

Original Research**A Cohort Study and Systematic Literature Review of the Prognostic Significance of PD-L1 Expression in Triple-Negative Breast Cancer**Daniel Schmolze, MD¹; Carolyn E. Behrendt, PhD²; Peter P. Lee, MD³; Sophia Apple, MD^{1,4*}¹Pathology, City of Hope National Medical Center, Duarte, CA, USA²Biostatistics, Beckman Research Institute of the City of Hope, Duarte, CA, USA³Immuno-Oncology, Beckman Research Institute of the City of Hope, Duarte, CA, USA⁴Pathology, David Geffen School of Medicine, University of California, Los Angeles, CA, USA***Corresponding author****Sophia Apple, MD**Emerita, Pathology, David Geffen School of Medicine, University of California, Los Angeles, CA, USA; E-mail: sapple@coh.org**Article information****Accepted:** May 6th, 2024; **Published:** May 18th, 2024**Cite this article**Schmolze D, Behrendt C, Lee PP, Apple S. A cohort study and systematic literature review of the prognostic significance of PD-L1 expression in triple-negative breast cancer. *Pathol Lab Med Open J.* 2024; 5(1): 12-19.**ABSTRACT****Context**

The prognostic value of programmed death ligand-1 (PD-L1) in triple-negative breast cancer (TNBC) is unclear.

Objective

To investigate PD-L1 expression for association with survival.

Design

Surgical specimens of primary TNBC were linked to data on recurrence-free survival (RFS). Tissue microarray averaging 3 tumor cores per case underwent immunohistochemical staining. Within each core, PD-L1 positivity was scored separately for tumor, stromal, and immune cells by averaging readings by two board-certified pathologists. Having at least one core with high (above 10%) PD-L1 staining (on tumor cells as primary risk, on stromal and immune cells as secondary risks) was evaluated for association with RFS using proportional hazards regression. Comparable survival analyses were identified by systematic search of MEDLINE-indexed journals through March 2019.

Results

Patients (n=112) contributed 308 cores of TNBC. During follow-up, TNBC recurred in 24 subjects; another 4 subjects died without recurrence. Ten (8.9%) subjects had High PD-L1 expression on tumor cells, a feature associated with better RFS [hazards ratio 0.44 (95% Confidence Interval 0.19-0.97)] independently of age, distant metastasis, tumor size, lymphovascular invasion, and Ki-67. In contrast, High PD-L1 in stromal cells or immune cells was not associated with RFS. Among 22 similar studies, high PD-L1 on tumor cells was associated with survival in 16 studies: 7 favorably, 8 unfavorably, and 1 with mixed results.

Conclusions

In this TNBC cohort, High PD-L1 on tumor cells conferred favorable prognosis. The lack of consensus across similar studies suggests that future studies should explore how timing, microenvironment, and glycosylation influence PD-L1's association with survival.

Keywords

Breast; PD-L1; PD-1; Triple negative; Immune; Immuno-oncology; TNBC; TMA; Prognosis.

INTRODUCTION

The programmed death (PD)-1 protein has potent inhibitory effects on T-lymphocytes and other immune cells, including B-lymphocytes and monocytes.¹ PD-1 is activated by its two major

ligands: PD-L1/CD274, expressed on the surface of various immune cells including T- and B-lymphocytes, macrophages, and dendritic cells, and PD-L2/CD273, expressed on antigen-presenting cells such as macrophages and dendritic cells. PD-L1 may also be expressed by tumor cells to evade immune killing,² thereby in-

creasing tumorigenesis and invasiveness.

Accordingly, PD-L1 has been associated with poor prognosis in non-small cell lung carcinoma, melanoma, renal cell carcinoma, and esophageal/gastric carcinoma.³⁻⁶ Antibodies to PD-L1 (atezolizumab and durvalumab) have been approved by the FDA to treat certain solid tumors (e.g. advanced urothelial carcinoma, non-small cell lung carcinoma) and are under development to treat other malignancies.⁷

Overall, PD-L1 is expressed by a minority of human breast tumors,⁸ and there are conflicting reports regarding the prognostic import of PD-L1 in breast cancer: some studies have associated overexpression of PD-L1 with inferior overall survival, while other studies showed improved prognosis.⁹⁻¹¹ A recent meta-analysis of PD-L1 expression in breast cancer concluded that expression of PD-L1 is associated with lymph node positivity, higher histologic grade, hormonal receptor-negative status, and shorter overall survival.¹²

