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REVIEW

PD-L1 expression in breast cancer: expression in subtypes and prognostic significance: a systematic review

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Abstract

Purpose To systematically review the literature on the expression of PD-L1 in primary BC, variation of expression between subtypes and effect on overall survival (OS), disease-free survival (DFS), and recurrence-free survival (RFS). Additionally, for studies in the neoadjuvant setting, we have reviewed the ability of PD-L1 to predict pathological complete response (pCR).

Methods Articles included in this review were retrieved by searching PubMed (1966–2018) and EMBASE (1980–2018). The following search terms were used: "PD-L1 expression" and "breast cancer" (PubMed234; EMBASE 161).

Results Thirty-seven articles were found relevant to this study. We summarize important findings from these works, and show that the observed PD-L1 expression in the studies varies greatly, with expression rates ranging from 0 to 83% across subtypes. PD-L1 expression in relation to prognosis both in the adjuvant and neoadjuvant chemotherapy setting remains controversial, with studies finding better, worse, or no effect on prognosis. We also show that a wide variety of strategies are used when evaluating PD-L1 immunohistochemically, e.g., different cut-off points, different cell types evaluated, and different perceptions of when a cell is positive for PD-L1 (cytoplasmic vs membrane staining).

Conclusion Further investigation of PD-L1 expression in breast cancer and its effect on prognosis is required. There is little consensus on the methods used to evaluate PD-L1 expression immunohistochemically, and this may contribute to the diverging results found in this study.

Keywords Breast cancer · PD-L1 · Biomarker · Prognostic · Immunohistochemistry

Introduction

In recent years, the treatment of breast cancer (BC) has made considerable progress, leading to the development of individualized and targeted treatment, delaying disseminated disease, and prolonging life. However, there is still a need for new treatment options for disseminated or aggressive disease, particularly for the triple-negative subset, which lacks targeted treatment options.

In this context, the recent developments in immunotherapy have given hope that this form of treatment may be effective in BC. Immunotherapy with checkpoint inhibitors has already shown unprecedented clinical activity in a range of tumor types, e.g., melanoma and non-small cell lung cancer and is rapidly changing the practice of medical oncology [1, 2]. In BC, clinical trials on monotherapy have demonstrated modest efficacy of PD-L1/PD-1 blockade in primarily triple-negative breast cancer (TNBC). The obtained overall response rate varied from 5 to 30% in heavily pretreated patients. The median duration of progression-free survival and overall survival are either not reported or short [3]. Still, responses were of long duration in a subset of patients. In the neoadjuvant setting, preliminary results including a very limited number of patients suggest high pathological response rates after combined blockade of the PD-1/PD-L1 pathway and chemotherapy. Multiple trials have been initiated to evaluate the combination of other anticancer agents and checkpoint inhibitors, especially in TNBC [3–5].

As immunotherapy gains momentum, the need increases for prognostic and predictive biomarkers of the interaction between tumor and immune system. PD-L1 expression in the tumor-immune microenvironment is considered one of the most likely candidates for this. However, several issues

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remain to be resolved before PD-L1 can be used as a biomarker in clinical practice.

Background

Heterogeneity in breast cancer

When considering treatment of BC with immune checkpoint inhibitors and possible prognostic and predictive markers of the immune microenvironment in BC, it has become increasingly evident that it is necessary to factor in the high molecular heterogeneity of BC. As BC is a heterogenous disease, there may be differences in the composition of the tumor-immune microenvironment, and also possible differences in effect of immune checkpoint inhibitors, between molecular subtypes. Gene expression profiling of BC has led to the identification of 5 intrinsic subtypes, luminal A and B, HER2 enriched, claudin-low, and basal-like (in the literature often used interchangeably with TNBC) [6]. These subtypes have been shown to vary in incidence, outcome, and response to chemotherapy [6]. Marked differences in the tumor-immune microenvironment and prognostic effect of immune-related markers have also been shown, with the HER2-positive and basal-like/triple-negative subsets often being the types with the most pronounced prognostic effect of immune-related markers [7–11].

As molecular profiling of larger numbers of BCs usually is not economically or logistically feasible in neither a clinical nor research setting, immunohistochemical (IHC) surrogate markers have been developed as a means of identifying intrinsic subtypes (e.g., estrogen/progesterone receptor, HER2, and Ki-67 for luminal A, B and HER2 positive, EGFR and CK5 for basal-like). However, several studies have shown discordance between IHC and gene expression classifications, with Prat et al. [12] finding discordance in 1 in 3 patients in a combined analysis of studies including a total of 5994 samples [12–14]. As most studies evaluating PD-L1 expression in intrinsic subtypes of BC have used the IHC surrogate markers for subtyping, this discordance should be taken into account when evaluating results.

PD-1/PD-L1

PD-1 is an inhibitory surface receptor primarily expressed by cytotoxic effector T-cells, but also by B-cells, natural killer cells, activated monocytes, and dendritic cells [15]. CD8+ cytotoxic T-cells are considered to be the primary T-cell subtype for direct antitumor activity. The ligands of PD-1 are PD-L1 and PD-L2, which can be expressed by tumor cells, but also by other cells in the tumor microenvironment, such as tumor-infiltrating lymphocytes (TILs), macrophages, and fibroblasts [15, 16]. Upon binding to its ligands, PD-1 renders the T-cell less active, and less able to respond to foreign antigens. This mechanism is beneficial for preventing autoimmune reactions to normal cells, but in the tumor microenvironment this process can lead to tumor cells escaping detection and elimination by the immune system.

