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ORIGINAL RESEARCH

Unveiling the Landscape of PD-L1 Expression and Tumor-Infiltrating Lymphocyte Subtypes in Advanced Triple-Negative Breast Cancer in Brazil

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Purpose: This study aimed to assess the frequency and prognostic significance of programmed cell death ligand 1 (PD-L1) expression and tumor-infiltrating lymphocyte (TIL) subtypes in advanced triple-negative breast cancer (TNBC).

Patients and Methods: A database search was conducted to identify women with previously untreated locally recurrent inoperable or metastatic TNBC treated between January 2018 and December 2022. The inclusion criteria required formalin-fixed paraffin-embedded samples aged less than four years. PD-L1 expression was evaluated using the PD-L1 IHC 22C3 pharmDx assay, and the combined positive score (CPS) was calculated. TIL subtypes were assessed using immunohistochemical staining.

Results: The study included 150 patients, with a median age of 51.5 years. The majority of patients were younger than 65 years, postmenopausal, non-white, and had metastatic TNBC. CPS≥10 was observed in 20.9% of cases, mainly in postmenopausal women. No significant differences were found in demographic characteristics and clinicopathological variables across PD-L1 subgroups. Tumors with PD-L1 CPS≥10 had higher expression of CD3+, CD4+, and CD8+ TIL subtypes. Most patients received first-line chemotherapy, with smaller proportions undergoing second, third, and fourth-line treatments. No statistically significant differences were observed in median progression-free survival (PFS) or overall survival (OS) across PD-L1 subgroups in this cohort of chemotherapy-treated patients.

Conclusion: This study provides insights into the expression profiles of PD-L1 and TIL subtypes in advanced TNBC. The PD-L1 CPS status did not significantly affect survival outcomes, but variations in TIL subtype composition were observed based on PD-L1 CPS status.

Keywords: triple-negative breast cancer, PD-L1 expression, tumor-infiltrating lymphocytes, immunohistochemistry, molecular epidemiology, progression-free survival

Introduction

Triple-negative breast cancer (TNBC) represents a diverse pathological entity defined by the absence of estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2) protein over-expression.[1] It accounts for approximately 10–20% of all breast cancer cases and is distinguished by its aggressive clinical course, propensity for distant metastasis, and unfavorable survival prospects.[1–3] TNBC predominantly affects younger females and exhibits an elevated occurrence in non-white racial and ethnic groups, notably Black and Hispanic populations.[4,5]

Studies conducted in Brazil revealed the unfavorable prognosis associated with TNBC compared to other breast cancer subtypes.[6] Brazilian TNBC patients experience higher rates of recurrence and shorter overall survival (OS), with

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a notable prevalence observed in the northern region of the country.[4,7] Furthermore, despite receiving treatment with standard chemotherapy, a significant proportion of women experience tumor recurrence and succumb to metastatic disease within the first few years after diagnosis.[5,7] With an aggravating factor of no availability of anti-PD1/PDL1 therapy at public health institutions.

Standard first-line treatment for advanced TNBC involves chemotherapy with taxanes or anthracyclines, as recommended by international guidelines[8,9] and more recently immunologic checkpoint blockade with antibodies against the programmed cell death protein-1 (PD-1) or its ligand (PD-L1) combined with chemotherapy for patients with PD-L1 positive status.[9–11] Furthermore, targeted therapy with poly-ADP ribose polymerase (PARP) inhibitors, such as olaparib or talazoparib, can be considered for patients with germline BRCA mutations.[9]

The tumor microenvironment (TME), consisting of tumor cells, stromal cells, and immune cells, including tumor-infiltrating lymphocytes (TILs), plays a crucial role in breast cancer development, progression, and response to therapy.[5] Clinical and pre-clinical studies have indicated that higher TILs levels within the tumor site are associated with improved treatment response, including to immunotherapy, chemotherapy, and radiation therapy.[12] TNBC often exhibits higher TILs levels, which may indicate an adaptive immune anti-tumor response.[13,14] TILs in TNBC predominantly consist of memory CD4+ and CD8+ T cells,[15] which are essential for eliminating cancer cells and are dynamically balanced by regulatory T cells, which instead express FOXP3+. Moreover, the composition of TILs has been associated with survival outcomes in TNBC and the expression of PD-L1.[15]