However, there is still a lack of consensus regarding the prognostic value of PD-L1 in triple-negative breast cancers (TNBC, heterogeneous tumors that do not express estrogen receptor, progesterone receptor, or HER2).¹³⁻¹⁵ Because TNBC represent 10-20% of new diagnoses of breast cancer, have inferior clinical outcomes, and lack molecularly targeted therapies, there is intense interest in emerging immunotherapeutic approaches such as agents that target PD-L1. Increased levels of tumor-infiltrating lymphocytes (TILs) have been observed in TNBC,^{16,17} leading to the hypothesis that these tumors may be particularly susceptible to immune-modulating therapies.

While PD-L1 is more commonly expressed in TNBC than in hormone receptor-positive cancers, most TNBC do not express PD-L1.^{8,18,19} Nevertheless, clinical trials are underway, particularly in the metastatic setting where the need for novel therapies is most acute. For example, a recent phase 1 trial of atezolizumab in TNBC reported a 19% objective response.²⁰ Another potential therapeutic target in TNBC is PD-L1's receptor, PD-1. In a phase 1b trial of anti-PD-1 pembrolizumab in metastatic TNBC positive for PD-L1 (defined as >1% in tumor cells or stroma), clinical benefit was observed in 20% of subjects.²¹ The relatively low response rates observed in these trials suggest that current methods for patient selection are suboptimal.

To better understand the prevalence of PD-L1 positivity and its association with survival in TNBC, we undertook a retrospective cohort study at a major academic medical center and a systematic review of comparable studies. Both activities aimed to investigate a pair of research questions: In primary TNBC, is tumor expression of PD-L1 prognostic of survival? If so, is PD-L1 expression in stromal and immune cells as prognostic as PD-L1 in tumor cells?

METHODS

Subjects

Because the study utilized routinely archived specimens only and involved no contact with patients, the institutional review board granted a waiver of informed consent for this study. Women with newly-diagnosed primary TNBC who underwent surgery during 2002-2010 were eligible for study. Subjects had archived tissue containing invasive ductal carcinoma (IDC) and corresponding clinical data available from the academic medical center's tissue procurement laboratory. Patients who lacked data on RFS or tumor dimensions or whose sample did not yield a core of tumor tissue were excluded. Patients who had received neoadjuvant treatment prior to surgery were included, but their neoadjuvant history was considered in the statistical analysis. Follow-up for recurrence-free survival (RFS) continued through September 2016 or last contact.

Tissue Acquisition

All samples were tissue from breast tumors that had been excised, processed into formalin-fixed, paraffin-embedded blocks, and subjected to routine pathologic examination. The latter examination had included immunohistochemistry (IHC) and fluorescent in situ hybridization (FISH) to determine endoplasmic reticulum (ER), per rectum (PR), and HER2/neu status. To meet the definition of TNBC, tumors had to be negative for ER and PR (0% to <1% staining by IHC) and negative for HER2/neu (defined as a score of 0 and 1+ by IHC and Her2/CEP17 ratio <2.0 by FISH). Ki-67 level, obtained from routine pathologic examination of the tumor, was classified as high when above 25%.²²

Construction and Staining of Tissue Microarray (TMA)

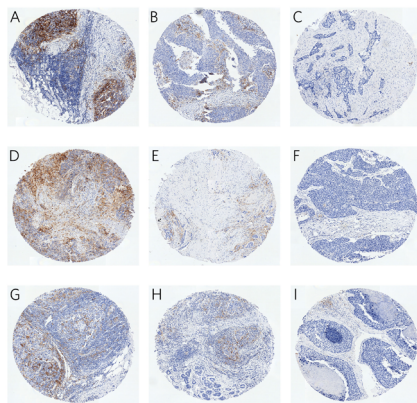
For each tumor, several tissue blocks were selected containing representative areas of normal tissue, ductal carcinoma in situ (if present), and invasive ductal carcinoma. A tissue microarray (TMA) was then constructed containing three 2 mm cores from each of the representative blocks, sampled from distinct areas to capture possible tumor heterogeneity. These cores (9 per tumor) were deposited into a recipient paraffin block using a TMA workstation (TMA builder 20010.02, Histopathology Ltd., Hungary). Four TMA blocks were ultimately prepared. From the TMAs, serial 3 µm tissue sections were cut and adhered to charged glass slides for subsequent hematoxylin and eosin (H&E) and immunohistochemical staining. PD-L1 staining was performed using the Agilent FDA-approved kit incorporating Dakoclon 22C3 (Agilent North America, Inc., Carpinteria, CA, USA). Following deparaffinization and antigen retrieval using sodium citrate buffer (pH 6.0), staining for CD8 was performed using clone 144B at 1:200 dilution, incubated overnight at 4°C (Abcam, San Francisco, USA).

