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Clinicopathological and Prognostic Significance of Programmed Death Ligand-1 SP142 Expression in 132 Patients With Triple-negative Breast Cancer

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Abstract. *Background/Aim: The prognostic value of programmed death ligand-1 (PD-L1) expression in triple-negative breast cancer (TNBC) has not been sufficiently investigated. In this study, we examined whether PD-L1 expression status is associated with clinicopathological features and outcomes of patients with TNBC. Patients and Methods: Immunostaining for PD-L1 SP142 was performed on tissue microarrays containing 132 TNBC samples. High PD-L1 expression was defined as ≥10% of the tumor area occupied by PD-L1-expressing cells. Results: Thirty-five (26.5%) patients showed high PD-L1 SP142 expression on immune cells (ICs). High IC PD-L1 expression was significantly correlated with smaller tumor size (p=0.030), absence of lymphovascular invasion (p=0.024), and fewer lymph node metastases (p=0.002). Multivariate survival analysis revealed that high IC PD-L1 expression independently predicted better disease-free survival (DFS) of TNBC patients. Conclusion: High PD-L1 SP142 expression on ICs was significantly associated with favorable clinicopathological parameters and better outcomes in patients with TNBC. Our observations suggest that high IC PD-L1 expression can be used as an independent prognostic marker for predicting better DFS in patients with TNBC.*

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Triple-negative breast cancer (TNBC) is an aggressive type of breast cancer that lacks expression of estrogen and progesterone receptors and amplification or over-expression of human epidermal growth factor receptor 2 (HER2) (1-3). TNBC accounts for nearly one-fifth of all cases of breast cancer (4, 5), and more frequently affects young women (6). TNBC patients exhibit adverse clinicopathological features and unfavorable prognoses compared to patients with other types of breast cancer (7, 8). Particularly, early-stage TNBC is associated with a higher risk of recurrence than other types, and advanced-stage disease has a poor prognosis with a median survival of 18 months (9). The absence of hormone receptors renders endocrine and human epidermal growth factor receptor 2-targeted therapies ineffective, leaving cytotoxic chemotherapy as the standard treatment option (10). However, chemotherapy for TNBC patients results in unsatisfactory long-term results, such as suboptimal response rates and short overall survival and response durations (11-13). Therefore, novel therapeutic strategies to improve outcomes are urgently needed.

Recent advances in the immune landscape of the tumor microenvironment have shed light on novel targeted opportunities for TNBC. TNBC possesses enriched tumor-infiltrating lymphocytes (TILs) compared to other types of cancer (10, 14), suggesting that a subset of TNBCs is immunogenically active. A higher level of TILs, an important component of the local tumor microenvironment, has been reported to be significantly associated with better cancer-specific survival in patients with TNBC (15). Furthermore, TNBC has a greater number of somatic mutations due to genomic instability, leading to an abundant load of neoantigens (14). The presence of TILs and tumor neoantigens correlates with programmed death-1 (PD-1)/programmed death ligand-1 (PD-L1) expression in patients with TNBC (16, 17). Moreover, the PD-1/PD-L1 pathway plays a critical role in regulating the immune response. TNBC has a unique microenvironment with a higher level of PD-L1 expression than normal breast tissue and other breast cancer types (17). Additionally, The Cancer Genome Atlas RNA sequencing data showed significant over-expression of the *PD-L1* mRNA in TNBC compared to non-TNBC (17). Thus, the emergence of immunotherapy targeting the PD-1/PD-L1 pathway will change the future treatment landscape for TNBC.

Atezolizumab, a monoclonal antibody targeting PD-L1, received accelerated approval from the US Food and Drug Administration (FDA) to be combined with nab-paclitaxel for patients with metastatic or unresectable locally advanced TNBC whose tumors express PD-L1. The IMpassion130 trial strongly confirmed that atezolizumab plus nab-paclitaxel improved both progression-free survival and overall survival in PD-L1-positive TNBC patients compared to nab-paclitaxel monotherapy (18, 19). Simultaneously, the Ventana PD-L1 SP142 assay (Ventana Medical Systems, Oro Valley, AZ, USA), was approved as a companion diagnostic device.