PD-L1 expression on tumor cells promotes immunosuppression and facilitates tumor growth by evading the anticancer immune response. PD-L1 expression and the presence of TILs have emerged as potential predictors of response to immune checkpoint inhibitors in advanced TNBC.[16] Notably, clinical trials have demonstrated improved survival in advanced TNBC patients with PD-L1 positive tumors treated with immune checkpoint inhibitors. Studies such as IMpassion130[2] and KEYNOTE-355[10] have reported significant benefits in terms of prolonged PFS and OS when pembrolizumab or atezolizumab is combined with chemotherapy, highlighting the increasing importance of immunotherapy in TNBC.[10]

However, the prevalence of PD-L1 expression in advanced TNBC patients in Brazil remains unclear, and its association with survival outcomes in this patient population is not well-established. Considering the differences observed in PD-L1 expression prevalence in other cancers in Brazil,[17] further investigation is warranted to understand the potential impact of pre/analytical issues and population differences on PD-L1 expression in TNBC.

This paper aimed to address these knowledge gaps by examining the role of PD-L1 and TILs subtypes in Brazilian patients with advanced TNBC, providing insights into the prevalence of PD-L1 expression and its association with survival outcomes.

Material and Methods

Study Design and Ethical Considerations

This was an observational, retrospective study in a single public institution (Brazilian National Cancer Institute – INCA) in Rio de Janeiro (Brazil). The study was approved by the Ethics in Human Research Committee and conducted following the Good Clinical Practice guidelines. Patients provided their informed consent prior to study procedures or fulfilled one of the criteria to waive the consent: those who were no longer being treated at the institution or lost to follow-up or those who died.

Patient Selection

Eligible patients had to be female and aged ≥18 years at the time of advanced TNBC diagnosis. Furthermore, their diagnosis of advanced TNBC needed to be histologically confirmed as ER, PR, and HER2 negative. The TNBC stage should either be locally advanced and unresectable (stage III) or metastatic (stage IV). Patients were required to have a biopsy sample with up to four years available for PD-L1 testing. Additionally, the biopsy must have been taken before starting any chemotherapy or systemic therapy for advanced TNBC. Individuals were excluded from the study if they had previously received, or were currently undergoing treatment with, anti-PD-1 or ant-PD-L1 agents. Additionally, exclusion was applied to those whose biopsy samples failed the quality check.

Study Design

Demographic and pathological aspects, treatments, and clinical outcomes were collected from the patient's medical records. Before completing the Electronic Case Report Forms (e-CRF), the investigator or local team filled out a small patient screening form to ensure that the cases met the study's inclusion criteria and that the tumor samples passed the quality check, being in good condition for immunohistochemistry (IHC) tests.

The primary outcome of this retrospective study was to estimate the frequency of PD-L1 expression in tumor samples of patients with advanced TNBC. PD-L1 protein expression was evaluated using PD-L1 IHC 22C3 pharmDx assay (Agilent Technologies, CA, USA) using the combined positive score (CPS), which is the number of PD-L1 staining cells (tumor cells, lymphocytes, macrophages) divided by the total number of viable tumor cells, multiplied by one hundred. The results of CPS expression were then classified into three categories: CPS≥10 vs CPS 1–10 vs CPS<1 (Figure 1).

The secondary outcomes included an assessment of cells infiltrating the TME in a tissue microarray (TMA), evaluated at INCA Division of Pathology (DIPAT), using the following antibodies: CD3 (MRQ-39, Sigma-Aldrich, CA, USA), CD4 (SP35, Sigma-Aldrich, CA, USA), CD8 (SP16, Sigma-Aldrich, CA, USA), CD56 (123C3.D5, Sigma-Aldrich, CA, USA), CD68 (Kp-1, Sigma-Aldrich, CA, USA), CD117 (c-kit, Sigma-Aldrich, CA, USA), FOXP3 (236/E7, Abcam, CBG, UK) and PD-1 (NAT105, Sigma-Aldrich, CA, USA). HER2 (SP3, Sigma-Aldrich, CA, USA), ER (EP1, Agilent Technologies, CA, USA), and PR (PgR636, Agilent Technologies, CA, USA) were also confirmed as negative.

All IHC techniques were performed with positive and negative controls. Evaluation and quality control of immunohistochemical reactions were performed by two experienced pathologists blinded to the patient's data. The PD-L1 expression was evaluated in a reference laboratory by trained pathologists. The quantitative and qualitative analyses of the TILs were done through a standardized test at the local laboratory.

The study also assessed on an exploratory basis the expression of PD-L1 (CPS≥10) as a predictive and prognostic factor for patients with TNBC receiving local standard treatment, consisting of approved regimens in the institution at the discretion of the attending physician.

