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Breast Cancer

Programmed Cell Death Ligand 1 in Breast Cancer: Technical Aspects, Prognostic Implications, and Predictive Value

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Key Words. Programmed cell death ligand 1 • Immunotherapy • Immune checkpoint inhibitors • Breast cancer • Biomarkers • Patient selection

Abstract

In the light of recent advances in the immunotherapy field for breast cancer (BC) treatment, especially in the triple-negative subtype, the identification of reliable biomarkers capable of improving patient selection is paramount, because only a portion of patients seem to derive benefit from this appealing treatment strategy. In this context, the role of programmed cell death ligand 1 (PD-L1) as a potential prognostic and/or predictive biomarker has been intensively explored, with controversial results. The aim of the present review is to collect available evidence on the biological relevance and clinical utility of PD-L1 expression in BC, with particular emphasis on technical aspects, prognostic implications, and predictive value of this promising biomarker. ***The Oncologist*** 2019;24:e1055–e1069

Implications for Practice: In the light of the promising results coming from trials of immune checkpoint inhibitors for breast cancer treatment, the potential predictive and/or prognostic role of programmed cell death ligand 1 (PD-L1) in breast cancer has gained increasing interest. This review provides clinicians with an overview of the available clinical evidence regarding PD-L1 as a biomarker in breast cancer, focusing on both data with a possible direct impact on clinic and methodological pitfalls that need to be addressed in order to optimize PD-L1 implementation as a clinically useful tool for breast cancer management.

Introduction

In the constantly evolving era of immunotherapy, the blockade of the programmed cell death 1 (PD1)/programmed cell death ligand 1 (PD-L1) immune checkpoint pathway represents one of the most promising strategies to revert immune evasion in the cancer immunoediting process. PD1 is a cell surface membrane protein, member of the B7 family of immune checkpoints, which is activated by its ligands PD-L1 and PD-L2. Activated lymphocytes induce the expression of PD-L1 on the surface of T cells, Natural Killer cells (NK), macrophages, and, most importantly, tumor cells, through different mechanisms, among which the secretion of interferon gamma (IFN-gamma) is the most important. Once engaged, the PD1/PD-L1 pathway leads to the mitigation of T-cell-mediated immune response through the inhibition of T-cell activation and the promotion of the regulatory function of T lymphocytes. In solid tumors, this process may be exploited by the tumor microenvironment to silence or at least attenuate the antitumor immune response [1–3].

The use of immune checkpoint inhibitors led to striking results in several solid tumors such as melanoma, bladder cancer, and non-small cell lung cancer (NSCLC). Although breast cancer (BC) is not traditionally considered immunogenic, a growing body of evidence suggests that certain BC subtypes, namely, triple-negative (TN) and human epidermal growth receptor 2 (HER2)-positive (HER2+), may exhibit a strong infiltration by immune cells with prognostic and even predictive implications [4, 5]. This evidence also fostered the evaluation of immune checkpoint inhibitors in BC, with promising results, especially in the TN subtype. Importantly, a statistically significant progression-free survival

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(PFS) benefit with the combination of atezolizumab plus nab-paclitaxel compared with placebo plus nab-paclitaxel in patients with TN metastatic BC (MBC) has recently been reported [6]. In this context, as a result of the growing interest in the identification of reliable prognostic and/or predictive biomarkers for patients treated with immunotherapy, the scientific interest focused on PD-L1 expression. However, the clinical relevance of PD-L1 as a biomarker in BC remains to be clearly defined.

We therefore conducted a review on the role of PD-L1 expression in BC with the aim of collecting available evidence coming from clinical studies.

Materials and Methods

Relevant studies were searched in the PubMed database with the following keywords: "breast cancer," "breast tumor," "breast neoplasm," "programmed cell death ligand 1," "programmed cell death 1," "PD-L1," "PD-1," "B7-H1," "CD274," "CD279," and "immune checkpoint."

In addition, reference lists of retrieved articles were manually reviewed.

In order to include the most recent data, we also searched for relevant studies presented in the form of abstracts in major international medical oncology conferences (American Society of Clinical Oncology 2016, 2017, 2018; San Antonio Breast Cancer Symposium 2016, 2017, 2018; European Society for Medical Oncology 2016, 2017, 2018).

The language was restricted to English.

PD-L1 Expression in BC

PD-L1 expression by immunohistochemistry (IHC) or gene expression has been observed in approximately 20%–40% of all BCs across different studies, and it has been shown to be significantly higher in invasive disease compared with normal breast tissue [7–12] and premalignant lesions as in situ carcinoma [13].

In addition, it has been reported that PD-L1 is differentially expressed across different BC subtypes. In particular, available evidence consistently reports greater expression of PD-L1 in the TN subtype (up to 60% of PD-L1 expression) compared with non-TNBC [14–21]. These data appear to be coherent with the observation that PD-L1 tumor expression is positively associated with stromal tumor-infiltrating lymphocytes (TILs) in this BC subtype [22–29], which is known to be more frequently infiltrated by stromal TILs than non-TNBC [5], as summarized in Table 1, thus possibly suggesting that these two immune biomarkers tend to run parallel.

Data on PD-L1 expression in HER2-positive BC are more controversial. In fact, whereas in some studies HER2 positivity has been correlated with higher expression of PD-L1 (up to 50%) compared with HER2-negative BC [10, 14, 18, 20], others failed to report any difference [9, 15–17, 19, 30].

Results from two meta-analyses including partially overlapping studies confirmed the greater PD-L1 expression in TNBC [31, 32] compared with non-TN subtypes but were not consistent in reporting the association between HER2 status and PD-L1 expression.

However, when considering molecular intrinsic BC subtypes by gene expression profiling rather than IHC, both basal-like and HER2-enriched subgroups were found to be enriched in PD-L1 expression with respect to luminal BC [10, 25, 33–35], thus possibly highlighting that a more subtle classification in BC subtypes may help better capture the relevance of immune microenvironment for TN and HER2-positive BC.

Association with Clinicopathological Characteristics

Several studies evaluated PD-L1 expression according to baseline clinicopathological features of patients with BC, consistently reporting a correlation between higher PD-L1 expression and unfavorable classic prognostic factors, particularly poorer histological grade [10, 11, 14, 18, 30, 36–45], higher proliferative index [10, 38, 39, 46, 47], more advanced N stage [14, 30, 44, 48], larger tumor size [10, 11, 14, 30, 44, 48], and younger age at BC diagnosis [14, 39, 40, 48].

A possible explanation for such observations may be attributable to the immune escape phenomenon. Indeed, a high expression of PD-L1 may reflect the activation of the immune checkpoint PD1/PD-L1 pathway leading to the mitigation of the host's antitumor immune response, thus ultimately resulting in increased tumor aggressiveness [31]. Although intriguing, further evidence is needed in order to confirm this hypothesis. In addition, it should be considered that aggressive clinicopathological characteristics are typical features of the TN subtype, thus possibly posing a bias in the interpretation of these data.

Association with Prognosis

Several authors explored the possible prognostic role of PD-L1 expression in early BC, reporting conflicting results, as shown in Table 2.