While the predictive value of PD-L1 expression has been documented, its prognostic role has not been sufficiently investigated in patients with TNBC. Particularly, differences in the prognostic significance have been observed depending on which clones were used, where PD-L1 expression was evaluated, or which cutoff values were used. The purpose of this study was to evaluate the prevalence of positive PD-L1 SP142 expression in patients with TNBC and to examine its association with clinicopathological features and outcomes of patients with TNBC.

Patients and Methods

Patient selection. The Institutional Review Board of Kangbuk Samsung Hospital reviewed and approved this study (approval number: 2022-05-030). We collected 132 consecutive cases of primary TNBC. The final diagnoses were established based on the fifth edition of the World Health Organization Classification of Breast Tumours (20). Pathologists inspected the resected specimens prior to fixation in 10% neutral-buffered formalin. The tissues were macroscopically examined and sectioned after fixation for 12-24 h. The sections were embedded in paraffin blocks after being processed with an automatic tissue processor. Using a rotary microtome, each formalin-fixed, paraffin-embedded (FFPE) tissue block was cut into 4 μm-thick slices, which were subsequently stained with hematoxylin and eosin in an automatic staining instrument. All available hematoxylin and eosin-stained slides were examined by two board-certified pathologists. The following clinicopathological information was obtained from electronic medical records and pathology reports: patients' age, histological grade (21), pathological tumor (pT) and nodal (pN) stage, lymphovascular invasion (LVI), tumor multiplicity, extensive intraductal component, first post-operative recurrence or metastasis, and follow-up period. The modified Bloom-Richardson grading system was applied to assign histological grades. The eighth edition of the American Joint Committee on Cancer Staging Manual was used for pathological staging (22). To estimate disease-free survival (DFS), patients were followed-up from the date of surgery to the date of death or other events, such as locoregional recurrence or distant metastasis. The development of recurrence or metastasis was confirmed by computed tomography or magnetic resonance imaging.

Tissue microarray. Tissue microarray blocks were constructed as previously described (1, 23). In brief, the two most representative tumor areas were marked on all hematoxylin and eosin-stained slides, and the corresponding FFPE tissue blocks. Two 2-mm diameter tissue cores were taken from marked tumor areas in each block and manually assembled into recipient tissue microarray blocks. Each core had more than 70% of tumor volume. For recipient blocks, holes for array cores at 1-mm intervals were created using an X-Y position guide and the appropriate instruments. The obtained tissue core was transferred into holes in the recipient block. Finally, for each case, a pair of tissue microarray blocks was constructed.

Immunostaining. The 4-μm slices from FFPE blocks were subjected to a series of processes including deparaffinization, dehydration with xylene, and rehydration in a graded series of alcohol solutions. Immunostaining was performed using an automatic immunostainer and a compact polymer method (Bond Intense Detection Kit, Leica Biosystems, Newcastle upon Tyne, UK) (1, 21, 23-27). We used a rabbit recombinant monoclonal PD-L1 antibody (clone SP142, prediluted, Ventana Medical Systems) as the primary antibody. After chromogenic visualization (EnVision+ Detection Systems, Dako, Glostrup, Denmark), the slices were counterstained with hematoxylin and coverslipped. PD-L1 expression was evaluated in both immune cells (ICs) and tumor cells (TCs) (28, 29). The scoring of PD-L1 expression on ICs was evaluated as the percentage of the tumor area covered with any detectable PD-L1 staining of any intensity. The tumor area was defined as the region occupied by TCs with intratumoral and peritumoral stroma. The ICs included macrophages, dendritic cells, and granulocytes as well as lymphocytes. For TCs, PD-L1 expression was scored as the proportion of tumor area with any intensity. The ICs and TCs were scored using continuous scores (0-100%) and categorical scores (<1%, 1-9%, 10-49%, and ≥50%). High PD-L1 expression was defined as ≥10% of the tumor area occupied by PD-L1-expressing cells at any staining intensity.

