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REVIEW

CTLA-4 Expression and Its Clinical Significance in Breast Cancer

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Abstract

Breast cancer is the leading cause of women's death among all cancers. The main reason associated with this is the development of metastasis and therapy-resistant breast carcinoma (BC), which pose the main challenge of oncology nowadays. Evidence suggest that these tumors seem to have inhibitory mechanisms that may favor their progression and surveillance. Cancer cells can evade antitumor T cell responses by expressing some immune inhibitory molecules such as the cytotoxic T-lymphocyte antigen-4 (CTLA-4), whose clinical meaning has emerged in the last few years and is poorly understood in the BC context. This systematic literature review aims at identifying studies on CTLA-4 expression in BC, and address what is known about its clinical meaning. A literature search was performed in PubMed and LILACS databases, using the MESH terms "breast cancer"; "CTLA-4 Antigen/antagonists and inhibitors"; and "Lymphocytes, Tumor-Infiltrating/immunology", published in the last 10 years. In total, 12 studies were included in this review. Systematic review used the Preferred Reporting Items for Systematic Reviews and Meta-Analyses. Despite the small number of eligible studies, the literature reports some associations between CTLA-4 expression in the tumor microenvironment and worse BC outcomes, regardless of its molecular subtype**.** CTLA-4 expression in BC is a putative marker of clinical significance and a rationale therapeutic target in the emerging field of immunotherapy.

Keywords Breast neoplasms · CTLA-4 antigen/antagonists and inhibitors · Lymphocytes · Tumor-infiltrating/immunology · Ipilimumab · Immunohistochemistry · Flow cytometry

Abbreviations

BC	Breast cancer
CTLA-4	Cytotoxic T-lymphocyte antigen-4
DFS	Disease-free survival
HER2	Human epidermal growth factor receptor 2
IHC	Immunohistochemistry
TIL	Tumor-infiltrating lymphocytes
OS	Overall survival
PD-1	Programmed cell death-1
PD-L1	Programmed cell death ligand-1
pCR	Pathological complete response
RT-qPCR	Reverse transcription-quantitative polymerase chain reaction
TNBC	Triple negative breast cancer
Treg	Regulatory T lymphocytes

Introduction

Breast cancer (BC) is the most common female cancer worldwide and has the fifth highest mortality rate of all cancers (McGuire 2016; Siegel et al. 2019). It is a heterogeneous disease consisting of subgroups with different molecular features and clinical outcomes, and the discovery of intrinsic subtypes improved the understanding of BC biology, which has influenced treatment conducts (Sørlie et al. 2001; Voduc et al. 2010).

The current treatments consider the molecular differences among the BC subtypes; however, treatments must also consider specific conducts for each metastatic subtype, since they have distinct characteristics (Valiente et al. 2018). Although BC treatment has increased its success in recent years, more aggressive phenotypes such as the triple negative BC (TNBC) still lacks targeted treatment options (Bianchini et al. 2016; Khosravi-Shahi et al. 2018). Furthermore,

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a significant percent of BC patients will not respond to the available treatment, reinforcing the need to understand the molecular mechanisms implicated in treatment failure.

Translational studies have suggested that BC infiltration by tumor-infiltrating lymphocytes (TIL) and regulatory T (Treg) cells might have a great significance in the final clinical outcomes (Demaria et al. 2001). In this sense, the emerging field of immunotherapy rises with promising drugs for aggressive BC, such as checkpoint inhibitors (de la Cruz-Merino et al 2019; Ribas and Wolchok 2018; Vikas et al 2018). These drugs target the programmed cell death protein 1 (PD-1) and/or the cytotoxic T-lymphocyte associated protein 4 (CTLA-4), known as immune checkpoints, which have the function of reducing or limiting the cellular immune responses in a physiological scenario (Brunet et al. 1987). They are proteins on T cells or on cancer cells that need to be activated/inactivated to start/stop an immune response. Physiologically, PD1:PD1-ligand interactions inhibit the activation of trained killer T lymphocyte in peripheral tissues. Differently, CTLA-4:B7 interactions inhibit the early activation of T lymphocytes in secondary lymphoid organs (Buchbinder and Desai 2016; Buhlmann et al. 2003). Tumors may co-opt these immunoregulatory pathways to decrease T-cell function (Chen et al. 2017; Emens and Middleton 2015; Makkouk and Weiner 2015). For example, CTLA-4-positive BC cells suppress dendritic cells maturation and function or PD-L1 overexpression in cancer leads to exhaustion or anergy in TIL (Barber et al. 2006; Chen et al. 2017; Dong et al. 2002).