PD-L1 expression evaluated by IHC on untreated primary BC has been associated with both better and poorer clinical outcome. In particular, several authors reported poorer disease-free survival (DFS)/recurrence-free survival (RFS) and/or overall survival (OS) in cases of higher PD-L1 expression on primary BC [14, 19, 30, 49–53], especially in the TN subtype [19, 50, 53]. These results seems to be consistent with the previously mentioned correlation between PD-L1 expression and unfavorable clinicopathological BC features, thus possibly indicating that PD-L1 expression may be part of the immune evasion process taking place in the context of tumor microenvironment [14].

Counterintuitively, a prognostic role in the opposite direction has also been suggested.

In particular, PD-L1 expression has been positively and independently associated with DFS/RFS and/or OS in several unselected primary BC cohorts [16, 21, 22, 24, 25, 40, 44, 46, 48, 54–58]. In addition, when considering specific BC subtypes, it has been suggested that PD-L1 protein expression may retain a positive prognostic role in TNBC [21, 22, 24, 46, 55, 56] and in HER2+ BC, in both trastuzumab-treated and untreated patients [44, 57].

Such inconsistency may reflect the fact that in the above-mentioned studies, PD-L1 expression was assessed at the protein level by IHC. However, it should be considered that the assessment of PD-L1 by IHC on BC tissue lacks standardization, thus possibly impairing the reproducibility of results across different studies. Interestingly, when considering the prognostic role of PD-L1 assessed at mRNA expression level, available evidence is consistent in suggesting an association

Table 1. Studies reporting a correlation between PD-L1 and TILs

Study (design)	No. of patients included (BC subtype)	PD-L1 positivity cutoff	TILs	Conclusions
Arias-Pulido 2018 [16] (retrospective)	221 (IBC)	PD-L1 on TC and IC (positive: ≥5%)	TILs on H&E, according to international guidelines [102] LPBC status: ≥50% tumor stromal area occupied by TILs	Positive correlation between high sTILs and PD-L1 on TC IC Positive correlation between LPBC status and PD-L1 on TC and IC
Emens 2018 [84] Impassion130 (phase III)	902 (TNMBC)	PD-L1 on IC (positive: ≥1%)	Intratumoral CD8 by IHC sTILs on H&E	Positive correlation between intratumoral CD8 and PD-L1 in TNBC NO correlation between sTILs and PD-L1
Wimberly 2015 [17] (retrospective)	94	Continuous quantitative score on TC and IC Dichotomized PD-L1	TILs on H&E, according to international guidelines [102] LPBC status: ≥50% tumor stromal area occupied by TILs	Positive correlation between PD-L1 as continuous quantitative score in the epithelium and stroma and high TILs
Okabe 2017 [51] (retrospective)	97	H-score on TC (low: 0–99; high: 100–300)	CD3+ or CD8+ TILs by IHC in intratumoral and peritumoral compartments	Positive correlation between CD3+ TIL density and intratumoral PD-L1 NO correlation between CD8+ TIL density and PD-L1
Mardones 2016 [22] (retrospective)	59 primary BCs (16 HR+, 16 HER2+, and 27 TNBC)	% positive TC (positive: ≥1%)	CD8 expression by IHC within tumor and stroma (average number of CD8+ cells within 10 high-power fields determined separately for invasive tumor cell nests and for stroma) CD68 expression by IHC within tumor and stroma (Nonlymphocyte mononuclear cells in tumor and stroma were used in counting CD68+ TAMs) Positivity: number of cells positive in the sample greater than the median	Positive correlation between CD8-TILs and PD-L1 in TNBC Positive correlation between CD68-TAMs and PD-L1 in TNBC
Schalper 2014 [59] (retrospective)	636	Average QIF score of DapB (positive: mRNA signal > DapB)	TILs on H&E using a four-tiered scale based on the visual estimation of the amount of lymphocytes in each histospot (0 = virtual absence of TILs, 1 = low TILs <30%, 2 = moderate 30%–60%, 3 = >60%)	Positive correlation between PD-L1 mRNA expression and elevated TILs (scores 2 and 3)
Aierken 2017 [24] (retrospective)	215 (TNBC)	Semiquantitative score (negative: ≤1; positive: >1)	sTILs and iTILs on H&E semiquantitatively (score 1 = TILs-low 0%–10%, 2 = TILs-moderate 11%–40%, 3 = TILs-Marked 41%–100%)	Positive correlation between sTILs scores and PD-L1 NO correlation between iTILs and PD-L1 Positive correlation between LPBC status and PD-L1
Ali 2015 [25] (retrospective)	3,916	TC and IC PD-L1 scored separately (0: <1%, 1: 1%–5%, 2: 6%–10%, 3: >10%)	Intratumoral CD8 and FOXP3 expression by IHC	Positive correlation between PD-L1 and CD8+ and FOXP3+
Cerbelli 2017 [26] (retrospective)	54 (TNBC)	% positive TC and IC PD-L1	sTILs on H&E, according to international guidelines [102]	Positive correlation between high sTILS and PD-L1 on ≥25% of TC and ≥ 10% of IC
Denkert 2015 [27] Geparsixto (RCT)	580 (TNBC)	NA	sTILs on H&E, according to international guidelines [96]. LPBC status: ≥60% of either intratumoral or stromal TILs	Positive correlation between PD-L1 and TILs

(continued)

Table 1. (continued)

Study (design)	No. of patients included (BC subtype)	PD-L1 positivity cutoff	TILs	Conclusions
Beckers 2016 [21] (retrospective)	161 (TNBC)	% staining positive (≥1% and 5% TC or ≥ 1% and 5% IC) H-score (low: 0–99; high: 100–300)	sTILs on H&E semiquantitatively (0 = virtual absence of TILs, 1 = low TILs <30%, 2 = moderate 30%–60%, 3 = >60%)	Positive association between TILs score ≥ 2 and PD-L1
Kim 2017 [57] (retrospective)	176 (HER2+ BC)	TC PD-L1 (Allred score) IC PD-L1 (low vs. high: the threshold was the mean value of PD-L1 immunoscore)	NA	Positive correlation between TILs and PD-L1
Choi 2016 [23]	39 (14 HER2+ and 25 TNBC)	NA	CD4. CD8 and FOXP3 expression by IHC	Positive correlation between PD-L1 and CD8+ T cells in TNBC
Cimino-Mathews 2016 [36] (retrospective)	45	% TC exhibiting clear membrane PD-L1 expression scored in 5% increments from 0% to 100% (negative <5%)	TIL included lymphocytes and macrophages TIL intensity scored as none (0), mild (1; rare TIL; 5% of tumor area), moderate (2; focal infiltrate; 5%–50% of tumor area), and diffuse/severe (3; diffuse infiltrate; >50% of tumor area) CD20, CD3, CD8, FOXP3 expression by IHC	Diffuse/severe TIL infiltration present in 89% of PD-L1+ tumors compared with 24% of PD-L1− tumors PD-L1+ tumors contained more CD3+, CD4+, CD8+, and FOXP3+ T cells than PD-L1− tumors
Mittendorf 2014 [35] (retrospective)	105 TNBC	% staining positive (>5%)	CD8 expression by IHC (CD8+ cells per 1 mm core)	Positive correlation between number of CD8+ T cells and PD-L1 in TNBC
Bertucci 2015 [9] (retrospective)	306 (112 IBC)	TC PD-L1 (PD-L1-high: T/NB ratio ≥ 2 vs. PD-L1-low: T/NB ratio <2. cutoff arbitrarily chosen)	TILs on H&E semiquantitatively (1 none, 2 small, 3 moderate, or 4 strong infiltrate) T-cell-specific, CD8+ T-cell-specific, and B-cell-specific gene expression signatures	Positive correlation between TILs and PD-L1 Positive correlation between PD-L1 and T-cell-specific, CD8+ T-cell-specific, and B-cell-specific gene expression signatures
Dieci 2018 [83] (retrospective)	94 (43 TN, 51 HER2+)	TC PD-L1 and IC PD-L1	TILs on H&E according to consensus guidelines [91, 102]	Positive correlation between TILs and PD-L1, especially in TNBC

