CME/SAM

Stromal PD-L1 Expression Is Associated With Better Disease-Free Survival in Triple-Negative Breast Cancer

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Key Words: Breast cancer; TNBC; PD-1; PD-L1; Disease-free survival; Overall survival; Full-face section; Stromal PD-L1 expression

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ABSTRACT

Objectives: *Triple-negative breast cancer (TNBC) is an aggressive subtype of breast cancer, and there is no approved targeted therapy. We studied the expression of programmed cell death protein 1 (PD-1) and its ligand (PD-L1) in TNBC.*

Methods: *Full-face sections from 136 TNBC cases without neoadjuvant therapy between 2004 and 2013 were stained and evaluated for immune cell PD-1 staining and stromal or tumoral PD-L1 staining using the H-score (staining percentage × intensity). Nottingham histologic grade, lymphovascular invasion (LVI), mitotic count, and tumor-infiltrating lymphocytes (TILs) were evaluated. Tumor size, lymph node status, Ki-67 score, metastasis, overall survival (OS), and disease-free survival (DFS) were retrieved from medical records.*

Results: *Of the 136 TNBC cases, 69 (51%) had any PD-L1 staining and 35 (26%) had PD-L1 staining with an H-score of 5 or more; 117 (86%) had any PD-1 staining and 68 (50%) had PD-1 staining with an H-score of 5 or more. Tumor size and LVI were significantly associated with worse OS and DFS, and TILs and LVI were significantly associated with metastasis in univariate analysis. Stromal PD-L1 expression was significantly associated with better DFS in multivariate analysis. PD-1 expression was not associated with DFS, OS, or metastasis.*

Conclusions: *PD-L1 expression is seen in a high proportion of TNBCs and associated with better DFS.*

Upon completion of this activity you will be able to:
- predict the proportion of triple-negative breast cancers that express PD-L1.
- outline basic mechanisms underlying the effect of PD-L1 expression on cancer progression.

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The programmed cell death protein 1 (PD-1) and its ligand PD-L1 have critical roles in tumor surveillance. PD-1 is an immune checkpoint protein expressed in mononuclear immune cells. It can bind its ligands, PD-L1 and PD-L2. PD-L1 is expressed on antigen-presenting cells such as B cells or macrophages. Tumor cells can also express PD-L1.[1] PD-L2 expression is seen on macrophages and dendritic cells. When PD-1 binds to PD-L1, PD-1 suppresses the T-cell immune functions by inhibiting expression of transcription factors of T cells such as GATA-3 and T-bet,[2] which leads to apoptosis of T cells and potentiates tumor progression. Immune checkpoint inhibitors, such as PD-L1 antibody, block the interaction of PD-1 and PD-L1. Several PD-L1 blocking antibodies have been approved by the US Food and Drug Administration to treat melanoma, Hodgkin lymphoma, and lung and bladder carcinomas.[3] PD-L1 expression has been identified in cancers of other organs such as kidney, pancreas, esophagus, ovary, and glioblastoma.[4-8] Few studies have

investigated PD-L1 expression in breast cancers, especially triple-negative breast cancer (TNBC).

Clinically, breast carcinomas are classified as estrogen receptor positive (ER+), human epidermal growth factor receptor 2 positive (HER2+), and TNBC. Different subtypes have different molecular and immunohistochemical profiles, prognosis, and response to treatments.[9-12] TNBC refers to breast cancers with negative expression of ER, progesterone receptor, and HER2.[13,14] TNBCs account for approximately 15% of breast cancers, with a high prevalence in African American and Hispanic women. Compared with ER+ or HER2+ breast carcinoma, TNBC has the worst prognosis, and there is no approved targeted therapy.[15,16]

In this study, we analyzed PD-1 and PD-L1 expression in full-face sections of 136 TNBC cases and correlated them with prognosis.

Materials and Methods

Patient Information

In total, 136 TNBC cases diagnosed from 2004 to 2013 were retrieved from the pathology archives after the protocol was approved by the institutional review board. All were from segmental mastectomies without exposure to neoadjuvant systemic therapies. All 136 cases were reviewed by a breast pathologist (X.L.) to evaluate Nottingham histologic grade, lymphovascular invasion (LVI), mitosis, and tumor-infiltrating lymphocytes (TILs). TILs were evaluated as the percentage of intratumoral stroma covered by mononuclear lymphocytes. One tumor block of each case was selected for immunohistochemical studies. Tumor size, lymph node status, Ki-67 score, and survival information were retrieved from pathology reports and patient medical records. Overall survival (OS) was defined as the length of time from the date of initial diagnosis to the date of last contact. Disease-free survival (DFS) was defined as the length of time from the date of initial diagnosis to the date of diagnosis of local and/or distant recurrence. DFS included distant recurrences (metastasis to other organs not including regional lymph nodes) and local recurrences.

