%| 56.7%| 60.0% | 86.7% | ND    | ND    | 30             | Whole     |
| Present study | ND   | ND   | 67.7%| ND    | 46.8% | 79.0% | 62             | TMA       |

ND, Not done; TMA, Tissue microarray
metastasis [22], and the present study included TNBC patients in various stages with or without metastasis. Moreover, the ratio of LPBCs in the cohort might have influenced the PD-L1-positive rate. This cohort comprised 30.6% LPBC cases, and this type of information is available in only one other study (28.9%) [23]. Thus, additional studies are needed to clarify the PD-L1 expression status in a larger patient population, which should also record the percentage of LPBC cases.

Although the positivity rates of PD-L1 on ICs were relatively different among the studies, the concordance among primary antibodies of PD-L1 was relatively high in these studies. Previous reports demonstrated 91.2% concordance between the SP263 and SP142 assays [21], 86.2% between the 28–8 and E1L3N assays, 78.0% between the E1L3N and SP142 assays [23], 95% between the 28–8 and 22C3 assays, 84% between the 28–8 and SP142 assays, and 85% between the 22C3 and SP142 assays [22]. The present study showed 85.5% concordance between the 73–10 and SP142 assays, and 67.7% between the 73–10 and E1L3N assays.

Moreover, the differences in the positivity rates of PD-L1 expression on TCs among different studies were less, and the concordance rate among primary antibodies of PD-L1 was also high. Previous reports demonstrated 70.0% concordance between the SP263 and SP142 assays [21], 92.9% between the 28–8 and 22C3 assays, 88.8% between the 28–8 and SP142 assays, and 89.8% between the 22C3 and SP142 assays [22], and the kappa value between the 28–8 and E1L3N assays was 0.752, and between the SP142 and E1L3N assays was 0.537 [23]. The present study showed 88.7% concordance between the 73–10 and SP142 assays (kappa coefficient: 0.485), and 95.2% between the 73–10 and E1L3N assays (kappa coefficient: 0.814).

This study also demonstrated substantial agreement between the 73–10 and SP142 assays on ICs. However, the present study provided no information to assess the predictive value of the efficacy of anti-PD-L1 immunotherapy because none of the patients in this cohort were treated with this therapy.

Moreover, the present study showed that the sample age and tumor diameter did not influence the PD-L1 expression rates on both ICs and TCs among the three primary antibodies for PD-L1. This was the first time that such an observation was made. Consistent with the results of this study, a previous study showed that sample age did not influence the PD-L1-positive ratio (28–8 and 22C3 assays) in non-small cell lung cancer [20].

As described earlier, there were some limitations to the present study. First, this study used a small sample size (approximately 50% patients had Nottingham histological grade 3) from a single institution, which could have led to the selection bias. Second, TMA was used to evaluate PD-L1 expression; this may have led to selection bias, although we selected regions that were the most representative of carcinoma tissue. In TNBC tissues, it is recommended that a whole section should be used for the evaluation of PD-L1 expression; however, in this study, we did not aim to assess prognostic or diagnostic significance of PD-L1 expression, instead we compared PD-L1 expression in the same sample using three different assays; thus, the use of TMA may be acceptable. Third, the present study provided no information to assess the predictive value of the efficacy of anti-PD-L1 immunotherapy. Thus, further studies are needed to

| Reference | 28–8 | 22C3 | SP142 | SP263 | E1L3N | 73–10 | No. of patients | Specimens |
|-----------|------|------|-------|-------|-------|-------|----------------|-----------|
| 21        | ND   | 50.5%| 16.8% | 52.6% | ND    | ND    | 95             | TMA       |
| 22        | 16.3%| 13.3%| 5.1%  | ND    | ND    | ND    | 98             | TMA       |
| 23        | 13.3%| ND   | 11.5% | ND    | 14.7% | ND    | 218            | TMA       |
| Present study | ND   | ND   | 6.4%  | ND    | 12.9% | 17.7% | 62             | TMA       |

ND, Not done; TMA, Tissue microarray

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clarify the PD-L1 expression status among various primary antibodies in a larger population of patients treated with anti-PD-L1 immunotherapy.

In conclusion, the present study demonstrated that the positivity rates of PD-L1 expression on ICs were the highest using the 73–10 assay, followed by the SP142 and E1L3N assays, and there was substantial agreement in the concordance rate between the 73–10 and SP142 assays. However, further studies are needed to clarify the PD-L1 expression status among various primary antibodies in a larger patient population treated with anti-PD-L1 immunotherapy [38]. This would be a prerequisite to the establishment of an effective evaluation method to assess the predictive value of anti-PD-L1 immunotherapies.