Abbreviations: BC, breast cancer; DapB, dihydrodipicolinate reductase; H&E, hematoxylin and eosin; HER2, human epidermal growth receptor 2; IBC, inflammatory breast cancer; IC, immune cells; IHC, immunohistochemistry; iTILs, intratumoral TILs; LPBC, lymphocyte-predominant breast cancer; NA, not available; PD-L1, programmed cell death ligand 1; QIF, quantitative fluorescence; sTILs, stromal TILs; TAM, tumor-associated macrophage; TC, tumor cells; TILs, tumor infiltrating lymphocytes; TN, triple-negative; T/NB ratio, tumor/normal breast ratio.

Table 2. Prognostic role of PD-L1 in untreated BC

Study (design)	No. of patients included (BC subtype)	PD-L1 assessment (assay)	PD-L1 positivity cutoff	Survival endpoints associated with PD-L1
Positive prognostic value				
Sabatier 2015 [10] (retrospective)	5,454	mRNA (Affymetrix U133 Plus 2.0 human microarrays)	Tumor/normal breast ratio Up ≥2 Non-up <2	MFS and OSS in basal-like BC
Schalper 2014 [59] (retrospective)	636	mRNA (fluorescent RNAscope paired-primer assay)	Average QIF score of DapB (negative control bacterial gene) Positive: mRNA signal > DapB Negative: mRNA signal ≤ DapB	RFS
Baptista 2015 [48] (retrospective)	192	Protein (IHC: Abcam, Cambridge, MA)	Allred score system Low vs. high: the threshold was the median of the scores	OS
Ali 2015 [25] (retrospective)	3,916	Protein (IHC: E1L3N)	TC and IC PD-L1 scored separately 0: <1% 1: 1%–5% 2: 6%–10% 3: >10%	BCSS in ER-negative BC (IC PD-L1 > 10%)
Bae 2016 [54] (retrospective)	465	Protein (IHC: clone E1L3N)	H-score (modified Muenst's scoring method) Low: 0–99 High: 100–300	DFS, OS Univariate analysis
Beckers 2016 [21] (retrospective)	161 (TNBC)	Protein (IHC: clone E1L3N)	% staining Positive: ≥1% and 5% TC or ≥1% and 5% IC H-score (modified Muenst's scoring Low: 0–99 High: 100–300	BCSS (≥5% TC PD-L1), OS (≥1% and 5% IC PD-L1)
Li 2016 [55] (retrospective)	136 (TNBC)	Protein (IHC: clone E1L3N)	H-score (modified Muenst's scoring 0–300	DFS (H score >0) Multivariate analysis
Sun 2016 [46] (retrospective)	218 (TNBC)	Protein (IHC: clone 28-8)	TC PD-L1: % staining (various cutoff: 1%, 5%, 10%, 50%) IC PD-L1: staining/HPF Negative: no immunostaining Low-positive: ≤30/HPF High-positive: >30/HPF	DFS (IC PD-L1), OS (IC PD-L1) Univariate analysis OS Multivariate analysis
Mardones 2016 [22] (retrospective)	59	Protein (IHC: clone 28-8)	% positive TC: Positive: ≥1%	3-years PFS and OS in TNBC
Wang 2017 [40] (retrospective)	443	Protein (IHC: clone SP142)	H-score on TC (modified Muenst's scoring Low vs. high: the threshold was the 75th percentile	RFS in basal-like BC
Aierken 2017 [24] (retrospective)	215 (TNBC)	Protein (IHC: Cell Signaling Technology, Beverly, MA)	Semiquantitative score (intensity of the staining and % positive cells) Negative: ≤1 Positive: >1	DFS, OS univariate analysis
Botti 2017 [56]	238 (TNBC)	Protein (IHC: clone SP142)	TC PD-L1:	DFS (TC PD-L1)

(continued)

Table 2. (continued)

Study (design)	No. of patients included (BC subtype)	PD-L1 assessment (assay)	PD-L1 positivity cutoff	Survival endpoints associated with PD-L1
(retrospective)			Qualitative criteria + quantitative criteria Stromal PD-L1: % positive cells Negative/low: ≤10% Positive/high: >10%	
Kim 2017 [57] (retrospective)	176 (HER2+ BC)	Protein (IHC: clone E1L3N) mRNA (Western blot, qRT-PCR)	TC PD-L1: Allred score IC PD-L1: low vs high: the threshold was the mean value of PD-L1 immunoscore	DFS (TC PD-L1) in HER2+/HR− Univariate analysis
Uhercik 2017 [12] (retrospective)	127	mRNA (qPCR)	Mean PD-L1 transcript copy number Low vs. high (no cutoff reported)	OS, DFS
Arias-Pulido [16] 2018 retrospective)	221 (IBC)	Protein (IHC: clone SP142)	TC and IC PD-L1 scored separately Positive: ≥5%	BCSS (stromal PD-L1), DFS (IC and TC PD-L1)
Li 2018 [44] (retrospective)	191 (HER2+ BC)	Protein (IHC: clones 28-8, 22C3)	% positive TC and IC: Positive: ≥1%	RFS (TC and IC PD-L1, clone 28-8, OS (tumor PD-L1, clone 28-8) Multivariate analysis
Barrett 2018 [58] (retrospective)	34 (TNBC)	Protein (IHC: clone 22C3)	Semiquantitative score on TC and non-TC: Negative: 0 Rare: 1 Low: 2 Moderate: 3 High: 4 Very high: 5	DFS (TC PD-L1), OS (TC PD-L1)
Dieci 2018 [29] (retrospective)	265 (TNBC)	Protein (IHC: clone 73-10 PharmDX Dako; software-assisted (Visiopharm)	% positive TC (any intensity) over total tumor cells % positive stromal cells (any intensity) over total stromal cells	DFS (PD-L1 as binary variable: PD-L1 > 20% vs. <20%, cutoff identified as showing the highest Harrell c-index of 0.567)
Negative prognostic value				
Muenst 2014 [14] (retrospective)	650	Protein (IHC: Abcam, Cambridge, U.K.)	H-score on TC (modified Muenst's scoring) Low: 0-99 High: 100-300	OS multivariate analysis
Qin 2015 [30] (retrospective)	870	Protein (IHC: Cell Signaling Technology, Beverly, MA)	% positive TC: Positive: ≥5%	DMFS, DFS, OS
Tsang 2017 [49] (retrospective)	1,090	Protein (IHC)	Immunoscore (staining intensity × % positive cells) Low vs. high: the threshold was the mean value of PD-L1	DFS in HER2+ BC
Mori 2017 [50] (retrospective)	248 (TNBC)	Protein (IHC: clone E1L3N)	% positive TC: Weak-positive: 1%–49% Strong-positive: ≥50% % positive IC: Positive: ≥5%	RFS, OS (only when PD-L1 was combined with TILs)