It is believed that PD-L1 expression in tumor cells can be constitutive through oncogenic processes [17, 18] or induced by activated tumor antigen-specific T-cells that produce interferons [19]. The regulation of PD-L1 is extremely complex and the PD-L1 gene is stimulated and tightly modulated by interferon-γ. In BC, PD-L1 transcript expression linearly correlates with that of interferon-γ and other inflammatory genes [20]. Thus, the expression of PD-L1 can be considered a dynamic process during effector T-cell antigen recognition. Data suggest that inducible PD-L1 expression may be most important for response to PD-1/PD-L1 pathway blockade [18]. In this context, the pre-existing presence of PD-1-positive T-cells with tumor antigen specificity is required for antitumor responses [17]. In several studies, elevated levels of TILs in the tumor correlate with PD-L1 protein/mRNA expression [7, 21–25]. Thus, Schalper et al. [22] suggest a functional link between TILs and tumor PD-L1 upregulation.

As antibodies targeting PD-1 or PD-L1 are the most promising immunotherapeutic treatment strategies in BC, it is of interest to explore the significance of PD-L1 expression in BC. This topic is a matter of debate, both as to what role PD-L1 plays as a prognostic marker, but also its role as a predictive marker for the effectiveness of PD-1/PD-L1 immune checkpoint inhibition.

With regard to PD-L1 as a prognostic marker, many studies have found a positive correlation between PD-L1 expression and a more favorable prognosis [7, 26–29], leading to hypotheses that PD-L1 expression is a sign of an effective immune response to tumor cells. However, a reverse relationship has also been found in several studies [8, 30, 31].

As to PD-L1 as a predictive marker of immunotherapy, there is still very little data for BC, but a recent meta-analysis of patients with malignant melanoma or non-small cell lung cancer demonstrated a significant association between the expression of PD-L1 and response to PD-1/PD-L1 blockade [32]. Nevertheless, high PD-L1 expression does not guarantee response and low or no PD-L1 expression does not exclude the possibility of response [33, 34].

Immunohistochemical evaluation of PD-L1

As tumor expression of PD-L1 is being considered as a predictive marker for immunotherapy with immune checkpoint inhibitors, there is a need to establish consensus on how to interpret immunohistochemical staining (e.g., membranous vs cytoplasmic, intensity of staining, counting only positive tumor cells or also including other positive cell types), and

which cut-off points to use. This has been further complicated by the fact that each of the anti-PD-1 and anti-PD-L1 agents in clinical/experimental use has their own accompanying IHC assay (Table 1). Additionally, several assays have been developed in laboratories independent of clinical trials. Yet, none of the mentioned assays have been approved as companion diagnostics for BC.

Taken together, this diversity potentially decreases comparability of PD-L1 as a biomarker between studies. However, several studies comparing PD-L1 antibodies for IHC in BC (mainly the assays used for companion or complementary diagnostics, Table 1) have found good concordance between different assays, with high concordance between SP263, 28-8, and 22C3, slightly less concordance for clone SP142, which generally stains less cells than the other assays [35–37]. This has also been the conclusion in other organ systems [37, 38], perhaps making comparability less of a problem than one could fear. In line with this, the SP263 clone (Roche, Ventana) has now been CE-approved (but not FDA-approved) for diagnostics for treatment with pembrolizumab and nivolumab in addition to durvalumab [39].

Objectives

The objectives of this study were to systematically review the literature on the expression of PD-L1 in primary BC and variation of expression between subtypes and effect on overall survival (OS), disease-free survival (DFS), and recurrence-free survival (RFS). Additionally, for studies in the neoadjuvant setting we have reviewed the ability of PD-L1 to predict pathological complete response (pCR).

Search strategy

Articles included in this review were retrieved by searching PubMed (1966–2018) and EMBASE (1980–2018). We searched for studies using the following search terms: "PD-L1 expression" and "breast cancer" (PubMed234; EMBASE 161). Eligibility criteria were clinical studies published in peer-reviewed journals, with the primary objective of investigating PD-L1 expression in human primary BC. Studies investigating PD-L1 expression only in the post-neoadjuvant setting were excluded, as were articles in other languages than English, and studies with fewer than 50 patients. No time limits were set as to age of the studies or length of follow-up. Titles and relevant abstracts were read. References for the selected articles were checked for additional relevant information. All searches were last updated July 2018.

The following data were extracted: Study design, patient characteristics, format of sampling, immunohistochemical manufacturer, methods of IHC evaluation and cut-off points, distribution in subtypes, and clinical outcomes.

Results

Study selection

Our search strategy yielded 245 articles after removal of duplicates. Of these, 209 were removed based on title or abstract. A total of 45 full text articles were retrieved, and 37 articles were found relevant to this study (Fig. 1). Of these, 31 were concerned with PD-L1 expression in the adjuvant setting, while 6 articles concerned the predictive value of PD-L1 in the neoadjuvant setting.