Supporting information

S1 File. Clinicopathological characteristics of patients with triple-negative breast cancer. (PDF)

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References

1. Cleator S, Heller W, Coombes RC. Triple-negative breast cancer: therapeutic options. Lancet Oncol. 2007; 8: 235–244. https://doi.org/10.1016/S1470-2045(07)70074-8 PMID: 17329194

2. Dent R, Trudeau M, Pritchard KI, Hanna WM, Kahn HK, Sawka CA, et al. Triple-negative breast cancer: clinical features and patterns of recurrence. Clin Cancer Res. 2007; 13: 4429–4434. https://doi.org/10.1158/1078-0432.CCR-06-3045 PMID: 17671126

3. Foulkes William D, Smith Ian E, Reis-Filho Jorge S. Triple-negative Breast Cancer. N Engl J Med. 2010; 363: 1938–1948. https://doi.org/10.1056/NEJMra1001389 PMID: 21067385

4. 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: 361–370. https://doi.org/10.1158/2326-6066.CIR-13-0127 PMID: 24764583

5. Tung N, Garber JE, Hacker MR, Torous V, Freeman GJ, Poles E, 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. https://doi.org/10.1038/npjbcancer.2016.2 PMID: 28721372
6. Ali HR, Gliont SE, Blows FM, Provenzano E, Dawson SJ, Liu B, et al. PD-L1 protein expression in breast cancer is rare, enriched in basal-like tumours and associated with infiltrating lymphocytes. Ann Oncol. 2015; 26: 1488–1493. https://doi.org/10.1093/annonc/mdv192 PMID: 25897014

7. Wang C, Zhu H, Zhou Y, Mao F, Lin Y, Pan B, et al. Prognostic value of PD-L1 in breast cancer: a meta-analysis. Breast J. 2017; 23: 436–443. https://doi.org/10.1111/tbj.12753 PMID: 28079291

8. Dill EA, Gru AA, Atkins KA, 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: 334–342. https://doi.org/10.1097/PAS.0000000000000780 PMID: 28195880

9. Mori H, Kubo M, Yamaguchi R, Nishimura R, Osako T, Arima N, et al. The combination of PD-L1 expression and decreased tumor-infiltrating lymphocytes is associated with a poor prognosis in triple-negative breast cancer. Oncotarget. 2017; 8: 15584–15592. https://doi.org/10.18632/oncotarget.14698 PMID: 28107186

10. Li Z, Dong P, Ren M, Song Y, Qian X, Yang Y, et al. PD-L1 expression is associated with tumor FOXP3(+) regulatory T-cell infiltration of breast cancer and poor prognosis of patient. J Cancer. 2016; 7: 784–793. https://doi.org/10.7150/jca.14549 PMID: 27162536

11. Schmid P, Adams S, Rugo HS, Schneweiss A, Barrios CH, Iwata H, et al. Atezolizumab and nab-paclitaxel in advanced triple-negative breast cancer. N Engl J Med. 2018; 379: 2108–2121. https://doi.org/10.1056/NEJMoa1809615 PMID: 30345906

12. Lawson NL, Dix CI, Scorer PW, Stubbs CJ, Wong E, Hutchinson L, et al. Mapping the binding sites of antibodies utilized in programmed cell death ligand-1 predictive immunohistochemical assays for use with immunono-cology therapies. Mod Pathol. 2020; 33: 518–530. https://doi.org/10.1038/s41379-019-0372-z PMID: 31558782

13. U.S. Food and Drug Administration. Dako PD-L1 IHC 22C3 pharmDx [cited]. Available from: https://www.fda.gov/medical-devices/recently-approved-devices/pd-l1-ihc-22c3-pharmdx-p150013s014.

14. Bristol-Myers Squibb. Opdivo® [cited]. Available from: https://news.bms.com/news/corporate-financial/2020/US-Food-and-Drug-Administration-Approves-Opdivo-nivolumab—Yervoy-ipilimumab-as-First-Line-Treatment-of-Patients-with-Metastatic-Non-Small-Cell-Lung-Cancer-Whose-Tumors-Express-PD-L1/default.aspx.

15. Rebeatto 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. https://doi.org/10.1186/s13000-016-0545-8 PMID: 27717372

16. Dirix LY, Takacs I, Jerusalem G, Nikolinas P, Arkenau HT, Forero-Torres A, et al. Avelumab, an anti-PD-L1 antibody, in patients with locally advanced or metastatic breast cancer: a phase 1b JAVELIN Solid Tumor study. Breast Cancer Res Treat. 2018; 167: 671–686. https://doi.org/10.1007/s10549-017-4537-5 PMID: 29063313

17. Mahoney Kathleen M, Heather Sun, Xiaoyun Liao, Ping Hua, Marcella Callea, EA Greenfield, et al. Antibodies to the cytoplasmic domain of PD-L1 most clearly delineate cell membranes in immunohistochemical staining. Cancer Immunol Res. 2015; 3: 1308–1315. https://doi.org/10.1158/2326-6066.CIR-15-0116 PMID: 26546452

18. Hirsch FR, McElhinny A, Stanforth D, Ranger-Moore J, Jansson M, Kulangara K, 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; 12:208–222. https://doi.org/10.1016/j.jtho.2016.11.2228 PMID: 27913228