Immunohistochemical Studies and Evaluation

Consecutive full-face 5-μm formalin-fixed, paraffin-embedded sections were stained using PD-1 mouse monoclonal antibody (clone NAT105, 1:100 dilution; Abcam, Cambridge, MA) and PD-L1 rabbit monoclonal antibody (clone E1L3N, 1:200 dilution; Cell Signaling, Beverly, CA). Citrate low pH antigen retrieval was used for 20 minutes with the Bond III (Leica, Bannockburn, IL). Positive controls for PD-1 and PD-L1 were tonsil and placenta, respectively. Negative controls had the antibody replaced by buffer only.

All stained slides were reviewed and scored by one breast pathologist (X.L.). Membranous staining of PD-1 **Image 1B** and **Image 1C** and PD-L1 **Image 1E** and **Image 1F** was regarded as positive. PD-L1 staining was evaluated in tumor and stromal cells. PD-1 staining was evaluated in mononuclear immune cells only. PD-1 and PD-L1 staining was evaluated only within the boundary of invasive carcinoma. Overall percentage of positive cells and their staining intensity (1-3) were evaluated. An H-score (PD-1 or PD-L1 staining intensity × percentage of positive cells with a range between 0 and 300) was used to correlate with OS, DFS, and metastasis.

Statistical Analysis

Tumor size, Ki-67 score, TILs, mitosis, lymph node status (positive or negative), Nottingham histologic grade (1 and 2 vs 3), and LVI (positive or negative) were evaluated for potential association with OS, DFS, and metastasis. Log-rank test and Cox regression model were used in univariate analysis of their relationships with OS and DFS. The relationships between the clinical parameters with metastasis were assessed using a two-sample t test and Pearson χ^2 test.

The associations between PD-L1 expression and OS and DFS were evaluated using a log-rank test with hazard ratio (HR), and the 95% confidence interval (CI) was calculated using a univariate Cox regression model. Considering the confounding effects of the potential parameters, all variables were considered for inclusion in a multivariate Cox model through the use of backward selection (α-to-stay was set as 0.1 to adjust for confounding). Univariate and multivariate logistic regression with similar backward selection was used to evaluate the association between PD-L1 expression and metastasis. Odds ratio (OR) and 95% CI were reported.

Results

PD-L1 Expression in TNBC

Of the 136 TNBCs evaluated for PD-L1 expression, the number of cases with any tumoral cell staining was 28 (21%). Of the 136 cases, 14 (10%) showed an H-score of 5 or more. The number of cases with any stromal cell staining was 55 (40%), and 32 (24%) cases showed an H-score of 5 or more. Some cases had PD-L1 expression in both tumoral and stromal cells **Image 1D** (Images 1E and 1F). In total, the number of cases with any PD-L1 staining in either the tumoral or the stromal cells was 69 (51%), and 35 (26%) cases showed an H-score of 5 or more. Any PD-L1 expression was noted at the periphery of tumor (tumor front at the interface between tumor and surrounding normal breast