Statistical Analysis

The normality of continuous variables (age and TILs expression) was assessed by using the Shapiro–Wilk test. PD-L1 CPS distribution was reported in terms of both absolute and relative frequencies as well as categorical demographic and clinical variables. The age difference between the PD-L1 groups was assessed using the Mann–Whitney test, and associations between the PD-L1 groups and demographic and clinical variables were evaluated using the Pearson chi-square test. The difference between the expressions of each TIL and the PD-L1 groups was assessed using the Mann–Whitney test.

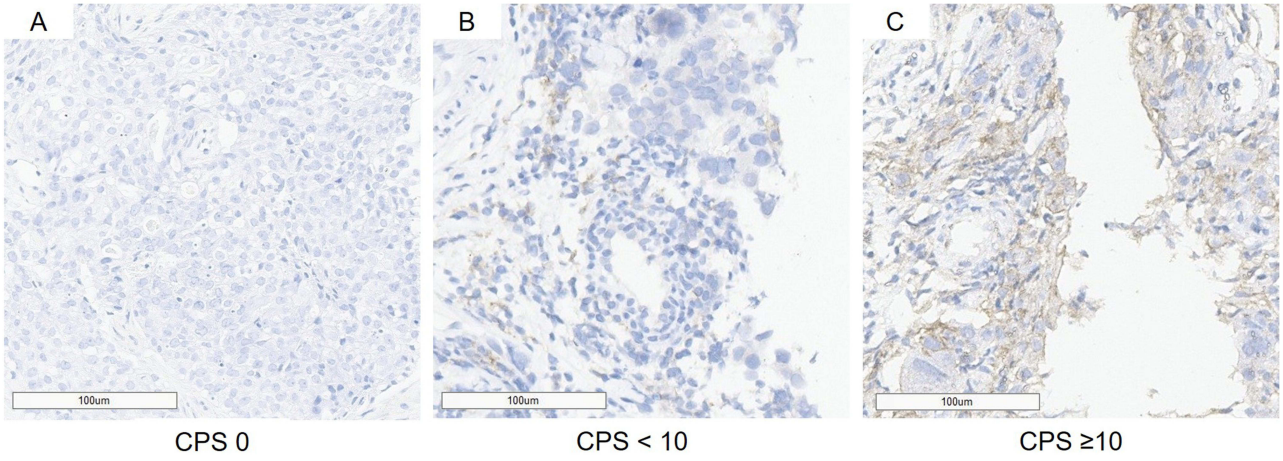

Figure 1 Representative pictures of different PD-L1 expression levels on immunohistochemistry staining. Images of immunohistochemical 22C3 pharmDx assay showing PD-L1 expression scored as CPS 0 (**A**), CPS < 10 (**B**) and CPS ≥10 (**C**). Each specimen was acquired at ×20 magnification.

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PFS was determined by measuring the interval from the date of diagnosis to the earliest occurrence of disease progression or death. OS was calculated based on the date of diagnosis, with events defined as death from any cause, and censored if the patient was known to be alive on the last day of data collection. Estimation of PFS and OS for PD-L1 CPS was done through the use of the Kaplan–Meier method, followed by comparison using the Log rank test. A multiple Cox proportional hazards model was then employed to estimate the adjusted Hazard Ratio and respective 95% Confidence Interval (CI 95%). To select confounding variables for the multiple models, the univariate Cox model was used for each potential confounder (age, Performance Status and metastatic site) and the variables with a p-value <0.2 were manually entered into the model. Statistical significance was set up at a threshold of p <0.05. Missing data were systematically excluded from the analysis. These statistical procedures were executed within the R environment version 3.5.3.

Results

A total of 150 patients were included in the study, with two cases in which samples have passed in the quality check having undetermined PD-L1 expression. Most patients were under 65 years old (73%), postmenopausal (56.8%), and non-white (70.3%). The median age of the patients was 51.5 years (IQR: 41.8–60.2), and 20.9% of the cases showed a CPS≥10, with postmenopausal women constituting the majority (74.2%) of this group (Table 1).

Regarding PD-L1 expression, no significant statistical differences were observed in demographic characteristics and clinicopathological variables: 79.1% of patients had a CPS<10 and 75.7% of them had de novo stage IV metastatic disease (Table 1). Tumors with PD-L1 CPS≥10 showed higher expression of CD3+ (p=0.037), CD4+ (p=0.005), and CD8+ (p=0.001) TILs (Table 2 and Figure 2).