Table I. *Baseline characteristics of 132 patients with triple-negative breast cancer.*

Parameter		Number of cases (%)
Age (years)	<53	58 (43.9)
	≥53	74 (56.1)
Histological grade	1	5 (3.8)
	2	43 (32.6)
	3	84 (63.6)
Pathological tumor stage (pT)	pT1a	3 (2.3)
	pT1b	5 (3.8)
	pT1c	47 (35.6)
	pT2	69 (52.3)
	pT3	8 (6.1)
Pathological nodal stage (pN)	pN0	89 (67.4)
	pN1	25 (18.9)
	pN2	9 (6.8)
	pN3	9 (6.8)
Lymphovascular invasion	Absent	98 (74.2)
	Present	34 (25.8)
Multiplicity	Absent	124 (93.9)
	Present	8 (6.1)
Extensive intraductal component	Absent	126 (95.5)
	Present	6 (4.5)

Statistical analysis. All statistical analyses were performed using STATA version 17.0 software (StataCorp, College Station, TX, USA). Independent two-sample *t*-tests, Pearson's Chi-squared test, Fisher's exact test, or linear-by-linear association test was used to determine the association between PD-L1 SP142 expression status and clinicopathological features of TNBC patients. Univariate and multivariate survival analyses were used to examine the prognostic significance of PD-L1 SP142 expression. Curves for DFS were drawn using the Kaplan-Meier method, and differences were analyzed by applying the log-rank test for univariate survival analysis. Multivariate survival analysis was performed using the Cox proportional hazards model (95% confidence interval) with the backward stepwise elimination method. Statistical significance was defined as a p-value <0.05.

Results

Baseline clinicopathological characteristics of TNBC. Table I summarizes the baseline clinicopathological characteristics of the 132 TNBC patients enrolled in this study. The mean age of patients at the time of surgery was 53 years (range=25-85 years). Approximately 63.6% of the TNBC cases (84/132) were classified as histological grade 3 tumors, while 3.8% (5/132) and 32.6% (43/132) of cases were diagnosed as histological grades 1 and 2, respectively. Additionally, more than half of the tumors (77/132; 58.3%) were staged as pT2 or higher, and 43 patients (32.6%) had lymph node metastases. Moreover, the pN stage distribution was as follows: pN0: 67.4% (89/132), pN1: 18.9% (25/132), pN2: 6.8% (9/132), and pN3: 6.8% (9/132). LVI was detected in 34 (25.8%) cases. Finally, only 8 patients (6.1%) had multiple tumors.

Table II. *Programmed death ligand 1 (PD-L1) SP142 expression on immune cells (ICs) and tumor cells (TCs).*

PD-L1 SP142 expression		Number of cases (%)	
		IC	TC
Low	<1%	51 (38.6)	90 (68.2)
	1-9%	46 (34.8)	36 (27.3)
High	10-49%	27 (20.5)	3 (2.3)
	≥50%	8 (6.1)	3 (2.3)

PD-L1 SP142 expression in ICs and TCs of TNBC. We conducted PD-L1 SP142 immunostaining on tissue microarray blocks containing 132 FFPE TNBC tissue samples. The PD-L1 expression status in ICs and TCs of TNBC is shown in Table II. Regarding ICs, 35 cases (26.5%) were classified as IC PD-L1-high TNBC. Among these, eight cases (6.1%) exhibited IC PD-L1 expression ≥50%. In contrast, 51 tumors (38.6%) showed IC PD-L1 expression <1%. Regarding TCs, only six cases (4.6%) expressed high TC PD-L1 levels. In most cases (95.5%), TCs showed low PD-L1 immunoreactivity. In particular, 68.2% (90/132) of TC samples exhibited <1% of PD-L1 expression. Representative photomicrographs depicting PD-L1 SP142 immunoreactivities in ICs and TCs of TNBC are demonstrated in Figure 1.