PD-L1 Expression

TMA slides stripped of clinical information were interpreted independently by two board-certified pathologists with breast expertise. For each core, the readers scored tumor cells, tumor-asso-

ciated immune cells (primarily lymphocytes and histiocytes), and tumor stromal cells separately on their percentage positive for PD-L1 staining (Figure 1). Within each core, each pair of scores per cell type was averaged. Each patient was assessed on whether any of her cores scored high (>10%) for PD-L1 in tumor, in stroma, or in immune cells.

Figure 1. Representative PD-L1 Staining in Tumor Cells (Top Row), Stromal Cells (Middle Row), and Immune Cells (Bottom Row) that Illustrates High (>10%, A, D, G), Intermediate (>5%-10%, B, E, H), and Low (0-5%, C, F, I) PD-L1 Expression



Statistical Analysis

Correlation between patient PD-L1 status (high/low) per tumor cells, stromal cells, and immune cells was evaluated by Fisher's exact test of proportions. For the survival analysis, proportional hazards regression was used to evaluate high PD-L1 in tumor cells as the primary risk factor, and high PD-L1 in stromal and in immune cells as secondary risk factors, for RFS. Covariates accounted for in the survival analysis were age at diagnosis, tumor's greatest dimension, tumor's Ki-67 level, distant metastasis, and lymphovascular invasion; history of neoadjuvant treatment was omitted as a covariate after it was found not to improve the models' fit to observed survival.

In two subgroups, the proportion of cores associated with an event (recurrence or death) was either 0 (observed with the primary risk factor: high PD-L1 in tumor cells) or 100% (observed with the covariate: distant metastasis at diagnosis). To accommodate these sparse data, data augmentation²³ was applied to the proportional hazards regression, specifying the following prior median hazards ratios: 0.5 (95% limits 0.2-1.2) for high PD-L1 in tumor cells and 4 (95% limits 1-16) for distant metastasis. The primary risk factor's approximate posterior median hazards ratio was interpreted as statistically significant ($p < 0.05$) if its 95% profile likelihood limits excluded 1.0.

Literature Review

A systematic search of MEDLINE-indexed journals was undertaken in April 2019 using the terms "PD-L1 expression AND (TNBC OR basal-like)". The resulting 100 publications were reviewed by one of the current authors (CEB) to identify and summarize the 22 studies that presented original analyses evaluating PD-L1 ex-

pression for association with overall or disease-free survival among patients with TNBC.

RESULTS

Table 1. Subjects with Primary Triple Negative Breast Cancer (N=112)

Characteristics	N
Age	
30-64 Years	88 (78.6)
65-87 Years	24 (21.4)
Menopausal Status	
Premenopausal	42 (37.5)
Postmenopausal	62 (55.4)
Status Unknown	8 (7.1)
Family History of Breast and Ovarian Cancer	
Breast Cancer Only	42*(37.5)
Ovarian Cancer Only	4 (3.6)
Both Cancers	12 (10.7)
Neither Cancer	52 (46.4)
Family History Not Available	2 (1.8)
Type of Surgery to Excise Tumor	
Lumpectomy	64 (57.1)
Mastectomy	44 (39.3)
Both	4 (3.6)
Number of Interpretable Cores of Tumor	
1	14 (12.5)
2	26 (23.2)
3	56 (50.0)
4, 5 or 6	15 (14.3)
TNM Stage	
I	27 (24.1)
II	66 (58.9)
III	16 (14.3)
IV	1 (0.9)
Stage Not Available	2 (1.8)
Largest Dimension of Tumor	
0.2-<2.0 cm	34 (30.4)
2.0-<4.0 cm	52 (46.4)
4.0-16.0 cm	26 (23.2)
Neoadjuvant Treatment	
Yes	13 (11.6)
No	99 (88.4)
Distant Metastasis	
Present	17 (15.2)
Absent	95 (84.8)
Ki67	
Above 25	94 (83.9)
1-25	18 (16.1)
Lymphovascular Invasion	
Present	39 (34.8)
Absent	73 (65.2)

*Includes 2 subjects whose family history was informative regarding breast but not ovarian cancer.