(continued)

Table 2. (continued)

Study (design)	No. of patients included (BC subtype)	PD-L1 assessment (assay)	PD-L1 positivity cutoff	Survival endpoints associated with PD-L1
Okabe 2017 [51] (retrospective)	97	Protein (IHC: clone EPR1161)	H-score on TC (modified Muenst's scoring) Low: 0–99 High: 100–300	DFS in HR+/HER2− BC)
Acs 2017 [52] (matched case-control)	42 (early-onset BC)	Protein (IHC: clone 28-8)	% positive TC, intratumoral lymphocytes, peritumoral lymphocytes (scored separately) ROC curves to define cutoffs	OS (TC PD-L1 ≥10%; intratumoral lymphocytes PD-L1 ≥1%), DFS (TC PD-L1 ≥1%)
Asano 2018 [19] (retrospective)	177	Protein (IHC: clone 27A2)	% positive TC in 3 FOVs: Negative: <10% Positive: ≥10%	DFS in TNBC Multivariate analysis
Adams 2018 [53] (retrospective)	183 (TNBC)	Protein (IHC: Cell Signaling Technology, Beverly, MA)	% positive cells Low vs. high: the threshold was the median value of PD-L1	OS

Abbreviations: BC, breast cancer; BCSS, breast cancer-specific survival; DapB, dihydrodipicolinate reductase; DFS, disease-free survival; DMFS, distant metastasis-free survival; EBC, early breast cancer; FOVs, fields of view; HER2, human epidermal growth receptor 2; HPF, high-power field; HR, hormone receptor; IBC, inflammatory breast cancer; IC, immune cells; IHC, immunohistochemistry; MFS, metastasis-free survival; OS, overall survival; OSS, overall specific survival; PD-L1, programmed cell death ligand 1; QIF, quantitative fluorescence; qPCR, quantitative polymerase chain reaction; qRT-PCR, quantitative reverse transcription polymerase chain reaction; RFS, recurrence-free survival; ROC, receiver operating characteristic; SISH, silver in situ hybridization; TC, tumor cells; TN, triple-negative.

between PD-L1 and better outcome in terms of both metastasis-free survival/DFS and OS, especially in TN/basal-like BC [10, 12, 59]. Actually, although the positive prognostic value of PD-L1 may appear paradoxical, it has been reported that mRNA expression of immunosuppressive checkpoint molecules such as PD-L1 strongly correlates with other immune markers with proimmune activity [27]. In addition, a positive correlation between higher PD-L1 mRNA expression and an immune signature of genes associated with a strong cytotoxic activity has been reported [10]. In this context, PD-L1 expression may reflect a negative feedback mechanism following the activation of cytotoxic antitumor immune response, rather than an isolated immunosuppressive process. Indeed, this hypothesis may also be biologically plausible if considering that the expression of immune checkpoint molecules is also triggered by activated T cells through an interferon-gamma-mediated feedback mechanism [60].

Although the positive prognostic value of PD-L1 may appear paradoxical, it has been reported that mRNA expression of immunosuppressive checkpoint molecules such as PD-L1 strongly correlates with other immune markers with proimmune activity. In addition, a positive correlation between higher PD-L1 mRNA expression and an immune signature of genes associated with a strong cytotoxic activity has been reported.

Association with Treatment Response

Immunotherapy

In the last decades, immune checkpoint inhibitors have emerged as a promising treatment strategy for metastatic BC, especially in the TN subtype, where the immune microenvironment is thought to play a major role in tumorigenesis and tumor progression. Indeed, the encouraging results from several early-phase trials testing immune checkpoint inhibitors in heavily pretreated MBC fostered the evaluation of such agents in phase II and III trials both as single agent and in combination with conventional treatment strategies, including targeted therapies. However, only a portion of patients seem to derive benefit from these agents. For this reason, a proper and reliable selection of patients is strongly needed in order to discriminate potential responders from nonresponders.

In this context, PD-L1 expression has been proposed as potentially capable of predicting the benefit from anti-PD1/PD-L1 agents in the context of several prospective trials, as summarized in Table 3.

In detail, the KEYNOTE-012 phase Ib trial tested pembrolizumab monotherapy in advanced TNBC. The enrollment of only PD-L1-positive patients did not allow for a conclusion on the predictive role of PD-L1 expression to be drawn; however, the authors reported a trend toward greater clinical benefit from pembrolizumab in cases of higher PD-L1 expression evaluated with a prototype scoring assay (IHC assay using clone 22C3) [61].

Table 3. Studies of immune checkpoint inhibitors: association with PD-L1 status

Study (design)	No. of patients included (BC subtype)[a]	Treatment arms (primary endpoints)	Study enrollment according to PD-L1 status	Centralized PD-L1 assessment (assay)	PD-L1 positivity cutoff	Main findings	Origin of samples
Nanda 2016 [61] Keynote-012 (phase Ib)	111 (TNMBC)	Pembrolizumab (ORR)	PD-L1 positive	Protein (prototype IHC assay: clone 22C3)	% positive TC and IC: Positive: ≥1%	Increased ORR (one-sided $p = .028$) and PFS (one-sided $p = .012$) with increasing expression of PD-L1	NA (archival)
Adams 2019 [62] Keynote 086 (phase II) Cohort A	170 (TNMBC)	Pembrolizumab (ORR)	Regardless of PD-L1 status	Protein (IHC: clone 22C3)	CPS: Positive: ≥1	ORR independent from PD-L1 status but trend of greater PD-L1-positive tumors in terms of DCR (ORR 5.3% in total population vs. 5.7% in PD-L1+ vs. 4.7% in PD-L1−; DCR 7.6% in total population vs. 9.5% in PD-L1+ vs. 4.7% in PD-L1−)	146 newly collected: mostly from metastatic sites 47 archival: mostly from primary breast tumors
Adams 2019 [63] Keynote II Cohort B	84 (TNMBC)	Pembrolizumab (ORR)	PD-L1 positive	Protein (IHC: clone 22C3)	CPS: Positive: ≥1	ORR 21% DCR 23.8%	
Tolaney 2017 [64] Keynote 150-Enhance 1 (phase Ib/II-interim analysis)	106 (TNMBC)	Pembrolizumab + eribulin (ORR)	Regardless of PD-L1 status	Protein (IHC: clone 22C3)	CPS: Positive: ≥1	No association between response and PD-L1 status (ORR for PD-L1+ vs. PD-L1−: 25.7% vs. 25%; median PFS for PD-L1+ vs. PD-L1− 19 months vs. 21 months)	NA
Loi 2018 [28] Panacea (phase Ib/II)	58 (HER2+ MBC)	Pembrolizumab + trastuzumab (ORR)	Phase Ib: PD-L1 positive Phase II: regardless of PD-L1 status	Protein (IHC: clone 22C3)	TC and IC PD-L1: Positive: ≥1% CPS: positive: ≥1	ORR for PD-L1+ vs. PD-L1−: 15% vs. 0% 1-year OS for PD-L1+ vs. PD-L1−: 65% vs. 12%	Metastatic lesions
Schmid 2017 [65] (expansion cohort phase Ia study)	112 (TNMBC)	Atezolizumab (ORR)	Initially limited to PD-L1 positive, then opened also to PD-L1 negative	Protein (IHC: clone SP142)	Staining on IC Negative: 0/1 Positive: 2/3 (≥ 5%)	ORR for PD-L1 2/3 vs. PD-L1 0/1: 17% vs. 8%	NA
Adams 2016 [66]; Pohlmann 2018 [67] (phase Ib; 2-years update)	32 (TNMBC)	Atezolizumab + nab-paclitaxel (ORR)	Regardless of PD-L1 status	Protein (IHC: clone SP142)	% positive TC and IC: 0: 0% (negative) 1: 0%–4% (positive) 2: 5%–9% (positive) 3: ≥10% (positive)	ORR for PD-L1+ (PD-L1 1/2/3) vs. PD-L1− (PD-L1 0): 42% vs. 33% Secondary endpoints: longer PFS (PD-L1+ vs. PD-L1−: 6.9 months vs. 5.1 months) and OS (PD-L1+ vs. PD-L1−: 21.9 months vs. 11.4 months) with higher PD-L1	NA
Dirix 2017 [68] Javelin (expansion cohort phase I trial)	168 (MBC)	Avelumab (ORR)	Regardless of PD-L1 status	Protein (IHC: clone 73-10)	% positive TC: different thresholds for positivity: 1% and 5% % positive IC: positive: ≥10%	TC PD-L1: no efficacy trends in subgroups defined by PD-L1 expression in tumor cells at different thresholds IC PD-L1: ORR for PD-L1+ vs. PD-L1− 16.7% vs. 1.6% in the overall group, and 22.2% vs. 2.6% in TNBC	The most recent suitable biopsy or surgical specimen available
Schmid 2018 [6] Impassion130 (phase III)	902 (TNMBC)	nab-paclitaxel + atezolizumab /placebo (PFS, OS)	Regardless of PD-L1 status (PD-L1 status was a stratification factor)	Protein (IHC: clone SP142)	PD-L1 on IC (percentage of tumor area): positive: ≥1%	PFS for PD-L1+ in control vs. experimental arm: 7.5 vs. 5.0 months OS for PD-L1+ in control vs. experimental arm: 55 vs. 15.5 months	NA