Association between PD-L1 expression status and clinicopathological characteristics of patients with TNBC. A total of 132 TNBC patients were divided into two groups according to their IC PD-L1 expression status, with a cutoff value of 10%. Table III summarizes the results of the statistical analysis of the clinicopathological significance of IC PD-L1 expression in TNBC. High IC PD-L1 expression was significantly associated with lower pT (smaller tumor size; p=0.030), fewer lymph node metastases (p=0.002), and the absence of LVI (p=0.024). Most cases (62/77; 80.5%) of pT2-3 TNBC (>2 cm) showed low IC PD-L1 expression, while more than half (20/35; 57.1%) of the patients with high IC PD-L1 expression had pT1 (≤2 cm) tumors. The mean number of metastatic lymph nodes was significantly lower in IC PD-L1-high patients (0.43) than in IC PD-L1-low patients (2.78). The majority of IC PD-L1-high patients (31/35; 88.6%) did not have LVI. Additionally, although the presence of lymph node metastasis was not significantly associated with IC PD-L1 expression, the frequency of LVI was significantly different according to IC PD-L1 expression status. The differences in age, tumor multiplicity, and the presence of an extensive intraductal component between IC PD-L1-high and -low patients were not statistically significant. Finally, no significant difference was observed in clinicopathological characteristics of TNBC patients according to TC PD-L1 expression status (data not shown).

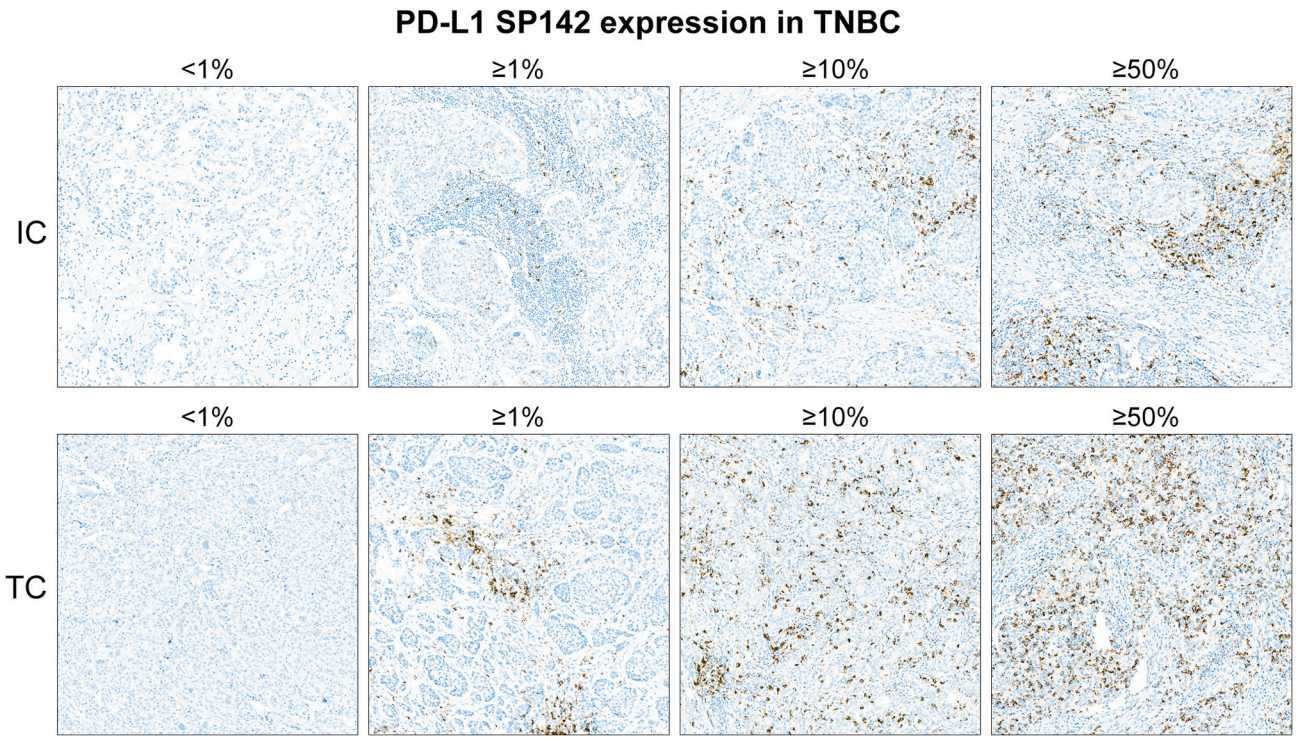

Figure 1. *Programmed death ligand-1 (PD-L1) SP142 immunoreactivity in immune cells (ICs) and tumor cells (TCs) of triple-negative breast cancer (TNBC). Original magnification, 100×.*