Study characteristics

Results for PD-L1 expression in the adjuvant setting are given in Table 2. Number of patients included varied between 64 and 3916. A total of 14 studies registered PD-L1 expression for several different subtypes (either hormone receptor and HER2 status or intrinsic subtypes) [7, 8, 21, 26, 30, 40–48], 10 included only TNBC [9, 28, 31, 49–55], one included only estrogen receptor (ER)-positive, HER2-negative patients [5],

Table 1 PD-L1 assays with coupled treatment agents

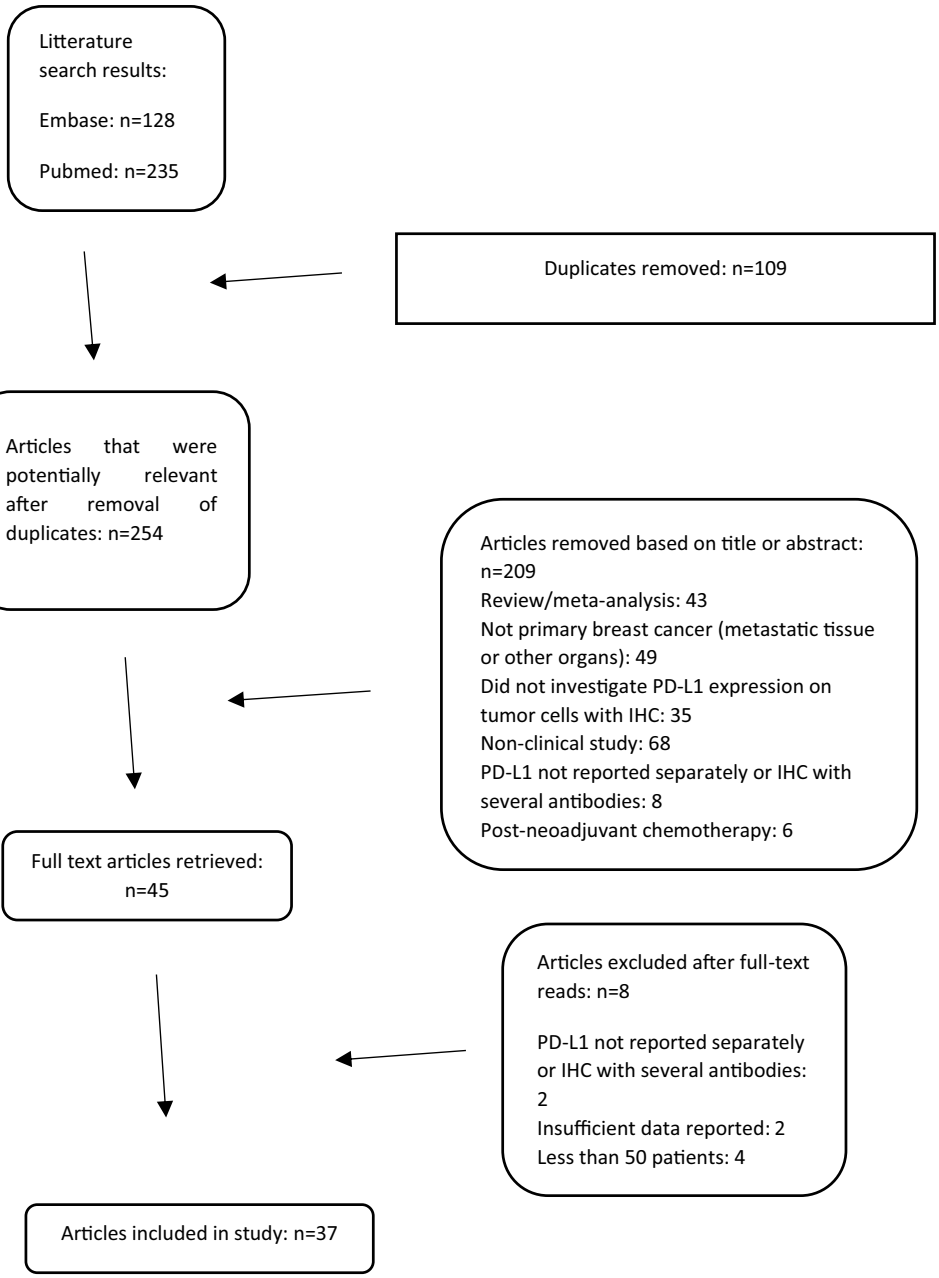
	FDA-approved as companion diagnostic	CE-approval	Platform
22C3 pharmdX	Pembrolizumab, NSCLC[a], gastric and gastroesophageal junction carcinomas[b]		DAKO
28-8 pharmdX	Nivolumab, non-squamous NSCLC, and melanoma[b]		DAKO
SP263 (Ventana)	Durvalumab, urothelial carcinoma[c]	Pembrolizumab, nivolumab, non-squamous NSCLC, and NSCLC	Roche/Ventana
SP142 (Ventana)	Atezolizumab, urothelial carcinoma[c]		Roche/Ventana

[a]Non-small cell lung cancer

[b]*Source* http://www.agilent.com

[c]*Source* http://diagnostics.roche.com

Fig. 1 Study selection



and 6 included BC, not otherwise specified (NOS), or did not register PD-L1 expression for subtypes [10, 24, 27, 56–58]. All studies used immunohistochemistry for PD-L1 evaluation, but at least 8 different IHC clones were used. The two most commonly used PD-L1 antibodies were the E1L3N clone (Cell Signaling Technology, Danvers, MA, USA) and the SP 142 clone (Spring Bioscience, Pleasanton, Canada, FDA-approved). Different evaluation strategies were used, with some studies only registering membranous staining as positive [9, 30, 41, 43, 47, 54, 58], some registering both membranous and cytoplasmic staining as positive [10, 24, 26, 28, 40, 56]. There were also differences in which cells were evaluated, and while all registered positivity in tumor cells, some also registered positivity in tumor-infiltrating immune cells [7, 9, 21, 47], and some also included positivity in other stromal cells [5, 51]. Additionally, different cut-off points for PD-L1-positive tumors were utilized, ranging from ≥1% to ≥50% positive cells, while others used a composite score of staining intensity and percentage of positive tumor and/or immune cells. Sixteen studies used Tissue MicroArrays (TMA), while fourteen used full tissue slides. Two studies also incorporated pre-operative core-needle biopsies (CNB). Other supplementary diagnostic methods used were flow cytometry [40, 54] and mRNA sequencing data [50, 54, 56]. All studies registering results for intrinsic subtypes used IHC surrogate markers for classification, while none used gene expression profiling.