[a]Patients included in the translational analysis.

Abbreviations: BC, breast cancer; CPS, combined positive score; DCR, disease control rate; HER2, human epidermal growth receptor 2; IC, immune cells; IHC, immunohistochemistry; ITT, intention-to-treat population; MBC, metastatic breast cancer; NA, not available; ORR, overall response rate; OS, overall survival; PD-L1, programmed cell death ligand 1; PFS, progression-free survival; TC, tumor cells; TNMBC, triple-negative metastatic breast cancer.

Pembrolizumab monotherapy has also been tested in the context of the KEYNOTE-086 phase II trial, which included two cohorts of patients: cohort A enrolled previously treated patients with TNMBC irrespective of PD-L1 status, whereas cohort B included previously untreated PD-L1-positive (IHC assay using clone 22C3) TNMBC. In cohort A, overall response rate (ORR) appeared to be modest and independent from PD-L1 status; however, a trend toward a greater clinical benefit from pembrolizumab in terms of both disease control rate (DCR) and duration of response was observed in PD-L1-positive

versus PD-L1-negative patients [62]. In cohort B, higher ORR (21.4%) and longer duration of responses (median 10.4 months) were observed as compared with cohort A, thus strengthening the hypothesis that patients with BC harboring PD-L1 positivity may show good responses to pembrolizumab monotherapy, especially in the earliest lines of treatment for metastatic disease [63].

The combination of pembrolizumab plus eribulin has been evaluated in the context of the KEYNOTE-150 phase IB/II trial, which included TNMBC regardless of PD-L1 status. No association between treatment response and PD-L1 status (IHC assay using clone 22C3) was reported [64].

The association of PD-L1 protein expression with treatment response has also been evaluated in HER2+ BC. In the phase Ib/II PANACEA trial, the combination of pembrolizumab plus trastuzumab was explored in HER2+ MBC in both PD-L1-positive and -negative patients (IHC assay using clone 22C3). The authors reported higher ORR (15.2% vs. 0%) and longer 1-year OS (65% vs. 12%) for PD-L1-positive as compared with PD-L1-negative patients [28].

Atezolizumab monotherapy was tested in an expansion cohort of a phase Ia trial of both PD-L1-positive and PD-L1-negative TNMBC. The authors reported that PD-L1 positivity was associated with higher response rates [65].

In the phase Ib trial of atezolizumab in combination with nab-paclitaxel for TNMBC (regardless of PD-L1 status), the biomarker analysis revealed that PD-L1 expression on either tumor or immune cells (by IHC, clone SP142;) correlated with ORR [66, 67].

The subsequent phase III trial—Impassion130—randomized patients with TNMBC to receive atezolizumab + nab-paclitaxel versus placebo + nab-paclitaxel. Stratification factors included PD-L1 status assessed on TILs by IHC (clone SP142; intention-to-treat [ITT] population = 902, PD-L1-positive patients = 369). The results showed only a slight PFS improvement associated with atezolizumab (median 7.2 vs. 5.5 months; hazard ratio [HR] 0.80; 95% confidence interval [CI] 0.69–0.92; p = .002) and no effect on OS (median 21.3 vs. 17.6 months; HR 0.84; 95% CI 0.69–1.02; p = not significant) in the ITT population. However, when considering only PD-L1-positive patients, a significant PFS benefit (median 7.5 vs. 5.0 months; HR 0.62; 95% CI 0.49–0.78; $p < .001$) and a trend in improved OS (25 vs. 15.5 months; HR 0.62; 95% CI 0.45–0.86; no formal testing performed) were observed in the atezolizumab arm compared with the placebo arm, thus demonstrating for the first time in a randomized clinical trial the possible predictive value of PD-L1 in TNMBC [6].

Single anti-PD-L1 agent avelumab has been evaluated in a cohort of MBC in the context of the phase Ib Javelin trial. In the biomarker analysis, different compartments for PD-L1 evaluation (tumor cells vs. tumor-associated immune cells) and different PD-L1 positivity thresholds (for tumor cells: ≥1% vs. ≥5% with any staining intensity and ≥ 25% with moderate-to-high staining; for tumor-associated immune cells: ≥10% at any staining) were evaluated, reporting a trend toward higher ORR in the overall population and TN subgroup when PD-L1 positivity was determined on tumor-associated immune cells (≥10%) rather than on tumor cells [68].

Neoadjuvant Chemotherapy

In the last decades, neoadjuvant chemotherapy (NACT) has been increasingly used in the management of locally advanced BC, especially in the TN and HER2+ subtype, where the achievement of a pathological complete response (pCR) after NACT represents a strong positive prognostic factor [69]. For this reason, the identification of reliable biomarkers capable of identifying the subset of patients more likely to obtain a pCR after NACT is of great interest in BC translational research.

In this context, the possible association between baseline PD-L1 expression and efficacy of conventional neoadjuvant treatments has been recently evaluated. Studies addressing this issue have reported partially conflicting results, as shown in Table 4.

In detail, PD-L1 mRNA upregulation has been associated with increased pCR rates in two cohorts of patients with BC treated with anthracycline-based chemotherapy (CT) [10] in a large retrospective study and anthracycline-taxane ± carboplatin [27] in the context of the GeparSixto randomized trial. The association between PD-L1 and pCR was only confirmed for basal-like/TN and HER2-enriched/HER2-positive subsets.