Association between PD-L1 expression status and patient outcome. Univariate and multivariate survival analyses were performed to investigate the prognostic significance of IC PD-L1 expression in TNBC patients (Table IV). The median post-operative follow-up time was 32 months. Moreover, 26 (20.0%) patients developed post-operative locoregional or metastatic recurrences. Univariate analysis revealed that pN1 (p=0.018) and pN2-3 (p=0.005) significantly predicted worse DFS. Moreover, high IC PD-L1 expression was significantly associated with favorable DFS with a hazard ratio of 0.12 (p=0.020). Kaplan-Meier plots showing the difference in DFS according to the expression status of IC PD-L1 in patients with TNBC demonstrated that patients whose tumor exhibited high IC PD-L1 expression showed better DFS than those with low PD-L1 expression (Figure 2). In addition to these three significant clinicopathological parameters, tumor multiplicity, which showed a marginal significance in univariate analysis (p=0.092), was entered into the multivariate analysis with a backward stepwise elimination method. As a result, pN1, pN2-3, and high IC PD-L1 expression maintained their statistical significance at the multivariate level (p=0.015, p=0.014, and p=0.038, respectively), indicating that these parameters were independent prognostic factors for predicting worse DFS in patients with TNBC. The hazard ratio for IC PD-L1-high patients compared to IC PD-L1-low patients was 0.10 in multivariate analysis. Tumor multiplicity of TNBC was not an independent predictor of DFS (p=0.908) in this study. Finally, no significant differences were observed in DFS according to TC PD-L1 expression status (data not shown).

Discussion

PD-L1 is expressed in TCs, as well as ICs. The binding of PD-L1 to its receptor, PD-1 prevents autoimmunity by the suppression of T cell effector functions (30, 31). In tumors, where PD-L1 expression is upregulated, the immunosuppressive effect of PD-1/PD-L1 signaling helps the tumor escape from the anti-tumor immune response (32). Immune checkpoint inhibition by blocking the PD-1/PD-L1 axis has emerged as an effective treatment for various solid tumors (33). PD-L1 expression has been evaluated as a biomarker for predicting the therapeutic response to anti-PD-1/PD-L1 drugs. Since TNBC was found to exhibit a higher tumor mutational burden, TILs, and PD-L1 expression than other types of breast cancer, immune checkpoint inhibitors have been considered an effective strategy for the treatment of TNBC (34). Previous studies of TNBC have shown that the expression of PD-L1 occurs mainly on tumor-infiltrating ICs, rather than TCs (17, 35). The IMpassion130 trial confirmed the overall survival benefit of

Table III. *Relationship between programmed death ligand-1 (PD-L1) SP142 expression on immune cells (ICs) and clinicopathologic parameters of 132 patients with triple-negative breast cancer.*

Parameter		IC PD-L1 SP142 expression		*p*-Value
		Low (<10%)	High (≥10%)	
Age (years; mean±SD)		54.13±12.77	50.43±10.24	0.125
Histological grade	1	5 (100.0)	0 (0.0)	0.056
	2	36 (83.7)	7 (16.3)	
	3	56 (66.7)	28 (33.3)	
Pathological tumor stage (pT)	pT1	35 (63.6)	20 (36.4)	0.030*
	pT2-3	62 (80.5)	15 (19.5)	
Lymph node metastasis	Absent	62 (69.7)	27 (30.3)	0.152
	Present	35 (81.4)	8 (18.6)	
Number of lymph node metastasis (mean±SD)		2.78±6.99	0.43±0.95	0.002*
Lymphovascular invasion	Absent	67 (68.4)	31 (31.6)	0.024*
	Present	30 (88.2)	4 (11.8)	
Multiplicity	Absent	89 (71.8)	35 (28.2)	0.109
	Present	8 (100.0)	0 (0.0)	
Extensive intraductal component	Absent	92 (73.0)	34 (27.0)	1.000
	Present	5 (83.3)	1 (16.7)	

SD: Standard deviation. *Statistically significant.