Evidence has emerged showing the importance of TIL in the clinical evolution of many cancer types (Luen et al. 2017). In BC field, a growing body of evidence has also shown the clinical relevance of TIL (Solinas et al. 2017a, b). Despite the fact that BC has not previously been considered particularly immunogenic, molecular subtypes as TNBC have generally higher TIL levels than estrogen-positive BC (Loi et al. 2013; Stanton et al. 2016). At the moment, TILs have not reached a clear clinical utility in any BC molecular subtype, and thus should not be used alone as a biomarker to adapt clinical therapies in daily practice. Further studies are required to document the subtype-specific immune dynamics in advanced disease (Luen et al. 2017). Standardized methodologies are expected to help clinicians, researchers and pathologists to evaluate the utility of TILs assessment with a view to both prognosis and prediction of response to treatment (Hendry et al. 2017). Moreover, TIL evaluation might be useful in the selection of candidates to immunotherapy. For instance, patients with low levels of TIL may probably require a boost of their baseline anti-tumor immune response, and thus, they might benefit from combinations of immune checkpoint blockade (Solinas et al. 2017a, b).

By mechanisms of immune checkpoint molecules, tumor cells can circumvent immune surveillance and promote their growth and progression (Sharma and Allison 2015). So, checkpoint inhibitor-based treatments aim at stimulating and/or magnify a patient's endogenous antitumor immune response by blocking these checkpoints (Barber et al. 2006; Buchbinder and Desai 2016). These treatments have changed the perspective for many aggressive tumors, particularly melanoma, lung carcinoma, urothelial carcinoma, renal cell carcinoma and microsatellite instability-high tumors. For these types of cancer, it has rapidly become a standard care therapy for patients (Bhandaru and Rotte 2019; Pardoll 2012; Reck et al. 2016). Nonetheless, the use of checkpoint inhibitors in BC still require additional clinical trials and discussion. In BC, single-agent immune checkpoint experience has shown modest but interesting responses through BC subtypes (Emens et al. 2019; Nanda et al. 2016; Rugo et al. 2018).

Despite encouraging initial results of checkpoint inhibitor-based treatments, the clinical impact of CTLA-4 expression on BC treatment is still not clear. Therefore, this systematic literature review aims at identifying studies on CTLA-4 expression in BC, and addressing the knowledge about its clinical meaning. Our study may support the rationale for new therapeutic associations and identify clinical indications for assessing CTLA-4 expression in BC.

Design of the Study

Search Strategy

We conducted a literature search using Preferred Reporting Items for Systematic Reviews and Meta-Analyses (Shamseer et al. 2015). On May 2020, we could identify 48 articles in PubMed database, and 13 publications in the Latin American and Caribbean Health Sciences Literature database (LILACS) using the following Medical Subject Headings: "breast cancer"; "CTLA-4 Antigen/antagonists and inhibitors"; and "Lymphocytes, Tumor-Infiltrating/immunology".

Study Selection and Data Extraction

Inclusion criteria were CTLA-4 expression evaluation on BC patients; publication year 2010 or later; English language. Data extraction from reports was done in duplicate. Two reviewers evaluated all abstracts independently to identify applicable studies. Subsequently and separately, two authors reviewed the full text of all studies selected to determine the eligibility for inclusion. Data extraction from all the included articles was performed independently. Exclusion criteria were lack of information about CTLA-4 evaluation method, the non-inclusion of BC participants, or if patients were not differentiated between treated and untreated subjects. Moreover, review articles were excluded. So, after

evaluated title and abstract, 29 publications were excluded. The 21 publications selected were read in full and nine articles were excluded. Thus, 12 publications remained in the study (Fig. 1).

Results

CTLA-4 expression and clinicopathologic factors were evaluated in BC patients. Table 1 presents the characteristics (population, intervention, comparisons, study design, and outcomes) for each of the 12 selected studies**.** Most studies used a cross-sectional design ($n=8$). Several methodologies were applied to evaluate CTLA-4, especially flow cytometry and immunohistochemistry techniques, alone or combined. Most of studies included a small number of patients (close to 100 or less) and a half considered the molecular status of breast tumors to evaluate CTLA-4 meaning.

Concerning the clinical significance of CTLA-4 expression, the articles reported associations on disease-free survival (DFS), overall survival (OS), and BC clinical stage. Moreover, articles included in this review reported CTLA-4 expression might be useful to predict the benefits of CTLA-4 blockade or to evaluate immune changes induced by neoadjuvant chemotherapy, suggesting that its blockade might be considered as an effective additional method in BC treatment.

The study from Verma et al. (2013) showed by reverse transcription-quantitative polymerase chain reaction (RT-qPCR) that CTLA-4$^+$ Tregs expression in the blood of BC patients was sevenfold higher when compared with healthy, age-matched female donors. Mao et al. (2010) identified that spontaneous expression of CD3$^+$CTLA-4$^+$ on peripheral blood mononuclear cells of tumor patients was also significantly higher than normal breast controls CTLA-4 expression.