A positive relationship between PD-L1 protein expression and pCR has been reported as well. In particular, two retrospective studies reported that higher levels of PD-L1 expression were independently associated with increased pCR rates after anthracycline-based CT in hormone receptor-positive/HER2-negative BC [17] and in TNBC [26], respectively.

In addition, the translational analysis of the phase II HER2+ hormone receptor-negative WSG-ADAPT trial revealed that baseline PD-L1 expression on infiltrating immune cells was positively associated with pCR in the Trastuzumab emtansine (T-DM1) arm [70]. A similar association between baseline PD-L1 protein expression and pCR has been reported in the HER2-negative subtype in the context of two prospective trials testing neoadjuvant anthracycline-based CT ± bevacizumab, where PD-L1 was reported as positively associated with better response to neoadjuvant therapy [71, 72].

On the other hand, PD-L1 protein expression has also been related to pCR in the opposite direction in the context of a retrospective study reporting that patients with TNBC with higher basal PD-L1 protein expression experienced lower rates of pCR after anthracycline-taxane NACT [19].

Although these contradictory results indicate that further study of the possible role of PD-L1 in affecting either response or resistance to conventional neoadjuvant treatments in the context of adequately powered clinical studies is needed, it must be noted that the most robust body of evidence supports the notion that baseline PD-L1 may be positively associated with pCR. The potential capability of baseline PD-L1 to predict pCR after NACT may gain further relevance when considering that CT could be strategically used with the aim of enhancing antitumor immune response, turning a cold tumor into a hot tumor, and ultimately boosting the efficacy of immunotherapy.

The strategy of combining immunotherapy and chemotherapy in the neoadjuvant setting is the subject of several clinical trials, some of which are ongoing ([73–76], NCT02620280). Moreover, post-neoadjuvant immunotherapy for patients with triple-negative breast cancer who did not achieve a pCR after NACT is being tested in randomized trials (NCT02954874, NCT02926196). As discussed further in this review, because PD-L1 is a dynamic marker, its expression can be further modulated by NACT, and ongoing adjuvant immunotherapy trials will

Table 4. Studies reporting an association between pretreatment PD-L1 and response to neoadjuvant therapy

Study (design)	No. of patients included (BC subtype)[a]	Neoadjuvant treatment	PD-L1 assessment (assay)	PD-L1 positivity cutoff	Main findings
Sabatier 2014 [10] (retrospective)	5454	Anthracycline-based CT	mRNA (Affymetrix U133 Plus 2.0 human microarrays)	Tumor/normal breast ratio Up: ≥2 Non-up: <2	pCR for PD-L1+ vs. PD-L1-: 50% vs. 21%
Denkert 2015 [27] Geparsixto (RCT)	580 (TNBC)	Anthracycline-taxane ± carboplatin CT	mRNA (RT-PCR)	NA	PD-L1 positively associated with pCR
Wimberly 2015 [17] (retrospective)	94	Majority received anthracycline-taxane-based CT	Protein (IHC: clone E1L3N, immunofluorescence Cy5)	Continuous quantitative score on TC and IC Dichotomized PD-L1 (Joinpoint software)	PD-L1 positively associated with pCR
Kitano 2016 [41] (retrospective)	180	Anthracycline-taxane-based CT	Protein (IHC: clone 4059)	TC and IC PD-L1: Positive: any staining cell	PD-L1 not associated with pCR (TC PD-L1 marginally correlated with pCR in HR −/HER2+ BC)
Harberk 2016 [70] ADAPT (phase II)	326 (HR+/HER2+)	TDM1 + ET vs. trastuzumab + ET	Protein (IHC)	TC and IC PD-L1: H score	IC PD-L1 associated with pCR in the TDM1 arm
Hou 2017 [45] (retrospective)	123 (HER2+ BC)	Anthracycline-taxane-based CT + trastuzumab	Protein (multicolor IHC multiplex assay)	NA	PD-L1 not associated with pCR in multivariate analysis
Cerbelli 2017 [26] (retrospective)	54 (TNBC)	Anthracycline-taxane-based CT	Protein (IHC: clone SP142)	% positive TC and IC PD-L1 (evaluated separately): Positive: ≥1%	TC PD-L1 positively associated with pCR in multivariate analysis
Waks 2017 [71] (prospective)	55 (HR+/HER2-)	Anthracycline-taxane-based CT + bevacizumab	Protein (IHC)	TC PD-L1: Negative: 0% Low: 0%–4% Intermediate: 5%–9% High: ≥10%	PD-L1 positively associated with RCB MP
Pelekanou 2018 [72] SWOG s0800 (phase II)	134	Anthracycline-taxane-based CT ± bevacizumab	Protein (IHC: clone 22C3)	% positive TC and IC: Positive: ≥ 1%	PD-L1 positively associated with pCR
Asano 2018 [19] (retrospective)	177	Anthracycline-taxane-based CT (+ trastuzumab in HER2+ BC)	Protein (IHC: clone 27A2)	% positive TC in 3 FOVs: Negative: <10% Positive: ≥10%	PD-L1 negatively associated with pCR

[a]Patients included in the PD-L1 analysis.

Abbreviations: BC, breast cancer; CT, chemotherapy; ET, endocrine therapy; FOVs, fields of view; HER2, human epidermal growth receptor 2; HR, hormone receptor; IC, immune cells; IHC, immunohistochemistry; MP, Miller-Payne; NA, not available; pCR, pathologic complete response, PD-L1, programmed cell death ligand 1; RCB, residual cancer burden; RCT, randomized clinical trial; RT-PCR, real-time polymerase chain reaction; TC, tumor cells; TNBC, triple-negative breast cancer.

possibly clarify whether post-treatment PD-L1 is able to predict immunotherapy efficacy.

PD-L1 Testing: Technical and Biological Heterogeneity

The implementation of PD-L1 as a reliable biomarker for the selection or exclusion of patients with BC for immunotherapy has thus far been complicated by several issues mainly attributable to technical and biological heterogeneity, as summarized in Figure 1.

Analytic Levels

Available studies on the potential predictive/prognostic role of PD-L1 in BC mainly focused on its expression at the protein level by IHC, frequently reporting conflicting and inconclusive results. Such inconsistency may reflect the current lack of standardization of PD-L1 testing techniques, particularly regarding the reproducibility and specificity of available PD-L1 antibodies and diversity of cutoff for positivity.

The comparison between different commercially available PD-L1 antibodies revealed a general good concordance in BC, especially between Ventana SP263, Dako 22c3, and rbMCAL10, and between Dako 28-8 and E1L3N [46, 77]. However, comparative analyses of different PD-L1 antibodies on NSCLC tissue samples consistently reported low sensitivity of SP142 antibody, because it was associated with significantly lower rates of PD-L1 detection on both tumor and immune cells [78, 79].

In addition, the adoption of different scoring systems and thresholds for PD-L1 positivity may contribute to further reducing the reproducibility of PD-L1 assessment. Indeed, in BC, discordant results were reported when different positivity cutoffs were applied and when tumor cells and tumor-infiltrating immune cells were differentially considered [46, 77].

In this context, the adoption of digital pathology and software-assisted methods may increase accuracy, reduce human error, and ultimately improve reproducibility of PD-L1 assessment and interpretation [80]. Recently, PD-L1 expression measured by IHC and assessed by digital pathology platforms has been positively associated with outcome in two cohorts of patients with TN early BC treated with surgery and standard CT [29, 81].