Table 2 PD-L1 expression in breast cancer

References	Format of sampling	Method	Number of patients	Distribution of subtypes	PD-L1 expression	Comment
Muenst [40]	TMA[a]	IHC, rabbit antihuman PD-L1 polyclonal Ab[b] (Abcam, Cambridge, UK); Membranous and/or cytoplasmic staining. Score 0 to 300; 0–99: negative; 100–300 positive	650	LUM A 13% LUM B (HER2−) 48% LUM B (HER2+) 11% HER2+ 9% Basal-like 20%[c]	Tumor cells: 23% LUM A: 12.1% LUM B (HER2−): 20% LUM B (HER2+): 29% HER2+: 34% Basal-like: 31%	PD-L1 associated with poor prognosis
Mittendorf [54]	TMA	IHC, 5H1, a mouse antihuman PD-L1 monoclonal Ab; membranous staining > 5% tumor cells, mRNA	836	TN[d] 14% Non-TN 86%	Tumor cells: TN 19%	PD-L1 gene higher in TNBC than non-TNBC
Tung [49]	TMA	IHC, not reported (NR)	193	TN 100% 40% germline BRCA1 mutations	Tumor cells ≥ 1%: 26%	PD-L1 does not differ with BRCA status
Beckers [28]	TMA	IHC, rabbit PD-L1 monoclonal Ab, clone E1L3N (Cell Signaling Technology, Danvers, MA, USA, clone E1L3N)	161	TN 100% Basal-like 81%	Tumor cells ≥ 1% (cell membrane): 64% Tumor cells ≥ 1% (cytoplasmic): 80% Stroma ≥ 1%: 93%	Membranous PD-L1 not associated with outcome Cytoplasmic PD-L1 correlated to lower risk of BC death
Ali [7]	TMA	IHC, rabbit PD-L1 monoclonal Ab, clone E1L3N (Cell Signaling Technology, Danvers, MA, USA, catalogue # 13684)	3916	TN 24% (calculated from Fig. 2)	Tumor cells > 1%: 2% Immune cells > 1%: 6% Immune cells > 1%: TN 19%	PD-L1 correlated with infiltration of cytotoxic, regulatory T-cells PD-L1 most frequent in basal-like tumors PD-L1 correlated to improved disease-specific survival in ER− BC
Bae [26]	TMA	IHC, rabbit PD-L1 monoclonal Ab, clone E1L3N (Cell Signaling Technology, Danvers, MA, USA); Membranous + cytoplasmic staining of tumor cells	465	TN 23%	PD-L1 positive Overall: 14% LUM A: 3% HER2+: 29% TN: 30%	PD-L1 + high TIL associated with HER2+ and TNBC PD-L1 associated with better DFS and OS[e]
Baptista [27]	TMA	IHC, rabbit anti-PD-L1 polyclonal Ab (Abcam, Cambridge, MA); weak positive defined as < 1% positive cells	192	NR	PD-L1 positive Tumor cells: 57%	Tumor cell PD-L1 associated with ER− PD-L1 associated with better OS
Ghebeh [10, 24]	Fresh tissue, whole tissue section	IHC, B7-H1 primary Ab, clone MIH1(Ebioscience); membranous and/or cytoplasmic staining	69	NR	PD-L1 positive Tumor cells: 34%	Tumor cell PD-L1 associated with grade, ER−, PGR-, high Ki-67 (proliferation rate) TILs
Buisseret [21]	Whole tissue section, FFPE[f]	IHC, PD-L1 monoclonal Ab, clone E1L3N (Cell Signaling Technology); ≥ 1% tumor/stromal cells or TILs	125 (110 investigated for PD-L1 status)	TN 13% (calculated from Fig. 4)	PD-L1 positive Tumor cells: 5.5% TILs: 20% Stromal cells: 3.6%	PD-L1 correlated with TILs and HR−[g]

Table 2 (continued)