The study included n=112 subjects (Table 1); their age at diagnosis was 54.7 ± 12.7 years. Median follow-up of subjects was 3.5 years (range 3 days-9.4 years). During follow-up, TNBC recurred in 24 subjects, and another 4 subjects died without a recurrence.

Subjects contributed 1-6 cores each, for a total of n=308 cores; most (87.5%) subjects were represented by multiple cores

(Table 1). All but 2 cores contained invasive ductal carcinoma (IDC); the exceptions were both adenoid cystic carcinoma. At least one core with high PD-L1 expression in tumor, stromal, and immune cells was found in n=10 (8.9%), n=11 (9.8%), and n=11 (9.8%) subjects, respectively. Within patients, PD-L1 status (high/low) per stromal cells was correlated with PD-L1 status per tumor cells and per immune cells; however, there was no correlation between PD-L1 status per tumor cells and immune cells (Table 2).

In the survival analysis, the primary risk factor, High PD-L1 in tumor cells, was associated favorably with RFS (Table 3, Model A), independently of age, distant metastasis, tumor size, lymphovascular invasion, and high Ki67. In contrast, neither secondary risk factor, high PD-L1 in stromal or in immune cells, was associated with RFS, either individually (Table 3, Models B and C) or in the same model with high PD-L1 in tumor cells (data not shown).

According to systematic review of the literature (Table 4), prior studies in TNBC found that high PD-L1 on tumor cells could be associated with better,²⁴⁻³⁰ worse,³¹⁻³⁸ or neutral³⁹⁻⁴⁴ outcome. A final study⁴⁵ associated PD-L1 in tumor cells with either better survival, given high numbers of TILs, or worse survival, given low numbers of TILs. Two more studies considered TILs in their evaluation of PD-L1 as a prognostic factor. According to Tomioka et al,³⁴ high PD-L1 exacerbated the worse survival associated with low TILs; moreover, according to Zhu et al,³¹ high PD-L1 overrode the favorable prognosis associated with high TILs. In contrast to the lack of consensus across studies on the prognostic significance of PD-L1 in tumor cells, not one prior study demonstrated prognostic significance for PD-L1 expression on stromal or immune cells (Table 4).

Table 2. Correlations among Patients' PD-L1 Status per Tumor, Stromal, and Immune Cells

A) PD-L1 Status per Tumor versus Stromal Cells

PD-L1 per Stromal Cells		
N (ROW %)	High	Low
PD-L1 per Tumor Cells		
High	5 (50.0)	5 (50.0)
Low	6 (5.9)	96 (94.1)

*Fisher's exact test, $p < 0.001$

B) PD-L1 Status per Tumor versus Immune Cells

PD-L1 per Stromal Cells		
N (ROW %)	High	Low
PD-L1 per Tumor Cells		
High	2 (20.0)	8 (80.0)
Low	9 (8.8)	93 (91.2)

*Fisher's exact test, $p = 0.25$ (not significant)

C) PD-L1 Status per Stromal versus Immune Cells

PD-L1 per Immune Cells		
N (ROW %)	High	Low
PD-L1 per Stromal Cells		
High	4 (36.4)	7 (63.6)
Low	7 (6.9)	94 (93.1)

*Fisher's exact test, $p = 0.01$

Table 3. Multivariable Association of Cell-Specific PD-L1 Positivity with Recurrence-Free Survival (N=112)

Approximate Posterior Median Hazards Ratio (95% Profile-Likelihood Limits)

	Model A: PD-L1 in Tumor Cells	Model B: PD-L1 in Stromal Cells	Model C: PD-L1 in Immune Cells
>10% PD-L1 Positivity in			
At Least One Core	0.44 (0.19, 0.97)*	0.99 (0.15, 3.67)	2.44 (0.56, 7.61)
Adjusted for Covariates:			
Per Year of Age Over 65	1.11 (1.03, 1.18)	1.11 (1.03, 1.18)	1.11 (1.03, 1.19)
Per Centimeter of Tumor's			
Greatest Dimension	1.54 (1.29, 1.87)	1.54 (1.29, 1.88)	1.56 (1.31, 1.91)
Distant Metastasis	10.26 (4.71, 22.67)	10.75 (4.87, 24.17)	11.64 (5.27, 26.11)
Lymphovascular Invasion	1.88 (0.80, 4.56)	1.85 (0.78, 4.52)	1.80 (0.77, 4.36)
Ki-67 Above 25%	2.37 (0.83, 8.55)	2.29 (0.80, 8.30)	2.16 (0.75, 7.88)