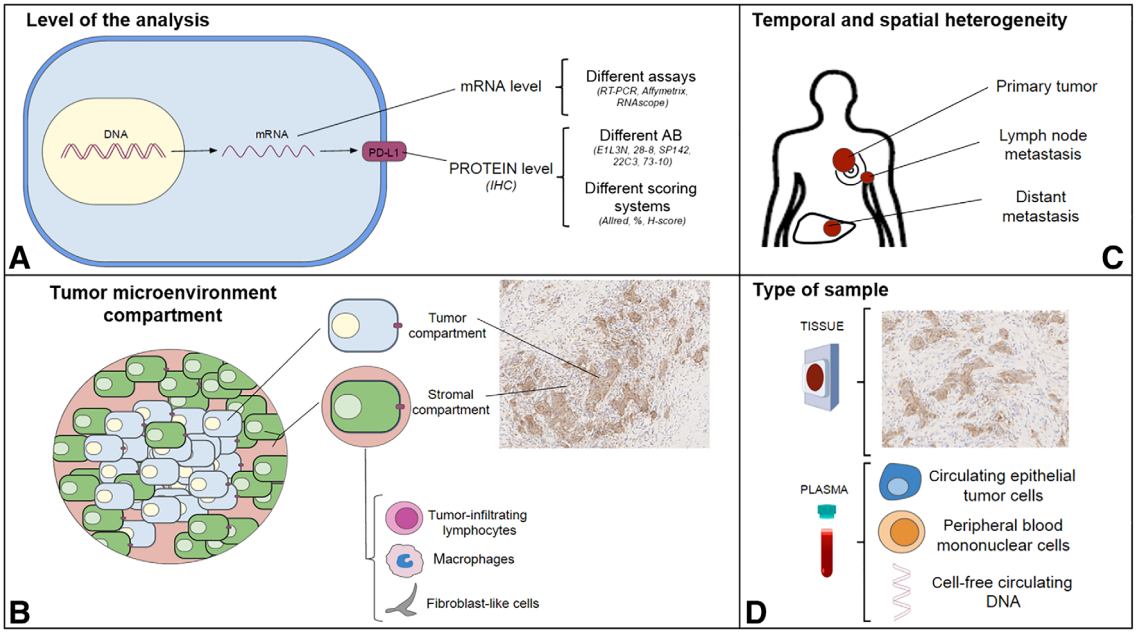

Figure 1. Programmed cell death ligand 1 (PD-L1) testing in breast cancer (BC): technical and biological heterogeneity. **(A):** Analytical level: PD-L1 can be assessed at both protein and mRNA level. **(B):** Tumor microenvironment compartment: PD-L1 expression can be detected on both tumor and stromal cells, such as tumor-infiltrating lymphocytes, macrophages, and fibroblast-like cells. **(C):** Temporal and spatial heterogeneity: PD-L1 expression has been evaluated on both primary BC and matched metastatic lesions (lymph node metastases and/or distant metastases). **(D):** PD-L1 has been mainly assessed on tumor tissue; however, it can also be detected on circulating tumor-related material.
Abbreviations: AB, antibodies; IHC, immunohistochemistry; RT-PCR, reverse transcriptase polymerase chain reaction.

In addition, alternative analytic methods for PD-L1 assessment have been also suggested.

In particular, the evaluation of PD-L1 at the mRNA level emerged as a method potentially capable of overcoming the major limitations of PD-L1 assessment by IHC, because it relies on an antibody-independent method. Indeed, the more consistent data on the possible positive prognostic role of PD-L1 have been derived from studies evaluating PD-L1 mRNA rather than protein expression. However, a possible limitation of mRNA evaluation may be that it does not discriminate between PD-L1 expression on tumor cells and nontumor cells, such as TILs [10].

Site of PD-L1 Expression

Cellular Compartment. Tumoral expression of PD-L1 encompasses both membrane and cytoplasm. It is unclear whether a differential evaluation of the two compartments may affect PD-L1 biological and clinical value in BC. However, preliminary data coming from a TNBC patient cohort suggest that cytoplasmic PD-L1 expression may be more biologically relevant compared with the membranous expression [21]. Of course, these data need to be confirmed in other clinical series.

Tumor Microenvironment Compartment. An additional source of variability in the assessment of PD-L1 protein expression is that PD-L1 may be evaluated in both tumor and stromal compartments.

In contrast to NSCLC, where regulatory approval for anti-PD1/PD-L1 therapy (pembrolizumab) is based on PD-L1 positivity assessed on tumor cells [82], in BC, PD-L1 seems to be predominantly expressed by stromal compartment [20, 25, 29, 38, 83, 84]. However, so far, no consistent data exist on the possible biological and clinical implications of a differential expression of PD-L1 by either tumor cells or tumor-infiltrating immune cells. Of note, it has been suggested that a significant proportion of PD-L1-negative tumors assessed on tumor cells may actually be classified as PD-L1 positive if assessed on stromal immune cells, thus enriching the subset of patients that might be candidates for immunotherapy [20, 25, 38]. However, Tawfik and colleagues also suggested that the adoption of a stricter cutoff for PD-L1 positivity (10% instead of 1%) may help increase the agreement between stromal and tumor compartments [38].

In contrast to NSCLC, where regulatory approval for anti-PD1/PD-L1 therapy (pembrolizumab) is based on PD-L1 positivity assessed on tumor cells, in BC, PD-L1 seems to be predominantly expressed by stromal compartment. However, so far, no consistent data exist on the possible biological and clinical implications of a differential expression of PD-L1 by either tumor cells or tumor-infiltrating immune cells.

Interestingly, results from the phase Ib Javelin trial with avelumab suggested that the predictive value of PD-L1 may be larger when PD-L1 is evaluated on TILs rather than tumor cells [68]. Indeed, the first phase III trial suggesting the predictive role of PD-L1 in TNMBC actually defined as PD-L1-positive tumors those expressing PD-L1 only on immune cells [6, 84].

It should also be noted that PD-L1 stromal expression encompasses not only TILs but also macrophages and fibroblast-like cells, thus further increasing the complexity of the biological role of PD-L1 in possibly affecting response to immunotherapy [85].

Temporal and Spatial Heterogeneity

In the last few years, several authors investigated the dynamic nature of PD-L1 by assessing its protein expression in primary and matched metastatic tumor samples.

In more detail, higher concordance rates between primary and secondary lesions have been reported when PD-L1 was assessed on tumor cells rather than on TILs [20, 38]. In addition, several authors reported that discordant cases in terms of PD-L1 status between primary and secondary lesions (encompassing both distant and local lymph node metastases) tended to exhibit a gain in PD-L1 expression rather than a loss [20, 36, 38, 42]. Finally, it has been reported that patients with TNBC with PD-L1 gain from primary to paired local lymph node metastasis experienced worse DFS compared with patients with PD-L1 negativity in both primary tumor and lymph node metastasis [42].

Although these findings seem to suggest that PD-L1 may increase from primary to secondary lesions, available evidence on its spatial and temporal heterogeneity remains limited as well as potentially biased by the fact that the majority of available data are derived from studies assessing PD-L1 on lymph node metastasis, where the reliability of immune-related biomarker detection is currently unclear.