References	Format of sampling	Method	Number of patients	Distribution of subtypes	PD-L1 expression	Comment
Gatalica [41]	Whole tissue section	IHC, Ab not reported; Membranous + cytoplasmic staining of tumor cells; weak cytoplasmic as positive	116	TN 46%	PD-L1 positive LUM A: 33% LUM B: 33% HER2: 20% TN 59%	PD-L1 higher in TNBC
Park [42]	Whole tissue section, FFPE	IHC, rabbit anti-PD-L1 polyclonal Ab (Abcam, Cambridge, MA)	333 (316 for PD-L1)	HR + 56%	PD-L1 positive HR+: 68% HR− :31%	PD-L1 more frequent in HER2+BC PD-L1 not associated with DFS or OS
Guo [50]	TMA	IHC, anti-PD-L1, clone SP142 (Ventana, Tuscon, AZ); negative: < 10% positive tumor or immune cells. mRNA for CD274	183	TN: 100%	PD-L1 positive: 13.7%	PD-L1 expression not associated with outcome. High concordance of PD-L1 expression with CD274 mRNA
Qin [30]	Whole tissue section, FFPE	IHC, rabbit PD-L1 Ab (Cell Signaling Technology, Beverly, MA); PD-L1 positive > 5% tumor cells membrane with or without cytoplasm staining	870	TN 26%	PD-L1 positive LUM A: 12% LUM B: (HER2−) 9% LUM B: (HER2+) 8% TN: 56%	High PD-L1 associated with poor DFS and decreased OS
Nanda [51] (Keynote-012)	Whole tissue section	IHC, antihuman PD-L1 Ab, clone 22C3 (Merck & Co, Kenilworth, NJ); any staining in stroma or ≥ 1% of tumor cells	111 (32 enrolled in study; 27 evaluable for antitumor efficacy)	TN 100%	PD-L1 positive: 59%	PD-L1 positive treated with pembrolizumab: ORR[h] 18.5%. Increasing PD-L1 expression associated with increased probability of response
Rugo [5] (Keynote 028)	Whole tissue section/ biopsy	IHC, antihuman PD-L1 Ab, clone 22C3 (Merck & Co, Kenilworth, NJ); any staining in stroma, ≥ 1% of tumor or immune cells	248 (48 PD-L1 positive, 25 evaluable)	ER+, HER2− 100%	PD-L1 positive: 19%	PD-L1 positive treated with pembrolizumab: ORR 12%. PD-L1 expression in relation to treatment response not reported
Li Z [43]	Whole tissue section, FFPE	IHC, PD-L1 Ab, clone 58810 (Abcam); cytoplasm and/or membrane. Tumor cell expression and staining intensity coupled for a semiquantitative H score	501	LUM A: 36% LUM B: 24% Luminal HER2: 14% HER2+: 8% Basal-like: 18% (calculated from Table 1)	PD-L1 positive LUM A: 37% LUM B: 46% Luminal HER2: 49% HER2+: 58% Basal-like: 58%	PD-L1 positive associated with worse DFS and OS
Tsang [8]	TMA	IHC, polyclonal PD-L1 ab (Novus); staining intensity 0–3. Immunoscore calculated from percentage of positive cells multiplied with staining intensity. Dichotomized into high/low	1091	LUM A: 44.9% LUM B: 30.7% HER2+: 10% TN: 14.4%	PD-L1 high LUM A: 34.1% LUM B: 29.7% HER2+: 15.7% TNBC: 8.3%	High PD-L1 associated with worse overall survival in HER2-positive cancers

Table 2 (continued)

References	Format of sampling	Method	Number of patients	Distribution of subtypes	PD-L1 expression	Comment
Okabe [44]	Whole tissue section	IHC, PD-L1 Ab, clone EPR1161 (Abcam, Cambridge, MA, USA); tumor cell expression and staining intensity coupled for a semiquantitative H score	97	ER+/HER2−: 56.7% HER2+: 21.6% TN: 21.6%	PD-L1 positive ER+/HER2−: 32.7% HER2+: 33.3% TN: 33.3%	Lack of PD-L1 expression associated with better survival
Mori [31]	Whole tissue section, FFPE	IHC, PD-L1 rabbit monoclonal Ab, clone E1L3N (Cell Signaling Technology); PD-L1 weak expression: 1–49% positive tumor cells	248	TN 100%	Tumor cells strong (≥ 50%) or weak positive (1–49%), or negative Strong positive: 15.3% Weak positive: 26.2% Negative: 58.5%	PD-L1+/TILs low had the poorest prognosis, PD-L1+/TILs high had the best prognosis in multivariate analysis
Botti [9]	TMA	IHC, PD-L1 Ab, clone SP 142 (Spring Bioscience, Pleasanton, Canada); Tumor cells: ≥ 10% membranous staining with/without cytoplasmic staining, TILs: ≥ 10% positive	238	TN 100%	PD-L1 high tumor cells Ductal: 37.1% Non-ductal: 21.4 PD-L1 high TILs 42.8%	PD-L1 high associated with greater disease-free survival, but not overall survival
AiErken [55]	TMA	IHC, PD-L1 ab (Cell, Signaling). Tumor cell expression and staining intensity coupled for a semiquantitative score	215	TN 100%	PD-L1 positive: 32.6%	PD-L1 positive associated with better DFS and OS
Joneja [45]	Whole tissue section, FFPE	IHC, PD-L1 Ab, clone SP 142 (Spring Bioscience, Pleasanton, Canada). Intensity 0–3, percentage of pos. tumor cells. ≥ 2 intensity in ≥ 5% tumor cells = positive	297	Metaplastic BC: 25% TN: 36% HER2+: 11% ER/PR+: 28% (calculated from text)	PD-L1 positive Metaplastic BC: 49% TN: 9% HER2+: 6% ER/PR+: 6%	PD-L1 expression significantly higher in metaplastic BC
Wang [46]	TMA	IHC, PD-L1 Ab, clone SP 142 (Spring Bioscience, Pleasanton, Canada). Percentage of positive cells multiplied with staining intensity. Dichotomized into high/low	443	LUM A: 44% LUM B: 11% HER2+: 15% TN non-basal: 8% Basal-like: 15% Unclassified: 7%	PD-L1 high LUM A: 12% LUM B: 21% HER2+: 9% TN non-basal: 31% Basal-like: 23%	PD-L1 high associated with better RFS[i] in triple-negative subset. No impact on outcome in other groups
Kim [48]	TMA	IHC, PD-L1 Ab. clone E1L3N (Cell Signaling Technology). Immunoscore calculated from percentage of positive cells multiplied with staining intensity. Dichotomized into high/low	167	ER/PR/HER2+: 53% ER/PR −/HER2+: 47%	PD-L1 high In TC: 49% In TILs: 51% Both TC and TILS: 32%	PD-L1 high associated with better DFS in ER/PR neg/HER2 + disease in univariate, but not multivariate analysis

Table 2 (continued)