Table 1 Clinical-Pathological Characteristics by CPS Status (N=148)

Characteristics		CPS<10 N (%)	CPS≥10 N (%)	Total N (%)	p
Total		117 (79.1)	31 (20.9)	148 (100)	
Age	Median (IQR)	49 (41–61)	54 (45.5–59.5)	51.5 (41.8–60.2)	0.546
Ethnicity	Black	27 (23.1)	9 (29.0)	36 (24.3)	0.778
	Brown	55 (47.0)	13 (41.9)	68 (45.9)	
	White	35 (29.9)	9 (29.0)	44 (29.7)	
Menopausal status	Premenopausal	42 (35.9)	7 (22.6)	49 (33.1)	0.072
	Perimenopausal	14 (12.0)	1 (3.2)	15 (10.1)	
	Postmenopausal	61 (52.1)	23 (74.2)	84 (56.8)	
Family history of cancer	No	51 (43.6)	14 (45.2)	65 (43.9)	1.000
	Yes	66 (56.4)	17 (54.8)	83 (56.1)	
PS ECOG	0	10 (8.5)	3 (9.7)	13 (8.8)	0.303
	1	72 (61.5)	15 (48.4)	87 (58.8)	
	2	23 (19.7)	12 (38.7)	35 (23.6)	
	3	9 (7.7)	1 (3.2)	10 (6.8)	
	4	2 (1.7)	0 (0.0)	2 (1.4)	
	Unknown	1 (0.9)	0 (0.0)	1 (0.7)	
Disease diagnosed as locally advanced or metastatic	Locally advanced/ unresectable	26 (22.2)	10 (32.3)	36 (24.3)	0.356
	Metastatic	91 (77.8)	21 (67.7)	112 (75.7)	
Bone metastasis	No	88 (75.2)	29 (93.5)	117 (79.1)	0.047
	Yes	29 (24.8)	2 (6.5)	31 (20.9)	
Brain metastasis	No	116 (99.1)	30 (96.8)	146 (98.6)	0.887
	Yes - Controlled neurological symptoms	1 (0.9)	1 (3.2)	2 (1.4)	
Liver metastasis	No	97 (82.9)	28 (90.3)	125 (84.5)	0.463
	Yes	20 (17.1)	3 (9.7)	23 (15.5)	

(*Continued*)

Table 1 (Continued).

Characteristics		CPS<10 N (%)	CPS≥10 N (%)	Total N (%)	p
Lung metastasis	No	67 (57.3)	20 (64.5)	87 (58.8)	0.600
	Yes	50 (42.7)	11 (35.5)	61 (41.2)	
Lymph nodes metastasis	No	66 (56.4)	19 (61.3)	85 (57.4)	0.776
	Yes	51 (43.6)	12 (38.7)	63 (42.6)	
Skin/Soft tissue metastasis	No	99 (84.6)	19 (61.3)	118 (79.7)	0.009
	Yes	18 (15.4)	12 (38.7)	30 (20.3)	

Abbreviations: CPS, Combined Positive Score; IQR, interquartile range.

Table 2 Tumor Infiltrating Lymphocytes Characterization (CD3, CD4, CD8, CD56, CD68, CD 117, FOXP3 and PD-1) and Its Correlation With CPS Status

Characteristics	Total N (%)	Missing N	CPS<10 (Median [IQR])	CPS≥10 (Median [IQR])	Total (Median [IQR])	p
CD3 expression	142 (95.9)	6	5.0 (1.0 to 20.0)	10.0 (4.0 to 35.0)	5.0 (1.0 to 27.5)	0.037
CD4 expression	142 (95.9)	6	5.0 (1.0 to 20.0)	20.0 (5.0 to 60.0)	10.0 (1.0 to 30.0)	0.005
CD8 expression	145 (98.0)	3	5.0 (1.0 to 15.0)	20.0 (5.0 to 50.0)	5.0 (1.0 to 20.0)	0.001
CD56 expression	145 (98.0)	3	0.0 (0.0 to 0.0)	0.0 (0.0 to 0.0)	0.0 (0.0 to 0.0)	0.883
CD68 expression	143 (96.6)	5	7.5 (1.0 to 40.0)	20.0 (1.0 to 30.0)	10.0 (1.0 to 40.0)	0.894
CD117 expression	145 (98.0)	3	0.0 (0.0 to 0.0)	0.0 (0.0 to 0.0)	0.0 (0.0 to 0.0)	0.683
FOXP3 expression	145 (98.0)	3	0.0 (0.0 to 2.0)	3.0 (0.0 to 10.0)	0.0 (0.0 to 3.0)	0.014
PD-1 expression	125 (84.5)	23	0.0 (0.0 to 1.0)	1.0 (0.2 to 13.8)	1.0 (0.0 to 1.0)	0.003

Abbreviations: CD3, Cluster of Differentiation 3; CD4, Cluster of Differentiation 4; CD8, Cluster of Differentiation 8; CD56, Cluster of Differentiation 56; CD68, Cluster of Differentiation 68; CD117, Cluster of Differentiation 117; CPS, Combined Positive Score; FOXP3, Forkhead Box P3; IQR, interquartile range; PD-1, Programmed Cell Death Protein 1.