19. Grote HJ, Feng Z, Schlichting M, Helwig C, Ruisi M, Jin H, et al. Programmed death-ligand 1 immunohistochemistry assay comparison studies in NSCLC: Characterization of the 73–10 assay. J Thorac Oncol. 2020; 15: 1306–1316. https://doi.org/10.1016/j.jtho.2020.04.013 PMID: 32353599

20. Saito T, Tsuta K, Ishida M, Ryota H, Takeyasu Y, Fukushima KJ, et al. Comparative study of programmed cell death ligand-1 immunohistochemistry assays using 22C3 and 28–8 antibodies for non-small cell lung cancer: Analysis of 420 surgical specimens from Japanese patients. Lung Cancer. 2018; 125: 230–237. https://doi.org/10.1016/j.lungcan.2018.10.005 PMID: 30429026

21. Lee SE, Park HY, Lim SD, Han HS, Yoo YB, Kim WS. Concordance of programmed death-ligand 1 expression between SP142 and 22C3/SP263 assays in triple-negative breast cancer. J Breast Cancer. 2020; 23: 303–313. https://doi.org/10.4048/jbc.2020.23.e37 PMID: 32595992

22. Huang X, Ding Q, Guo H, Gong Y, Zhao J, Zhao M, et al. Comparison of three FDA-approved diagnostic immunohistochemistry assays of PD-L1 in triple-negative breast carcinoma. Hum Pathol. 2021; 108: 42–50. https://doi.org/10.1016/j.humpath.2020.11.004 PMID: 33221342
23. Sun WY, Lee YK, Koo JS. Expression of PD-L1 in triple-negative breast cancer based on different immunohistochemical antibodies. J Transl Med. 2016; 14(1): 173. https://doi.org/10.1186/s12967-016-0925-6 PMID: 27286842

24. Rugo HS, Loi S, Adams S, Schmid P, Schneeweiss A, Barrios CH, et al. Performance of PD-L1 immunohistochemistry (IHC) assays in unresectable locally advanced or metastatic triple-negative breast cancer (mTNBC): post-hoc analysis of IMpassion130. Ann Oncol. 2019; 30: v858–v859. https://doi.org/10.1093/annonc/mdz394.009

25. Noske A, Ammann JU, Wagner DC, Denkert C, Lebeau A, Sinn P, et al. A multicentre analytical comparison study of inter-reader and inter-assay agreement of four programmed death-ligand 1 immunohistochemistry assays for scoring in triple-negative breast cancer. Histopathology. 2021; 78(4):567–577. https://doi.org/10.1111/his.14254 PMID: 32936950

26. Rakha EA, Allison KH, Bu H, Ellis IO, Foschini MP, Horii R, et al. Invasive breast carcinoma of no special type. In WHO Classification of Tumours. 5th Edition. Breast Tumours. Lyon. IARC; 2019. pp. 102–109.

27. Yoshikawa K, Ishida M, Yanai H, Tsuta K, Sekimoto M, Sugie T. Adipophilin expression is an independent marker for poor prognosis of patients with triple-negative breast cancer: An immunohistochemical study. PLoS One. 2020; 15: e0242563. https://doi.org/10.1371/journal.pone.0242563 PMID: 33201923

28. Yoshikawa K, Ishida M, Yanai H, Tsuta K, Sekimoto M, Sugie T. Prognostic significance of PD-L1-positive cancer-associated fibroblasts in patients with triple-negative breast cancer. BMC Cancer. 2021; 21: 239. https://doi.org/10.1186/s12885-021-07970-x PMID: 33676425

29. Vennapusa B, Baker B, Kowanetz M, Boone J, Menzl I, Bruey JM, et al. Development of a PD-L1 complementary diagnostic immunohistochemistry assay (SP142) for atezolizumab. Appl Immunohistoch Mol Morphol. 2019; 27: 92–100. https://doi.org/10.1097/PAI.0000000000000594 PMID: 29346180

30. Moehler M, Dvorkin M, Boku N, Özgüroğlu M, Ryu MH, Muntean AS, et al. Phase III trial of avelumab maintenance after first-line induction chemotherapy versus continuation of chemotherapy in patients with gastric cancers: results from JAVELIN Gastric 100. J Clin Oncol. 2021; 39: 966–977. https://doi.org/10.1200/JCO.20.00892 PMID: 33197226

31. Rimm DL, Han G, Taube JM, Yi ES, Bridge JA, Flieder DB, et al. Reanalysis of the NCCN PD-L1 companion diagnostic assay study for lung cancer in the context of PD-L1 expression findings in triple-negative breast cancer. Breast Cancer Res. 2019; 21: 72. https://doi.org/10.1186/s13058-019-1156-6 PMID: 31196152

32. Vranic S, Cyprian FS, Gatalica Z, Palazzo J. PD-L1 status in breast cancer: Current view and perspectives. Semin Cancer Biol. 2021; 72: 146–154. https://doi.org/10.1016/j.semcancer.2019.12.003 PMID: 31883913