References	Format of sampling	Method	Number of patients	Distribution of subtypes	PD-L1 expression	Comment
Dill [58]	TMA	IHC, clone SP 142 (Spring Bioscience, Pleasanton, Canada); Membranous staining ≥ 1% of tumor cells or stroma > 5% stromal cells	245	LUM A-like: 66.5% LUM B-like: 8.3% HER2 +: 1.7% TN: 23.6% (calculated from Table 1)	PD-L1 positive Tumor cells: 12.2% Stromal cells: 29%	
Lou [56]	Whole tissue section, FFPE	IHC, PD-L1 ab not reported; percentage of positive cells multiplied with staining intensity. Cytoplasmic staining included. mRNA	64	Invasive ductal carcinoma NOS	PD-L1 positive: 37,5%	
Polónia [47]	TMA	IHC, clone SP 142 (Spring Bioscience, Pleasanton, Canada); ≥ 1% membranous staining in both tumor cells and stromal TILs	440	LUM A: 65.4% LUM B: 7.5% HER2: 7.2% TN: 19.9%	PD-L1 positive LUM A: 2.3% LUM B: 13.3% HER2: 0% TN: 16.2%	PD-L1 associated with TN and basal cell markers PD-L1 expression associated with decreased overall survival only in vimentin-positive cases
Adams [52]	TMA	IHC PD-L1 ab (Cell signaling). High > 10% positive tumor cells, low ≤ 10%	128 (calculated from Fig. 2)	TN 100%	High PD-L1: 48% (calculated from Fig. 2 in article)	PD-L1 associated with decreased OS in univariate analysis
Choi [53]	Whole tissue section, FFPE	IHC PD-L1 ab (EMD Millipore, Temecula, USA). Positive if ≥ 5% positive tumor cells or TILs	117	TN 100%	PD-L1 positive: 83% (calculated from text)	PD-L1 associated with decreased DFS and OS with cut-off > 70% positive cells
Li F [57]	Whole tissue section, FFPE	IHC, PD-L1 polyclonal ab (Abcam). Cytoplasmic staining. Percentage of positive cells multiplied with staining intensity. Dichotomized into high/low	112	100% invasive ductal carcinoma	PD-L1 positive: 20%	

[a]Tissue microarray

[b]Antibody

[c]Luminal A and B subtype

[d]Triple-negative subtype

[e]Disease-free survival and overall survival

[f]Formalin fixed, paraffin embedded

[g]Hormone receptor negative

[h]Overall response rate

[i]Recurrence-free survival

The results for PD-L1 expression in the neoadjuvant setting are given in Table 3. Number of patients included ranged between 54 and 180. Three studies included both hormone receptor-positive and negative and HER2-positive and negative patients [59–61], two studies included only TNBC [62, 63], and one study included only HER2-positive patients [64]. None reported on intrinsic subtypes. One study used multiplex IHC, where several antibodies can be evaluated simultaneously on the same slide [64], and the others used conventional IHC. All used pre-operative CNB and two also included post-operative evaluation of tumor slides.

Neoadjuvant treatment regimens were all taxane- and anthracycline-based with the addition of anti-HER2 agents for HER2-positive BC and some also with the addition of carboplatin, cyclophosphamide, and bevacizumab. There were no studies on neoadjuvant immunotherapy.

PD-L1 expression in the adjuvant setting

PD-L1 expression in BC is given in Table 3. In general, the expression of PD-L1 across all subtypes varied between 0% and 83%, with the majority of studies (23 out of 31) finding values below 50%. This includes the 3 largest studies with a total of 6527 patients included [7, 8, 30, 40]. Eight studies investigated expression in intrinsic subtypes with results for luminal A tumors ranging between 2.3 and 37% [8, 26, 30, 40, 41, 43, 46, 47]; for luminal B subtype, values were 9–46% [8, 30, 40, 41, 43, 46, 47]. PD-L1 expression was 0–33% for the HER2-positive subtype [8, 30, 40, 44, 47]. For basal-like/triple-negative BC, values ranged between 5 and 80% [7–9, 26, 28, 30, 31, 40, 41, 44, 47, 49, 51, 54]. One study distinguished between triple-negative non-basal-like and basal-like BC and found 31% and 23% PD-L1 positivity in those groups [46].

PD-L1 in the adjuvant setting and relation to prognosis

Most studies, and in particular the largest, found a positive correlation between PD-L1 expression and a more favorable prognosis [7, 26–29]. However, a reverse relation between PD-L1 and prognosis was found by some authors [30, 40]. Others found no correlation between PD-L1 expression and prognosis [42, 50], while Mori et al. found correlation to prognosis only when PD-L1 expression was seen in combination with other TIL score [31].

PD-L1 expression in the neoadjuvant setting and predictive value

In the studies examining PD-L1 expression in core biopsies taken pre-treatment, 5 of 6 studies found PD-L1 to be associated with increased rate of pCR in univariate analysis [59–62, 64], but in multivariate analysis only two studies found an association [59, 62]. These two studies had a high percentage of hormone receptor and HER2-negative patients (100% TNBCs and 43% ER−/HER2−, respectively), and they were also the two studies with fewest patients (54 and 76). All patients in the studies who found higher rates of pCR in multivariate analysis were treated with regimens consisting of (1) a taxane and carboplatin/anthracycline + trastuzumab and/or pertuzumab for HER2-positive disease or (2) doxorubicin + cyclophosphamide + paclitaxel. Similar treatment regimens were employed in the studies finding no effect on pCR in multivariate analysis [63, 64], except that no other studies investigated cyclophosphamide.