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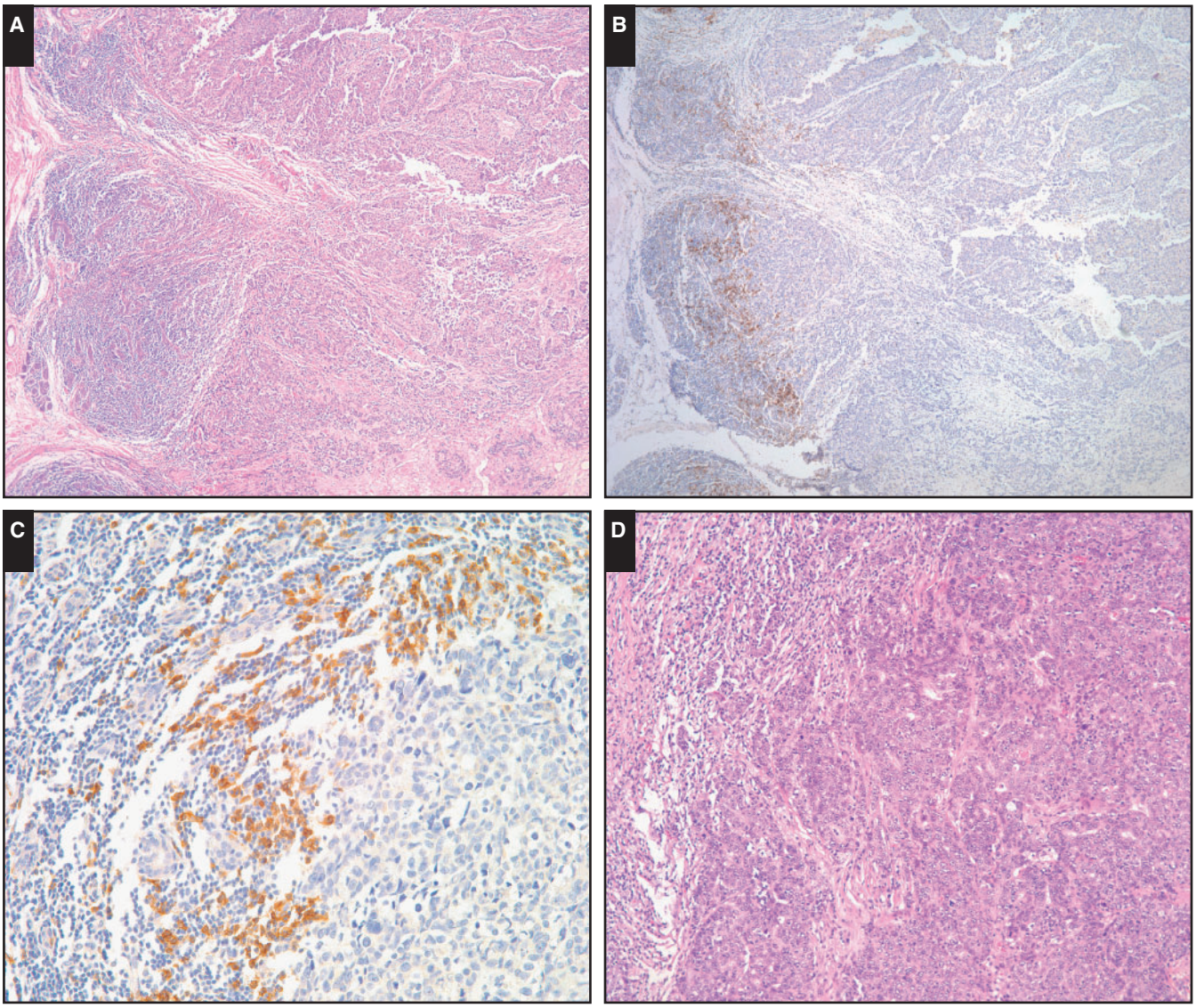

Image 1 Representative staining patterns of programmed cell death protein 1 (PD-1) and its ligand (PD-L1) in two representative cases. In case 1 (**A-C**), PD-1 expression was seen at the periphery of the tumor. Case 2 (**D-F**) had PD-L1 expression in both stromal and tumoral cells. **A**, Routine H&E staining of case 1 (×80). **B**, PD-1 expression in peripheral lymphocytes of case 1 (×80). **C**, PD-1 expression in peripheral lymphocytes of case 1 at a higher power (×800). **D**, Routine H&E staining of case 2 (×80).

tissue) **Image 1A** in seven (10%) of 69 cases. Among the 35 cases with a PD-L1 staining H-score of 5 or more, three (9%) had peripheral staining.

PD-1 expression was noted in the mononuclear immune cells in the tumoral stroma. The number of cases with any PD-1 staining was 117 (86%), and 68 (50%) cases showed an H-score of 5 or more.

Tumor Size and LVI Were Significantly Associated With Worse OS and DFS

Tumor size and LVI were significantly associated with worse OS (HR, 1.36; 95% CI, 1.23-1.51; $P < .0001$ and HR, 2.62; 95% CI, 1.15-5.93; $P = .0214$, respectively) and DFS (HR, 1.33; 95% CI, 1.21-1.47; $P < .0001$ and HR, 3.07; 95% CI, 1.48-6.39; $P = .0027$, respectively) in univariate analysis. Ki-67 score was significantly associated with worse DFS (HR, 1.02; 95% CI, 1.00-1.03; $P = .0144$) but not with OS. The results are summarized in **Table 1** and **Table 2**.