In terms of treatment, at the time of the analysis, approximately half of the patients (47%) underwent only first-line chemotherapy, 28.8% received second line, and 12.9% and 9.1% received third and fourth lines, respectively. Only three patients received more than four lines of palliative chemotherapy.

Survival outcomes, including median PFS and OS, showed no statistically significant differences between CPS≥10 and CPS<10 subgroups (PFS: 5.1 vs 5.0 months, p=0.88; OS: 8.7 vs 8.8 months, p=0.6) (Figures 3 and 4).

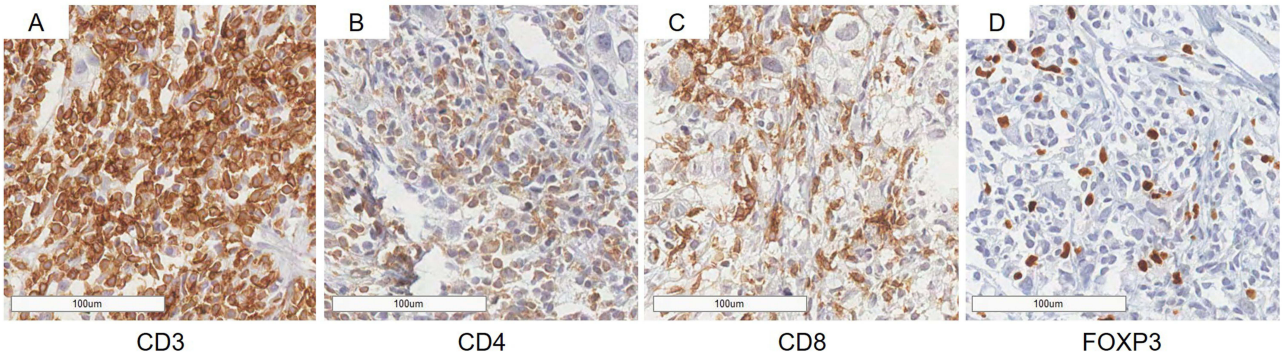

Figure 2 Representative images of tumor-infiltrating lymphocytes subtypes with high expression of CD3, CD4, CD8 and FOXP3 (**A**–**D**, respectively). Each specimen was captured at a ×20 magnification.

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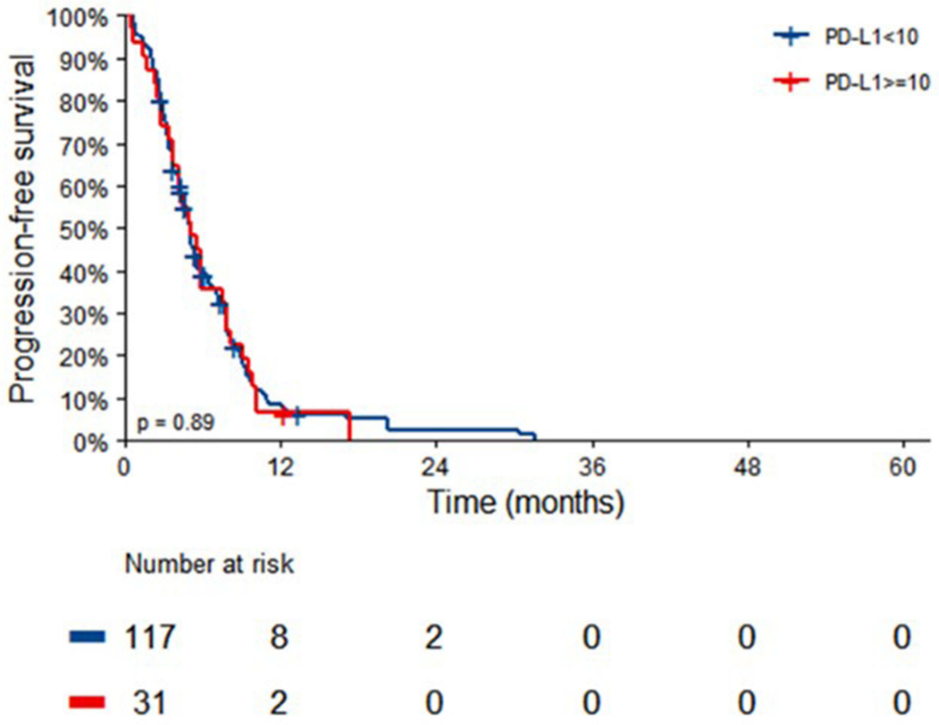

Figure 3 Progression-free survival (PFS) by PD-L1 expression status. Regarding the PD-L1 expression dichotomization, the Kaplan–Meier curve for PFS was stratified by the CPS cutoff of 10%. Tick marks indicate censored data.