*statistically significant ($p < 0.05$)

Table 4. Literature Review: Previous Survival Analyses of PD-L1 Positivity in TNBC

Association with Survival	Reference	Evaluable* TNBC Cases	Definition of PD-L1 Positivity	Frequency of PD-L1 Positivity	Survival Metric†	Hazards Ratio (95% CI) (if unavailable, then p value)
Protective	24	269	>=1% of tumor cells	26.5%	DFS	0.39 (0.20-0.76)
					OS	0.40 (0.18-0.86)
			Incremental RNA expression of CD274 (gene for PD-L1)	N/A	DFS	0.19 (0.04-0.97)
					OS	0.12 (0.02-0.81)
	25	64	H-score >=5 in tumor cells, in stromal cells	45.3%, 65.6%	DFS	p=0.001, p=0.002
	26	54	>=1% of tumor cells	55.6%	DFS	0.33 (0.14-0.80)
					OS	0.17 (0.05-0.63)
	27	329 basal-like	Per unit of fold change in normalized gene expression	N/A	DFS	p=0.01
					OS	p=0.02
	28	92, including 69 basal-like	>75 th quartile of H-score	38.0%	DFS	In Basal-like TNBC: 0.39 (0.22-0.86) In Nonbasal-like TNBC: Not significant
Harmful	29	215	>25% of tumor cells	32.6%	DFS	0.45 (0.21-0.96)
					OS	0.30 (0.13-0.72)
	30	>100	>=10% of tumor cells	29.4%	DFS	p=0.04
					OS	Not significant
	31	108	>=1% of tumor cells	22.2%	DFS	p=0.001
	32	61	>=10% of tumor cells	39.3%	DFS	9.48 (1.09-82.32)
None	33	117	>70% of tumor cells or TILs‡	31.6%	DFS	2.47 (1.12-5.43)
					OS	3.64 (1.46-9.07)
	34	22	>=50% of tumor cells	22.7%	DFS	15.4 (1.4-456)
	35	128	>10% of tumor cells	51.6%	OS	10.4 (3.6-29.6)
					DFS	2.32 (0.96-5.62)
	36	91	H-score >=100 in tumor cells	58.2%	OS	2.60 (1.02-6.65)
					DFS	p=0.005
	37	36	Amplification of chromosome 9p24.1 (genes for PD-L1, PD-L2, JAK2)	22.2%	OS	p=0.004
DFS					p=0.004	
38	127 basal-like	H-score >=100 in tumor cells	30.7%	OS	4.97 (2.94-8.43)	
Both Protective and Harmful	39	109	H-score >=100 in tumor cells	17.4%	DFS	No association
					OS	
	40	101	>5% of tumor cells or TILs	38.6% of primary tumors, 59.4% of paired tumor-positive axillary lymph nodes (LN)	DFS, OS	No association with PD-L1 or TILs in primary tumor; DFS association with PD-L1 in LN lymphocytes is nonsignificant after adjustment for MHT§
	41	103	>=10% of tumor cells, >=10% of TILs	23.4% per tumor cells, 26.2% per TILs	DFS, OS	No association with PD-L1 status per tumor cells or per TILs
	42	183	>=1% of tumor or immune cells	13.7%	OS	No association with PD-L1 status per tumor or immune cells
	43	136	H-score >=5 in tumor or in stromal cells	10% per tumor cells, 24% per stromal cells	DFS, OS	No association with PD-L1 status per tumor cells; DFS association with stromal PD-L1 is nonsignificant after adjustment for MHT
44	218	using clone 28-8: >5% of tumor cells, any positivity in immune cells	12.4% per tumor cells, 36.7% per immune cells	DFS, OS	No association with PD-L1 status per tumor cells; OS association with PD-L1 status per immune cells is nonsignificant after adjustment for MHT	
Both Protective and Harmful	45	248	>=1% of tumor cells	41.5%	DFS, OS	Given High TILs, ~0.5; Given Low TILs, ~2.0
*Cases with PD-L1 and Survival Data; †DFS=Disease-free Survival (also called Progression-free or Recurrence-free Survival); DSS=Disease-specific Survival; OS=Overall Survival; ‡ TILs=Tumor-infiltrating lymphocytes; § MHT=multiple hypothesis testing						