The remaining studies found no effect on pCR or only effect in univariate analysis. Interestingly though, no studies found PD-L1 to be associated with worse rates of pCR. None of the studies found PD-L1 expression to have effect on OS or RFS. Again, methods and cut-off points for evaluating PD-L1 expression differed widely.

Discussion

Especially in recent years, a large number of scientific papers have been published on PD-L1 expression in BC, as seen in this review where it has been possible to gather quite many studies with large and well-described study populations. TNBC has been the focus of many studies, as this patient group has been seen as the most likely candidate for immunotherapy, resulting in less available evidence for other large groups, such as the hormone-positive cancer groups. However, several larger studies in our review have investigated expression in some or all of the intrinsic subtypes, where the hormone receptor-positive subtypes are well represented. Subtype analysis has also allowed for investigation of expression in the less frequent subtypes, such as HER2-positive BC; however, case numbers for the infrequent subtypes are often low, and dedicated studies for these might be relevant to fully understand expression levels.

The results of our review show a large variance in PD-L1 expression between studies (from 0 to 83%). This may be a consequence of the previously mentioned lack of standardization for evaluation of PD-L1. In our study, we found widely varying differences in approach to analysis of tumor tissue, which may render comparisons between studies less certain.

One area of differing strategies is full tissue sections vs TMAs or core-needle biopsies in the pre-treatment setting. Wimberly et al. [23] showed that the level of PD-L1 expression within core biopsy material from one BC patient can vary up to 4 times between fields of view. Intratumoral heterogeneity has also been shown in other organ systems such as lung cancer with discordance of up to 48% between

Table 3 PD-L1 expression in the neoadjuvant setting

References	Format of sampling	Method	Number of patients	Distribution of subtypes	Neoadjuvant treatment	PD-L1 expression pre-neoadjuvant treatment	PD-L1 expression post-neoadjuvant treatment	Comment
McLemore [59]	Biopsy	IHC, PD-L1 ab[1] clone SP 142 (Spring Bioscience, Pleasanton, Canada); PD-L1 positive: membrane staining of ≥1% of tumor or immune cells	76	ER+/HER2−: 25% ER+/HER2+: 20% ER−/HER2-: 43% ER−/HER2+: 12%	Taxane + carboplatin/anthracycline (trastuzumab and/or pertuzumab for HER2+)	PD-L1 positive: 36% overall Subtypes ER+/HER2−: 16% ER+/HER2+: 7% ER−/HER2−: 61% ER-/HER2+: 33%		PD-L1 associated with excellent response to neoadjuvant treatment in uni- and multivariate analysis
Kitano [60]	Biopsy	IHC, PD-L1 ab (4059, Prosciense, Poway, California); Presence of any stained tumor cells = positive PD-L1 expression	180 (166 evaluated for PD-L1)	TN: 51% HR−/HER2+:23% HR+/HER2−: 26%	Anthracycline- and/or taxane based (no trastuzumab for HER2+)	PD-L1 positive: 34% overall Subtypes TN: 43% HR−/HER2+: 31% HR+/HER2−: 20%		PD-L1 expression associated with higher pCR[2] in univariate analysis. No effect on DFS and OS[3]
Hou [64]	Biopsy	Multiplex IHC, PD-L1 ab clone SP263 (Ventana Medical Systems, Tucson, AZ); PD-L1 positive: membrane staining of ≥1% of tumor cells or ≥10% immune cells	123 patients, 64 of which received neoadjuvant therapy	100% HER2+	Doxorubicin + cyclophosphamide, 4 cycles and ± subsequent paclitaxel/trastuzumab/pertuzumab/docetaxel	PD-L1 positive 72% in whole group 67% in neoadjuvant group		PD-L1 expression associated with higher pCR in univariate analysis, but not in multivariate analysis
Cerbelli [62]	Biopsy	IHC, PD-L1 ab clone SP 142 (Spring Bioscience, Pleasanton, Canada); PD-L1 positive: membrane staining of ≥1% of tumor and immune cells	54	100% TNBC	Doxorubicin + cyclophosphamide, 4 cycles, followed by 12 × paclitaxel	Tumor cells: 35% Immune cells: 81%		PD-L1 expression of ≥25% of tumor cells associated with pCR in multivariate analysis
Pelekanou [61]	Biopsy, Whole tissue section, FFPE[4]	IHC, PD-L1 ab, clone 22C3; PD-L1 positive: ≥1% positive tumor or stromal cells	120 pre-treatment 43 post-treatment	HR+: 69% HR−: 31%	Nab-paclitaxel + doxorubicin/cyclophosphamide ± bevacizumab	PD-L1 positive: 43%	PD-L1 positive: 33%	PD-L1 positive pre-neoadjuvant treatment associated with higher pCR rates, but not with DFS or OS

Table 3 (continued)

References	Format of sampling	Method	Number of patients	Distribution of subtypes	Neoadjuvant treatment	PD-L1 expression pre-neoadjuvant treatment	PD-L1 expression post-neoadjuvant treatment	Comment
Wang Y [63]	Biopsy, Whole tissue section, FFPE	IHC, PD-L1 ab (Abcam); Percentage of positive cells multiplied with staining intensity. Dichotomized into high/low	148 pre-treatment 114 post-treatment	100% TNBC	Anthracycline- and taxane based, 3–6 cycles	PD-L1 High: 32% Low: 68%	PD-L1 High: 38% Low: 62%	PD-L1 pre-neoadjuvant therapy not predictor of pCR in multivariate analysis. PD-L1 alone post-treatment showed no effect on prognosis, but did have significance in combination with other immunological markers

[1] Antibody
[2] Pathological complete response
[3] Disease-free survival and overall survival
[4] Formalin fixed, paraffin embedded

core-needle biopsies and full tumor sections in non-small cell lung cancer [65], and sensitivity of a single TMA of 87% [66]. The great advantage of using TMAs is the possibility of including a far greater number of patients at reduced costs, but our results should lead to consideration as to how comparable levels of expression are when using biopsy material, TMAs, or whole tissue sections. In our review, 2 out of 16 studies using TMAs found > 50% PD-L1-positive tumors in all of the cohort or in some subtypes [27, 28], whereas 6 out of 14 studies using whole tumor sections found the same [30, 41–43, 51, 53].