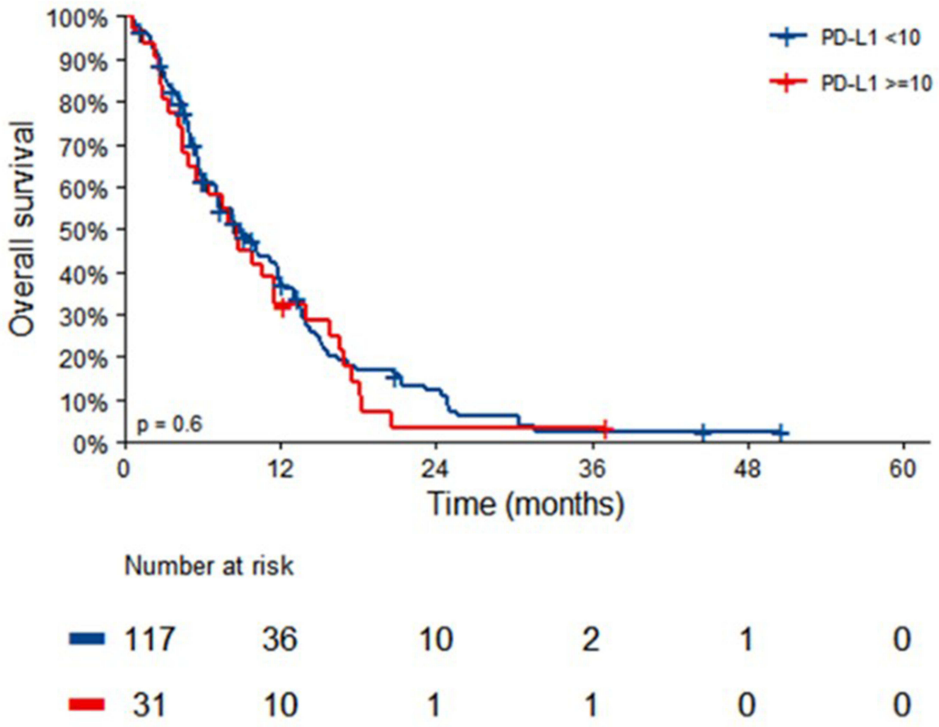

Figure 4 Overall survival (OS) by PD-L1 expression status. Regarding the PD-L1 expression dichotomization, the Kaplan–Meier curve for OS was stratified by the CPS cutoff of 10%. Tick marks indicate censored data.

Discussion

This study aimed to address the knowledge gap concerning PD-L1 expression and its association with survival outcomes in Brazilian patients with advanced triple-negative breast cancer (TNBC). A comprehensive description of PD-L1 expression, along with demographic and clinical characteristics was provided, revealing no statistically significant differences across combined positive score (CPS) groups, in this chemotherapy-treated cohort.

Emphasizing the clinical context, it is crucial to note that 75.7% of patients were already metastatic at the time of diagnosis, reflecting the prevailing scenario of breast cancer within the evaluated institution. This finding underscores the access barriers in Brazil's public health setting, potentially introducing bias. Most patients, upon diagnosis, exhibited exceptionally large tumors involving the skin and prominent lymphadenopathy. Notably, some patients, despite initially lacking detectable distant visceral or bone metastases, presented disease progression during first-line treatment, which was primarily targeted toward local disease control. Delineating between locally advanced and metastatic disease posed considerable challenges, impacting treatment decisions. In scenarios where traditional staging methods, such as tomography and bone scan, did not reveal distant metastasis, the primary treatment approach aimed at downstaging, despite the relatively unfavorable prognosis associated with such patients.