Table IV. *Univariate and multivariate survival analyses for prognostic significance of programmed death ligand-1 (PD-L1) SP142 expression on immune cells (ICs) and clinicopathologic parameters of 132 patients with triple-negative breast cancer.*

Parameter		Univariate		Multivariate	
		HR (95%CI)	*p*-Value	HR (95%CI)	*p*-Value
Age (years)	≥53 *vs*. <53	0.99 (0.96-1.04)	0.976	NA	NA
Histological grade	3 *vs*. 1-2	0.63 (0.26-1.49)	0.290	NA	NA
Pathological tumor stage (pT)	pT1c *vs*. pT1a-b	0.94 (0.38-2.34)	0.895	NA	NA
	pT2-3 *vs*. pT1a-b	1.68 (0.36-7.91)	0.512	NA	NA
Pathological nodal stage (pN)	pN1 *vs*. pN0	3.41 (1.24-9.42)	0.018*	3.80 (1.3-11.10)	0.015*
	pN2-3 *vs*. pN0	4.53 (1.57-13.08)	0.005*	6.16 (1.46-26.10)	0.014*
Lymphovascular invasion	Present *vs*. Absent	1.86 (0.77-4.49)	0.167	NA	NA
Multiplicity	Present *vs*. Absent	2.87 (0.84-9.76)	0.092	1.11 (0.18-6.70)	0.908
IC PD-L1 SP142 expression	High *vs*. Low	0.12 (0.02-0.92)	0.020*	0.10 (0.01-0.88)	0.038*

CI: Confidence interval; HR: hazard ratio; NA: not applicable. *Statistically significant.

atezolizumab plus nab-paclitaxel in IC PD-L1-positive TNBC patients (25.0 months; hazard ratio=0.62; 95% confidence interval=0.45-0.86), *versus* the placebo plus nab-paclitaxel (15.5 months) (18, 19). The US FDA approved atezolizumab combined with chemotherapy for PD-L1-positive metastatic TNBC and the PD-L1 SP142 assay as a companion diagnostic test to determine PD-L1 expression on ICs.

Considering PD-L1 expression as a prognostic marker for breast cancer patients, several studies have demonstrated that positive PD-L1 expression is associated with adverse clinicopathological features, such as more advanced stage and lymph node metastasis, as well as worse survival (36-38). However, in the case of TNBC, conflicting results have been documented. PD-L1 immunostaining has been a complex issue for the pathology laboratory as it requires an understanding of multiple clones and testing platforms, each with variable scoring criteria. The lack of standardization and reproducibility provide conflicting data regarding the clinicopathological significance of PD-L1 expression in TNBC. The utilization of various PD-L1 antibody clones has led to these controversies. In published data using E1L3N antibody in TNBC, the relationship between positive PD-L1 expression and patient outcome varied from better (39-41) to worse (42, 43) or insignificant (44, 45). Similarly, some previous studies regarding the SP142 antibody on TNBC revealed an association between positive PD-L1 expression