TILs and LVI Were Significantly Associated With Metastasis

The association between different parameters and metastasis was analyzed. Among all the parameters summarized in Table 3, TILs and LVI were significantly associated with a higher metastatic rate ($P = .0077$ and $P = .0029$,

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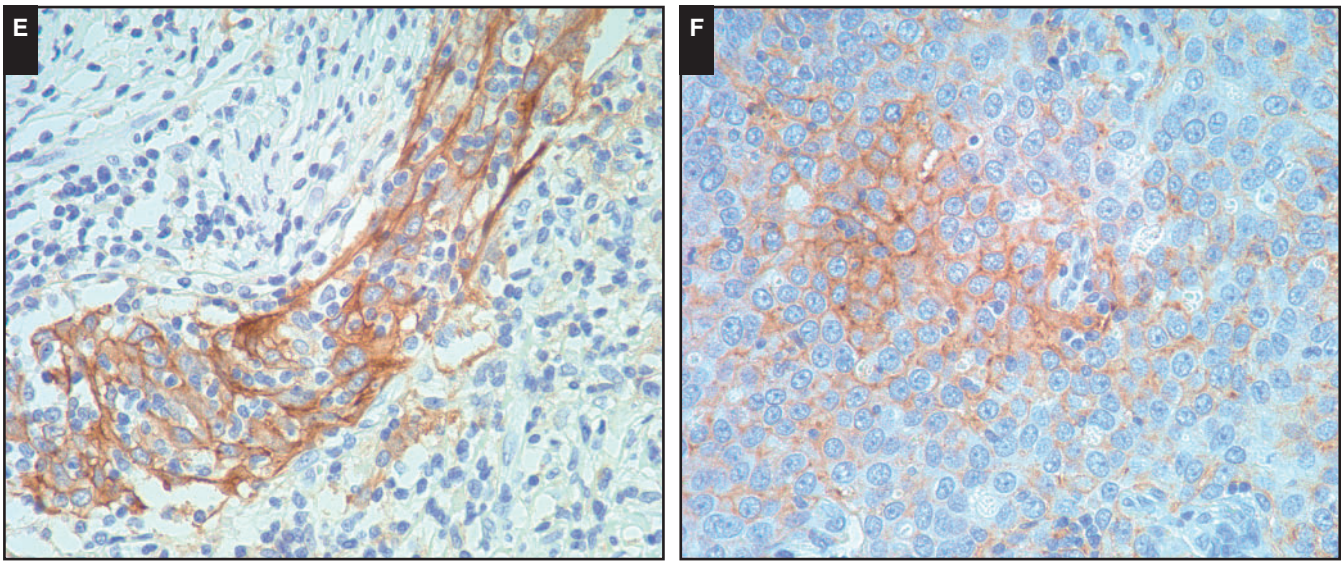

Image 1 (cont) **E**, PD-L1 expression in stromal cells in case 2 (×800). **F**, PD-L1 expression in tumor cells in case 2 (×800).

Table 1
Univariate Analysis of Association Between Confounders and Overall Survival

Characteristic	No.	HR (95% CI)	*P* Value	*P* Value for Log-Rank Test
Tumor size	136	1.36 (1.23-1.51)	<.0001	
Ki-67	116	1.01 (1.00-1.03)	.1357	
TIL	136	0.97 (0.92-1.01)	.1351	
Mitosis	136	1.01 (0.99-1.02)	.2423	
Lymph node (positive vs negative)	126	1.39 (0.46-4.22)	.563	.5617
Nottingham grade (1 and 2 vs 3)	136	0.30 (0.39-4.39)	.6688	.6677
LVI (positive vs negative)	136	2.62 (1.15-5.93)	.0214	.0169

CI, confidence interval; HR, hazard ratio; LVI, lymphovascular invasion; TIL, tumor-infiltrating lymphocyte.

Table 2
Univariate Analysis of Association Between Confounders and Disease-Free Survival

Characteristic	No.	HR (95% CI)	*P* Value	*P* Value for Log-Rank Test
Tumor size	136	1.33 (1.21-1.47)	<.0001	
Ki-67	116	1.02 (1.00-1.03)	.0144	
TIL	136	0.96 (0.92-1.00)	.0746	
Mitosis	136	1.01 (1.00-1.02)	.1437	
Lymph node (positive vs negative)	126	2.25 (0.93-5.48)	.0735	.0659
Nottingham grade (1 and 2 vs 3)	136	1.67 (0.51-5.54)	.4005	.3953
LVI (positive vs negative)	136	3.07 (1.48-6.39)	.0027	.0016

CI, confidence interval; HR, hazard ratio; LVI, lymphovascular invasion; TIL, tumor-infiltrating lymphocyte.

respectively) in univariate analysis. The results are summarized in **Table 3**.