*Cases with PD-L1 and Survival Data; †DFS=Disease-free Survival (also called Progression-free or Recurrence-free Survival); DSS=Disease-specific Survival; OS=Overall Survival; ‡ TILs=Tumor-infiltrating lymphocytes; § MHT=multiple hypothesis testing

DISCUSSION AND CONCLUSION

In our cohort of patients with TNBC, high PD-L1 on tumor cells is associated with superior survival. The definition of high PD-L1 that was optimal here, above 10% cells staining positive, accords with a number of previous studies.^{30,32,35,41} Limitations of the current cohort include its modest sample size, variable number of informative cores per patient, inability to compare PD-L1 expression pre- vs post-neoadjuvant therapy (for the minority of current subjects who were treated prior to surgery), and lack of data on TILs. Still, there is growing recognition that the bivariable approach to classifying TNBC, according to high/low levels of PD-L1 and TILs,^{31,34,45} is overly simplistic: reducing TILs to a dichotomous variable ignores the fact that TILs vary in density, location, and interaction with PD-L1.⁴⁶

The current association of high PD-L1 with favorable prognosis is consistent with findings from 7 similar studies²⁴⁻³⁰ and also from a recent spatial study of the tumor immune microenvironment in TNBC⁴⁷ period. The latter investigators observed better survival when TNBC exhibited an immunoreactive microenvironment they termed fully inflamed, characterized by tumoral infiltration of granzyme B cells, CD8+ T-cells, a type 1 interferon signature, and elevated expression of PD-L1 and indoleamine 2,3-dioxygenase (IDO) in tumor epithelium (but not in tumor stroma).

Yet as our systematic review demonstrates, the current literature includes a similar number of studies³¹⁻³⁸ that reached the opposite conclusion about the prognostic value of PD-L1 expression, namely that it augurs poor survival in TNBC. Support for the latter view also comes from a recent study by Keren et al,⁴⁸ who used a novel methodology to study how the spatial relationship between tumor cells, immune cells, and immune-related proteins bears on survival in TNBC. Using multiplexed ion beam imaging coupled with time-of-flight mass spectrometry, they imaged 36 proteins (including PD-L1 and IDO) in tumor sections from 41 TNBC patients. Along a continuum within TNBC tumors, Keren et al. identified “three archetypical subtypes of tumor-immune interactions: cold, with low immune infiltrate; mixed, with high mixing between tumor and immune cells; and compartmentalized, in which there are regions comprised predominantly of either immune or tumor cells.” Furthermore, mixed tumors had PD-L1 and IDO expression primarily on tumor cells, while compartmentalized tumors had PD-L1 and IDO expression predominantly on immune cells, particularly along the boundary between tumor and immune cells. Finally, patients whose TNBC showed compartmentalized organization had better survival than those whose TNBC showed mixing between tumor and immune cells (an archetype that was accompanied by PD-L1 expression on tumor cells).

Thus, even among studies^{47,48} with access to cutting-edge assessment of the spatial distribution of PD-L1 within TNBC tumors, there remains a lack of consensus on whether elevated PD-L1 expression on tumor cells predicts better or worse survival in TNBC. Perhaps the static design used by existing studies of PD-L1

in TNBC have failed to recognize what is essentially a dynamic process. Keren et al,⁴⁸ present evidence suggesting that specific subsets of immune cells migrate into the tumor in sequential order. To date, no study of TNBC has explored the dynamic interplay over time between subsets of immune cells in and around the tumor and the expression of PD-L1 and other immunomodulatory proteins.

Nor have existing studies of the prognostic value of PD-L1 in TNBC considered the glycosylation status of this protein. PD-L1 is functional (able to interact with its receptor, PD-1) only when it is glycosylated, and TNBCs have higher levels of glycosylated PD-L1 than other types of breast cancer do.⁴⁹ If, as is likely, the spatial and temporal expression and functional status of PD-L1 mediate its effects, then all previous studies on survival in TNBC and their discordant findings may have to be set aside in favor of more complex and informative studies that will explore how the timing, immune microenvironment, and glycosylation of PD-L1 influence its association with patient outcome.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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