The study found that 20.9% of patients with TNBC exhibited a CPS ≥10, indicating that at least 10% of the total cell population—including lymphocytes, macrophages, and tumor cells—demonstrated positive staining for PD-L1. This proportion is significantly lower than the rates observed in the KEYNOTE-355 study (38.13%)[18] and the Impassion 130 trial (40.9%).[2] Notably, the KEYNOTE-355 study employed the PD-L1 IHC 22C3 pharmDx assay for the evaluation of PD-L1 expression. On the other hand, the IMpassion130 trial employed the SP142 assay to determine PD-L1 status, designating positive cases as those displaying at least 1% PD-L1 immune cell positivity. In the IMpassion130 trial, IHC was conducted on samples derived from primary breast cancer (in 62% of enrolled patients), local recurrence biopsies, or biopsies of metastatic lesions in various organs, excluding bone. An exploratory analysis of these trial samples showed that primary breast tumor tissue exhibited a higher prevalence of PD-L1 positivity compared to samples obtained from distant metastatic sites.[19] This clear difference in PD-L1 positivity cannot be ignored and may indicate a peculiarity of the tested population. Furthermore, it is important to consider that the study was carried out in a single institution, which may explain the lower prevalence of CPS≥10 population compared to what was seen in KEYNOTE-355. In our study, out of the 150 samples analyzed, the majority originated from primary tumor sites, with only two samples obtained from metastatic sites.

Gelatti et al found a lower prevalence of high PD-L1 expression in Brazilian non-small-cell lung cancer patients than in other countries. The current study supports and reinforces these findings in a cohort of breast cancer patients, suggesting that differences in genetic background or environmental exposures may indeed influence tumor biology and PD-L1 expression in the Brazilian population. This emphasizes the significance of diversity in future clinical trials to accurately represent our population and potentially impact trial outcomes.[17]

Another significant observation is the lower proportion of patients with ECOG performance status 0–1 among those with CPS≥10, which may suggest a more aggressive disease course. Patients with CPS<10 presented a higher frequency of bone metastases, while those with CPS≥10 tended to have bilateral lung lesions, multiple hepatic lesions, distal nodal involvement, and soft tissue infiltration. These clinical patterns could potentially influence treatment approaches. It is conceivable that this distribution may be reflected in the number of treatment lines administered. The CPS<10 group appeared to receive a broader array of treatment modalities compared to the CPS≥10 group.

In terms of prognosis, PD-L1 expression did not significantly impact PFS or OS in advanced TNBC. Notably, no significant differences in response to cytotoxic chemotherapy were detected even within the CPS≥10 group. This finding aligns with previous studies that reported inconsistent results regarding PD-L1's prognostic role in TNBC. In those past cohorts, low PD-L1 expression was associated with higher complete response rates and served as an independent prognostic factor.[20,] Conversely, high PD-L1 expression has been correlated with improved outcomes in TNBC patients.[21,22] However, more recent cohorts,[23,] including this study, demonstrated no independent prognostic role for this biomarker in TNBC patients not exposed to immunotherapy. For instance, the median PFS reported in this study, regardless of CPS group (5.06 months for the CPS<10 group and 5.16 for the CPS≥10), is comparable to the placebo cohorts of IMpassion 130 trial (5.5 months for the total population and 5.0 months in the PD-L1-positive group) and KEYNOTE-355 trial (5.6 months for all subgroups analyzed: CPS≥10, CPS≥1 or the total population). The conflicting

observations may be attributed to several factors, including the different clinical outcomes measured, evolving methods for evaluating PD-L1 expression, complex interactions between checkpoint receptors and immune cells in the tumor microenvironment (TME), the definition of cutoffs, and the ongoing evolution of cancer therapy modalities.

On the other hand, as shown in KEYNOTE-086 and KEYNOTE-119 trials, TILs and PD-L1 expression have marked associations with survival outcomes in patients receiving checkpoint inhibitors.[23,24] In the KEYNOTE-119, where previously treated patients with metastatic TNBC received pembrolizumab or standard chemotherapy, higher PD-L1 expression was associated with longer median OS in the pembrolizumab group. Notably, a post-hoc analysis showed a pronounced benefit of pembrolizumab in patients with PD-L1-enriched tumors with CPS>20 (HR 0.58, 95% CI 0.38–0.88). However, in consonance with the current findings, the effect of chemotherapy on survival was independent of observed tumor PD-L1 expression.

Despite the conflicting prognostic implications, PD-L1 expression has firmly established its role as a biomarker guiding clinical indications for immune checkpoint inhibitors in advanced TNBC. As research progresses, the immune components of the TME continue to garner attention as potential predictors of response to checkpoint inhibitors. The complex and heterogeneous presence of TILs and other immune cells in the TME plays a crucial role in tumor response to different treatment strategies and may influence overall survival as well.[5,25]

It is noteworthy that in this study the composition of TILs within the TME appeared to vary based on PD-L1 expression. Tumors with PD-L1 CPS≥10 were correlated with the presence of cytotoxic T-cell subtypes (CD3, CD4, CD8). Conversely, the cellular immune profiling beyond TILs, such as CD56+ Natural Killer (NK) cells and FOXP3+ regulatory T lymphocytes, were not clearly associated with a PD-L1 CPS status.