Regarding which compartments were analyzed (tumor cells, stromal cells, immune cells), whether cytoplasmic or only membranous staining was considered positive and which cut-off points were used, there were also considerable variations in our study. These differences in approach may render comparisons between studies less certain.

Also, the effect of PD-L1 expression on prognosis remains unclear, as some studies show an inverse relationship with prognosis, while others show the opposite, and some only show effect in the triple-negative or ER-negative subset. This suggests that other factors might influence the prognostic effect of PD-L1 expression. Two studies found that PD-L1 was prognostic in the context of a high TIL count, suggesting that PD-L1 expression might have different prognostic significance for TIL high and TIL low cases.

Another issue is differences in expression between primary tumor and metastases, a subject that has only been covered in few publications. Dill et al. showed 94% fidelity between PD-L1 expression in primary tumors and metastases. However, they and others have also shown that metastases can express PD-L1 despite the primary tumor being negative, and vice versa [58, 67, 68]. These differences in expression may contribute to the variances found in prognostic effect of PD-L1 in primary tumors, as well as leading to considerations as to which tissues are relevant to test in the metastatic setting.

As such, it might be necessary to include other parameters in the evaluation of PD-L1 as a prognostic and predictive factor. A multifactorial approach could include characterization of the tumor-immune microenvironment, including the density of TILs and/or CD8 + T-cells, regulatory T-cells, and other cell types known to affect the tumor/immune interaction. Also, the proximity of TILs/CD8 + T-cells to tumor cells expressing PD-L1 has been considered as a relevant factor [44]. Other candidates might be tumor mutational burden, PD-1 and PD-L2 expression, and co-expression of other biomarkers of immune checkpoints such as TIM3 or LAG3. In addition, several newer studies are looking at gene expression profiles and microRNA expression with effects on PD-L1 expression to understand the mechanisms underlying the expression of this biomarker in tumor and immune cells

[20, 69]. Further knowledge in this area will potentially give a better understanding of biomarker expression and its effects on treatment susceptibility and prognosis.

We also looked at PD-L1 expression in pre-operative core-needle biopsies as a predictive factor in patients receiving neoadjuvant therapy. While only two studies found PD-L1 expression to be predictive of treatment effect, several found effect in univariate analysis. This could be due to PD-L1 being linked to other variables known to predict treatment outcome, such as more aggressive phenotype and triple-negative status; however, a study by Denkert et al. studying mRNA expression of PD-L1 expression in the neoadjuvant setting also found PD-L1 to be associated with higher rates of pCR in uni- and multivariate analysis [70]. The studies investigating this aspect generally contain a lesser number of patients. Larger studies would allow for subgroup analysis and validation of the results we found in this review. Studies evaluating PD-L1 expression in conjunction with neoadjuvant immunotherapy are yet to come, but it will be interesting to see if the predictive value of PD-L1 in this setting might be significant.

With the current evidence in mind, we believe that the most rational approach to PD-L1 evaluation must be to incorporate clinical evidence and current knowledge of the biologic mechanisms behind PD-L1 activation and effect. In this light, we would follow the strategies of some of the studies in our review, including the largest [7, 9, 21, 48], and report both membranous staining of tumor cells and immune cells separately. This would allow for prognostic evaluation of both types of expression both separately and together, which may in the future become an important factor. This especially in light of recently reported results from the IMpassion 130 study (published after literature search was completed). Here, the PD-L1 inhibitor atezolizumab in combination with nab-paclitaxel showed effect primarily in the PD-L1-positive subgroup, of which most were only PD-L1 positive on immune cells, not tumor cells (using the PD-L1 IHC clone SP142) [71].

The most rational choice of PD-L1 IHC clone in future studies would seem to be one of the three FDA-approved clones that have consistently shown comparable results (SP263, 28-8, or 22C3). However, as the status is now with every checkpoint inhibitor having its own companion PD-L1 IHC clone, the choice of PD-L1 clone may end up being guided more by which checkpoint inhibitors show effect in clinical trials than which clones actually show the most consistent results.

We also believe, if possible, that reporting the percentage of tumor/immune cells positive for PD-L1 would be beneficial, as this would allow for investigation of what the best cut-off point for PD-L1 positivity is in the prognostic or predictive setting.

Conclusion

With the advent of immunotherapy and the hopes that it will provide treatment options for many cancer forms that have previously lacked targeted therapies, interest in prognostic and predictive biomarkers related to the tumor-immune interaction has increased dramatically. However, many issues still remain before PD-L1 expression can be used as a reliable biomarker.

Consensus on diagnostic strategies is essential if clinical studies are to be comparable. In this context, an increased understanding of the biologic processes governing PD-L1 expression and its interaction with other factors in the tumor-immune microenvironment will be useful, as this will provide us with a more scientifically based foundation for establishing a unified approach. Further knowledge in this area is important, as there are hopes that PD-L1 will be a predictive biomarker for treatment with checkpoint inhibitors, enabling clinicians to select relevant patients for treatment and sparing others from the side effects of immunotherapy.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

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