The immune landscape of metastatic lesions may be even more complex when considering that a trend in the opposite direction with regard to TILs has been reported. In particular, results from two large retrospective cohorts of patients with MBC showed that TILs tended to decrease from primary to metastatic lesions in the TN subtype [36, 83], especially in patients receiving CT (for the advanced disease) prior to metastasis biopsy [83].

It remains therefore unclear whether PD-L1 expression assessed on secondary rather than primary lesions may provide additional and clinically relevant information. Indeed, Dieci et al. reported that whereas TILs assessed on metastatic lesions from patients with TNMBC were positively associated with outcome, stromal PD-L1 expression did not retain any prognostic value (with either 5% or 1% cutoff for positivity) [83].

The dynamic nature of PD-L1 has been further explored in the neoadjuvant setting, where modifications of PD-L1 expression from baseline to post-NACT BC samples were reported. In particular, it has recently been shown that PD-L1 significantly increased on residual disease after NACT compared with baseline in a large retrospective cohort of patients with TN early BC [29]. The induction of PD-L1 expression by chemotherapy is consistent with observations in other cancer types [85–89] and with the notion that chemotherapy is able to induce an adaptive immune response through various mechanisms, including immunogenic cell death and the activation of the damage response c-GAS/STING [90]. Indeed, it has been shown that CT may boost the immunogenicity of the tumor by increasing tumor immune infiltrate from baseline to post-NACT samples, with a high rate of conversion from low-TIL to high-TILs tumor [91]. Interestingly, in the advanced setting, results from the adaptive phase II randomized Tonic trial, testing the anti-PD1 agent nivolumab after an induction treatment in TNBC revealed that induction chemotherapy (with doxorubicin or cisplatin) resulted in T-cell and T-cell clonality increase from baseline to on-nivolumab biopsies of responders patients [92].

To conclude, although available data highlight the highly dynamic nature of PD-L1, robust evidence on its spatial and temporal heterogeneity is missing, and it is not currently possible to draw a conclusion on the ideal timing for PD-L1 testing.

Conclusion

Targeting the PD1-PD-L1 pathway is emerging as a promising treatment strategy for patients with BC, especially in the TN subtype. However, whereas some patients experience good response to immune checkpoint inhibitors, a subset of patients seem to derive little or no benefit.

Indeed, as already suggested by Adams et al. [93], who recently reviewed the current status of immunotherapy in BC, a closer understanding of tumor, microenvironment, and host factors that affect response to immunotherapy may help identify reliable biomarkers and thus ultimately optimize patient selection for immunotherapeutic strategies. In this context, PD-L1 evaluation represents a good candidate. However, many technical and biological issues need to be addressed. In particular, PD-L1 testing on BC tumor tissue currently lacks standardization in terms of diversity in assays (IHC, gene expression), antibodies for IHC testing, scoring systems and thresholds for PD-L1 positivity, compartments of the tumor microenvironment included in the analysis (tumor cells, immune cells, or both), and nature of tumor samples (primary, metastatic, or post-NACT), along with the lack of proper widespread resources in terms of PD-L1 antibody platforms for PD-L1 testing. In addition, no data are currently available on the effect of preanalytical variables (e.g., fixation time, type of fixative, storage, etc.) on the reproducibility of PD-L1 testing in BC. Moreover, selection of the optimal PD-L1 test and score to be incorporated in clinical trials is paramount in order to accurately understand the role of immunotherapy in selected patients. In this regard, practical risk-assessment recommendations have recently been suggested for effective integration of biomarkers in clinical trials [94].

Indeed, further efforts are needed to implement PD-L1 testing as a tool for properly selecting patients for immunotherapy. In this context, it is not acceptable that the same patient should be labeled as PD-L1 positive or negative depending on which PD-L1 assay or scoring system is used.

Furthermore, current scientific interest is pointed to the identification of alternative or complementary methods to improve patient selection for immunotherapy.

In this regard, TILs may provide additional information. In particular, translational analyses of three clinical trials of anti-PD1/PD-L1 agents revealed that patients with pretreatment higher TIL levels tended to derive a greater benefit from immune checkpoint inhibitors [28, 62, 63, 65, 95]. In detail, data from the KEYNOTE-086 trial that predominantly relied on newly collected metastatic samples revealed a significant correlation between PD-L1 and TILs. The latter were found

to be positively associated with greater responses to pembrolizumab, especially in the first-line setting (cohort A: ORR 6% vs. 2% for TIL ≥ vs. < median, respectively; median TILs 10% vs. 5% in responders vs. nonresponders, respectively; cohort B: ORR 39% vs. 9% for TIL ≥ vs. < median, respectively; median TILs 50% vs. 15% in responders vs. nonresponders, respectively) [62, 63]. In an expansion cohort of a phase Ia trial with atezolizumab in TNMBC, the presence of >10% TILs was associated with a trend toward higher ORR and longer OS [65]. In the PD-L1-positive cohort of the PANACEA Ib/II trial, higher baseline stromal TILs were significantly associated with better ORR (stromal TILs ≥5% vs. <5%: 39% vs. 5%) and DCR (stromal TILs ≥5% vs. <5%: 47% vs. 5%) [28].

However, preliminary translational analysis of the randomized phase III Impassion130 trial revealed that the evaluation of TILs did not provide additional predictive information beyond that provided by PD-L1 status [6, 84]. Nevertheless, it should be noticed that the cutoff for TIL positivity (low vs. intermediate/high) was set at 10%. In addition, as already mentioned, results from both retrospective and prospective studies showed that PD-L1 and TILs tend to be significantly associated with each other [22–29]. For these reasons, the evaluation of TILs as a predictive biomarker for immunotherapy deserves further investigation in the light of the recent publication of a consensus for standardized TIL assessment in metastatic lesions [96]. In addition, the quantification of TILs does not require any additional tissue availability or processing because it may be performed on diagnostic hematoxylin and eosin-stained slides, thus representing a more accessible and less expensive tool as compared with PD-L1 evaluation by IHC.

Recently, the evaluation of PD-L1 by liquid biopsy has emerged as a promising strategy potentially capable of better capturing the dynamic nature of this biomarker compared with its assessment on tumor tissue. Indeed, it has been stated that patients with BC frequently harbor PD-L1-positive circulating epithelial tumor cells [97, 98], peripheral blood mononuclear cells [99], or circulating tumor RNA. Interestingly, it has also been reported that serum PD-L1 is associated with tumor burden [97, 99] and outcome [100]. These preliminary data suggest that liquid biopsy may represent a noninvasive and feasible strategy for dynamic assessment and serial monitoring of PD-L1 of patients with BC, thus potentially providing a real-time picture of PD-L1 status.

The Cancer Genome Atlas data from more than 8,000 tumor samples (across 31 cancer types) revealed that PD1 mRNA may be a potential good predictor for anti-PD1/PD-L1 monotherapy activity [101].

Finally, although no data on patients with BC are currently available, gut microbiome and mutation burden have recently emerged as promising predictors of the benefit from immune checkpoint blockade in other solid malignancies, such as melanoma and NSCLC [4, 102–106]. These data fostered the conduction of several early-stage clinical studies—which are currently ongoing—on the possible association between response to immunotherapy and these pioneering biomarkers in patients with BC (mutational burden: NCT01375842; gut microbiome: NCT02079662, NCT03358511).

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