Targeting infiltrating immune cells other than TILs is of great significance since the effect of different cell types can lead to a variety of immunoregulatory results, from inhibiting cancer progression with cytotoxic cancer response to immune tolerance and escape from immune surveillance. While CD4+ helper and CD8+ cytotoxic T lymphocytes compose effector TILs and are associated with higher responses to chemotherapy, CD56+ NK cells' role is still to be defined in the anti-tumor response, as it did not seem to predict survival.[26,27] Nonetheless, regulatory T lymphocytes, FOXP3+ cells are critical for immune regulation and are known to maintain an immune tolerogenic microenvironment.[20,28,] In this cohort, the higher expression of FOXP3 in PD-L1 CPS≥10 group seemed not to elicit the worst outcomes in this group. Furthermore, the evaluation of another immune cell profile with the tumor-associated macrophages (TAMs) CD68+ showed no significant difference between PD-L1 CPS subgroups. This finding contrasts a prior report that associated TAMs high-density with worse survival outcomes, higher expression of inflammatory cytokines and greater risk of lymph nodes metastases in TNBC.[29,30]

The temporal changes and treatment effects on TME show that, instead of an intrinsic permanent characteristic, TILs can be dynamic in their conformation and clinical significance. In TNBC, core biopsy TME analysis pre-neoadjuvant chemotherapy did not correlate with response nor survival, although post-treatment tissue microarrays IHC immune cell profiling from surgical samples showed that TIL subtypes and its proportion analysis could play a key role in determining the prognosis of these patients.[31] Understanding the intricate interactions between immune checkpoint receptors and immune cells at the TME is essential to understand the conflicting observations on PD-L1 prognostic roles. Profound analysis of TILs levels on tumor site and dichotomization in high or low infiltration patterns was beyond the objective of this paper.

While this study provides valuable insights into the relationship between PD-L1 expression and the TME, several limitations warrant consideration. These include constraints imposed by limited biopsy material precluding the analysis of gene expression profiles. Losses in sample integrity may have occurred due to inadequately preserved specimens. Additionally, challenges arose in distinguishing intratumoral from stromal lymphocytic infiltration, with potential implications for the outcomes of TME analyses. Furthermore, the retrospective design, conducted within a single-center setting, may obscure regional disparities and limit the generalizability of findings. It is noteworthy that despite the majority of patients presenting with advanced disease at diagnosis, none received anti-PD1/PDL1 therapy, irrespective of CPS status, primarily due to the lack of access to these new treatments within the context of public healthcare institutions.

Conclusion

The present study elucidates that, in advanced TNBC, the presence of PD-L1 with CPS ≥ 10 does not exert an independent and statistically significant influence on survival outcomes in the context of standard chemotherapy. The findings underscore the imperative for a more profound comprehension of the intricate interplay among immune checkpoint receptors, immune cells, and the tumor microenvironment specific to TNBC. While isolated PD-L1 expression may not serve as a definitive prognostic marker, its significance persists in informing treatment decisions, particularly in the realm of immune checkpoint inhibitors. Ongoing and expanded research endeavors, encompassing larger patient cohorts, as well as a meticulous analysis of TME components, are essential for elucidating the intricate complexities inherent to TNBC. The refinement of personalized treatment strategies for this challenging disease needs the execution of international multicentric clinical trials with enhanced representation from the Brazilian population, thereby ensuring that real-world data results are more representative and possess increased clinical impact.

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Disclosure

At the time of the study, CPM, PMB, MACR, MACSMB were employees of Merck Sharp Dohme Farmacêutica Ltda, Sao Paulo, Brazil, a subsidiary of MERCK & CO., INC., RAHWAY, NJ, USA, who may own stock and/or hold stock options in MERCK & CO., INC., RAHWAY, NJ, USA ALSS, JLS, LZA, ALAN, CFS, LAMC, IAS, ACM were employees of the Division of Clinical Research and Technological Development, Brazilian National Cancer Institute, Rio de Janeiro, Brazil, which provided outsourcing services for the data collection, analysis and writing the manuscript. FRR, FCM were employees of the Division of Pathology, Brazilian National Cancer Institute, Rio de Janeiro, Brazil, which tissue sample analysis. The authors report no other conflicts of interest in this work.

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