Table 3
Association Between Confounders and Metastasis[a]

Characteristic	Metastasis		*P* Value
	No	Yes	
Tumor size, cm	2.5 ± 1.8	4.0 ± 4.1	.14
Ki-67, %	59.0 ± 35.3	68.2 ± 28.1	.39
TIL, %	11.1 ± 13.7	5.8 ± 5.8	.0077
Mitosis, 10 hpf	29.5 ± 23.6	40.6 ± 30.3	.0846
Lymph node			
Negative	95 (91.4)	9 (8.6)	.23
Positive	17 (81.0)	4 (19.0)	
Nottingham grade			
1 and 2	24 (96.0)	1 (4.0)	.2
3	93 (85.3)	16 (14.7)	
LVI			
Negative	89 (92.7)	7 (7.3)	.0029
Positive	28 (73.7)	10 (26.3)	

hpf, high-power field; LVI, lymphovascular invasion; TIL, tumor-infiltrating lymphocyte.
[a]Values are expressed as mean ± SD or number (%).

Stromal PD-L1 Expression but Not PD-1 Expression Was Significantly Associated With Better DFS

Both tumoral and stromal PD-L1 expressions were found to correlate with OS, DFS, and metastasis. Any stromal PD-L1 expression (>0 vs 0) was associated with better DFS in multivariate analysis ($P = .0458$) but not in univariate analysis **Table 4**. This was also true when comparing tumors with an H-score of 5 or more vs less than 5 for stromal PD-L1 expression (Table 4). However, positive stromal PD-L1 expression was not associated with OS **Table 5**. Positive tumoral PD-L1 expression was not associated with either OS or DFS (Tables 4 and 5).

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Table 4
Association Between PD-L1 Expression and Disease-Free Survival[a]

	Multivariate Analysis		Univariate Analysis	
PD-L1	**HR (95% CI)**	***P* Value**	**HR (95% CI)**	***P* Value for Log-Rank Test**
Tumoral (>0 vs 0)	0.21 (0.03-1.69)	.1438	0.87 (0.30-2.49)	.7909
Tumoral (≥5 vs < 5)	0.59 (0.08-4.48)	.6123	0.74 (0.18-3.13)	.685
Stromal (>0 vs 0)	0.28 (0.08-0.98)	.0458	0.45 (0.17-1.18)	.096
Stromal (≥5 vs < 5)	0.11 (0.01-0.83)	.0325	0.37 (0.11-1.23)	.0904

CI, confidence interval; HR, hazard ratio; PD-L1, programmed cell death protein 1 ligand.
[a]Multivariate analysis for tumoral (>0 vs 0): adjusting for tumor size, Ki-67, and lymph node. Multivariate analysis for tumoral (≥5 vs <5): adjusting for tumor size, Ki-67, and tumor-infiltrating lymphocytes. Multivariate analysis for stromal (>0 vs 0): adjusting for tumor size and Ki-67. Multivariate analysis for stromal (≥5 vs <5): adjusting for tumor size and Ki-67.

Table 5
Association Between PD-L1 Expression and Overall Survival[a]

	Multivariate Analysis		Univariate Analysis	
PD-L1	**HR (95% CI)**	***P* Value**	**HR (95% CI)**	***P* Value for Log-Rank Test**
Tumoral (>0 vs 0)	0.45 (0.06-3.49)	.4450	1.10 (0.37-3.23)	.8631
Tumoral (≥5 vs < 5)	0.69 (0.09-5.29)	.7181	0.94 (0.22-4.02)	.9345
Stromal (>0 vs 0)	0.53 (0.15-1.91)	.3317	0.47 (0.16-1.38)	.1598
Stromal (≥5 vs < 5)	0.23 (0.03-1.72)	.1509	0.32 (0.07-1.35)	.1014

CI, confidence interval; HR, hazard ratio; PD-L1, programmed cell death protein 1 ligand.
[a]Multivariate analysis adjusting for tumor size.