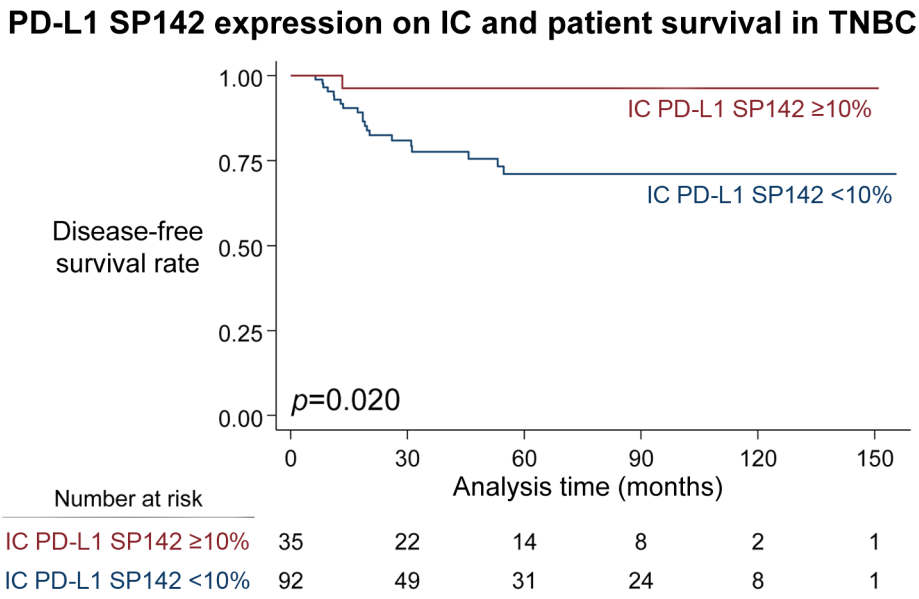

Figure 2. *Kaplan-Meier plots showing the difference in disease-free survival (DFS) according to the expression status of programmed death ligand-1 (PD-L1) SP142 in triple-negative breast cancer (TNBC). Patients whose tumor exhibits high PD-L1 expression (≥10%) show better DFS than those with low PD-L1 expression (<10%) TNBC (p=0.020).*

and better outcomes (21, 26, 46), whereas a recent study on 223 patients with TNBC showed that PD-L1 SP142 expression in ICs was independently prognostic of worse overall survival (47). Similarly, the cutoff scores for positive or high PD-L1 expression vary among studies. According to the results of previous studies evaluating PD-L1 SP142 expression in TNBC, the frequency of IC PD-L1 expression ≥1% ranged from 28% to 53% (21, 26, 46-48). In this study, the positive rate of PD-L1 expression ≥1% on ICs was relatively higher (61.4%; 81/132) than that of previous studies. However, unlike other studies using SP142 antibody (21, 26, 46-48), we could not find a statistically significant difference in survival between IC PD-L1 ≥1% and <1% tumors. Instead, when we applied a cutoff value of 10% for high IC PD-L1 expression, we observed that 26.6% (35/132) of the cases were classified as IC PD-L1-high tumors. Additionally, high IC PD-L1 expression was significantly associated with favorable clinicopathologic parameters, including smaller tumor size, fewer lymph node metastases, and the absence of LVI. Survival analyses also revealed that high IC PD-L1 expression was an independent prognostic factor of better DFS for patients with TNBC. Although the treatment-related cutoff value of PD-L1 SP142 (≥1% of ICs) is well established in TNBC, further investigations are warranted to determine its prognosis-related cutoff value.

This study had several limitations. First, we enrolled patients with TNBC who underwent surgery at a single institution. As this study did not include patients who received radiation therapy only or concurrent chemoradiation therapy, the cohort size was relatively small. Second, we did not analyze the statistical differences in locoregional recurrence-free survival or distant metastasis-free survival according to PD-L1 expression status. Further investigations using more detailed prognostic information obtained from larger cohorts are necessary. Third, we did not use morphometric or computer-assisted analyses for the quantification of PD-L1 expression. Finally, we did not measure PD-L1 expression in patients treated with anti-PD-1/PD-L1 therapies. Comparing the matched pre- and post-treatment expression levels of PD-L1 may better address its clinicopathological significance.

In conclusion, we demonstrated that when the cutoff value of 10% was applied, high IC PD-L1 expression was significantly associated with smaller tumor size, fewer lymph node metastases, the absence of LVI, and better DFS in patients with TNBC. In the multivariate analysis, IC PD-L1 expression status was an independent predictor of DFS. Although the impact of PD-L1 expression on TNBC outcomes had not been clearly established, our results indicate that IC PD-L1 SP142 expression is a significant marker of a better prognosis for patients with TNBC.

Conflicts of Interest

The Authors declare that they have no conflicts of interest in relation to this study.

Authors' Contributions

All Authors made substantial contributions to the conceptualization and design of the study, the acquisition, analysis, interpretation, and validation of the data, drafting of the article, critical revision of the article for important intellectual content, and the final approval of the version to be published.

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