Table 6
Association Between PD-L1 Expression and Metastasis[a]

	Multivariate Analysis		Univariate Analysis	
PD-L1	**OR (95% CI)**	***P* Value**	**OR (95% CI)**	***P* Value**
Tumoral (>0 vs 0)	3.26 (0.77-13.85)	.1098	1.81 (0.53-6.21)	.3456
Tumoral (≥5 vs < 5)	0.70 (0.08-6.61)	.7573	0.55 (0.07-4.50)	.5745
Stromal (>0 vs 0)	0.18 (0.02-1.65)	.1301	0.12 (0.02-0.94)	.0435
Stromal (≥5 vs < 5)	0.32 (0.04-2.98)	.3183	0.18 (0.02-1.43)	.1046

CI, confidence interval; OR, odds ratio; PD-L1, programmed cell death protein 1 ligand.
[a]Multivariate analysis adjusting for tumor-infiltrating lymphocytes and lymphovascular invasion. Model selection would remove all confounders.

Neither tumoral nor stromal PD-L1 expression was associated with metastasis **Table 6**. When PD-1 expression was analyzed, it was not associated with metastasis, DFS, or OS using H-scores of 0, 5, or 10 as cutoffs (data not shown).

Discussion

The PD-1 pathway plays a critical role in tumor surveillance. PD-L1 antibodies have been approved to treat several malignancies. However, few studies examined its role in breast cancer, especially in TNBC. TNBC is an aggressive breast cancer subtype with high metastatic rate and poor prognosis.[13,17]

In the studies of melanomas, Massi et al[18] reported increased aggressiveness and invasiveness in PD-L1–positive melanomas, and Madore et al[19] also showed that PD-L1 expression was associated with locoregional recurrence and melanoma-specific survival. Wang et al[20] found that positive PD-L1 expression was associated with poor OS in a meta-analysis. Bertucci et al[21] reported that in gastrointestinal stromal tumors, low expression of PD-L1 messenger RNA (mRNA) was associated with a higher risk of metastatic relapse. These studies showed the different relationship between PD-L1 expression and prognosis in various tumors.

Baptista et al[22] reported that PD-L1 expression was seen in 50.8% of breast cancers and associated with better OS, but the role of PD-L1 expression in TNBC was not further analyzed. Sabatier et al[23] reported that PD-L1 mRNA expression was associated with basal and HER2-enriched breast cancers. They also reported that increased PD-L1

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expression was associated with better metastasis-free survival and OS in basal cancers and higher pathologic complete response after neoadjuvant chemotherapy. Beckers et al[24] also reported that PD-L1 expression was seen in up to 93% of TNBCs. They reported that tumoral PD-L1 expression was associated with a lower risk of breast cancer–specific death, and stromal PD-L1 expression was associated with a lower risk of death from all causes. In contrast to this high expression rate of PD-L1 in TNBC, Ali et al[25] reported a much lower expression rate of PD-L1 in basal-like breast cancer (19%). This is consistent with the study from Mittendorf et al.[26]

We found that 51% of TNBCs have either tumoral or stromal PD-L1 expression. The different staining rate of PD-L1 may at least partially stem from different methods. Most of the aforementioned studies have used tissue microarray (TMA) for the immunohistochemical studies. One drawback of using TMA is the limited tissue sampling. PD-L1 expression can be focal and only seen at the periphery of the tumor. As shown in our study, 9% of tumors had peripheral PD-L1 expression. If only central tissue were used to construct a TMA, these 9% of tumors would be PD-L1 negative. Therefore, we recommend using full-face sections for PD-L1 expression studies. Consistent with other studies, we found stromal PD-L1 expression was associated with better DFS in TNBC in multivariate analysis.

TILs have been shown to be associated with better prognosis and response to neoadjuvant therapies in TNBC.[27] Both TILs and PD-L1 expression are immune responses in breast cancers. Few studies have investigated the relationship between TILs and PD-L1. Ali et al[25] reported a positive correlation between TILs and PD-L1 in breast cancer. Such positive correlation was not seen in ovarian cancers,[7] indicating possible different immune responses in different tumors. We observed a significant positive correlation between TILs and metastasis and a positive but not significant correlation between TILs and DFS. The correlation between TILs and DFS may reach a significant number if the sample size were increased.

Large tumor size and LVI are associated with poor prognosis.[28] Ki-67 is a nuclear protein associated with cellular proliferation.[29] Our results showed that tumor size and LVI were significantly associated with worse OS and DFS and that Ki-67 score was significantly associated with worse DFS in univariate analysis.

In conclusion, our study examined the expression of PD-L1 and PD-1 in full-face sections of a large TNBC cohort. We showed a 51% positive staining rate for PD-L1 expression, and stromal PD-L1 expression was associated with better DFS. The high expression rate of PD-L1 in TNBC indicates a promising role for PD-L1 antibody in treatment of TNBC.

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