Using different methods, Yu et al. (2015), Plitas et al. (2016), Lu et al. (2017), Khaja et al. (2017) and Lan et al. (2018) investigated CTLA-4 expression on BC tissue. Plitas et al. (2016) and Khaja et al. (2017) found that CTLA-4 was more highly expressed in tumor-resident Treg, cells. Yu et al. (2015) and Lu et al. (2017) identified that high CTLA-4 levels were associated with poor OS. Moreover, Lan et al. (2018) described DFS of the tumor CTLA-4 positive group as significantly shorter when compared with patients with tumor CTLA-4 (mean, 89.070 vs. 39.022 months; $p<0.0001$). Finally, Solis-Castillo et al. (2020) recent study also found high expression of CTLA-4 on CD4$^+$ TIL and CD8$^+$ TIL when compared with blood T lymphocytes. Likewise, Gu-Trantien et al. (2017) reported that intracellular CTLA-4$^+$ (iCTLA-4$^+$) CD4$^+$ TIL subpopulation were associated with extensive infiltration in BC. Therefore, TILs have their effector capacity or state of anergy partially modulated by immune checkpoints in BC patients.

Regarding the cellular type, Chen et al. (2017) have shown that there is a significant increase of CTLA-4 expression not only by TIL but also by BC cells themselves. CTLA-4 expression on BC cell lines was detectable by fluorescence-activated cell sorting flow cytometry and

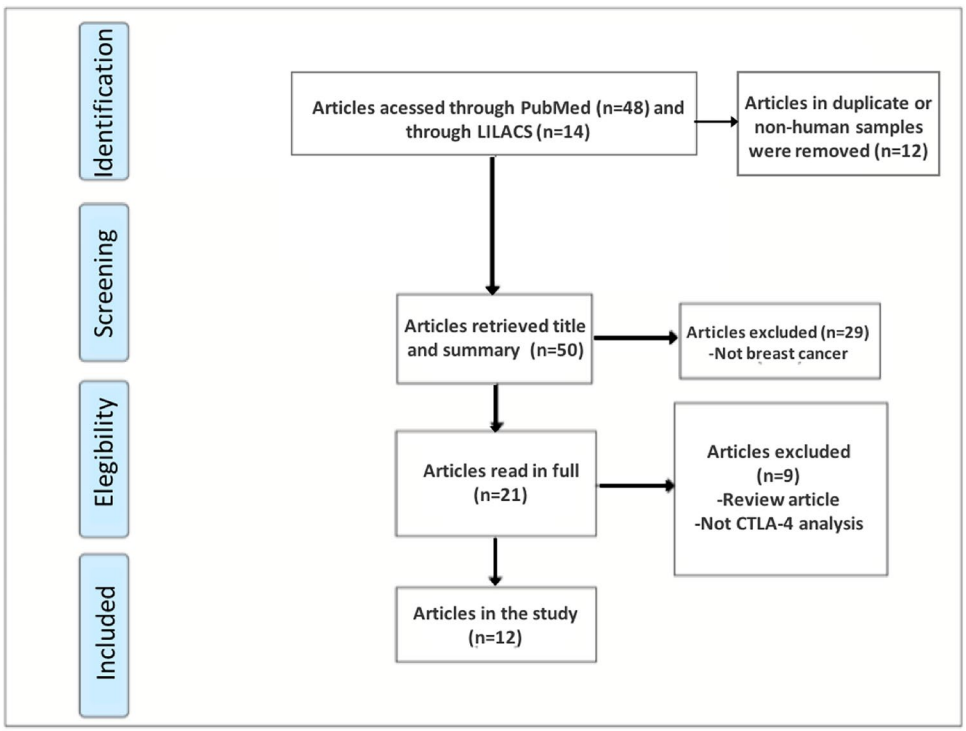

Fig. 1 Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) flow diagram

Table 1 Systematic review results–population, intervention, comparisons and outcomes

	References/location	CTLA-4 evaluation method	Design of the study	Number of patients	BC molecular Subtypes	Outcomes: CTLA-4 potential clinical significance in BC
1	Mao et al. 2010 China	IHC RT-qPCR Flow cytometry (blood T lymphocytes)	Case–control study	60 BC patients 30 healthy	Ø	Patients with higher mRNA level of CTLA-4 had obvious axillary lymph nodes metastases and higher clinical stage Appeared to be no correlation between CTLA-4 level and BC molecular subtypes or age of onset
2	Verma et al. 2013 England	Flow cytometry (blood T lymphocytes) RT-qPCR	Case–control study	16 BC patients 8 healthy	Ø	RT-PCR analysis: CTLA-4$^+$ *Tregs* expression in the blood of BC patients was sevenfold higher when compared with healthy age-matched females donors
3	Yu et al. 2015 China	IHC	Retrospective study / cross-sectional study	130 BC patients	Ø	Associated higher density of CTLA-4$^+$ *tumor cells* expression with shorter OS Elevated soluble CTLA-4 in tumor microenvironment associated with poor prognosis Patients' CTLA-4 profiles might be used to predict the benefits of CTLA-4 blockade
4	Plitas et al. 2016 USA	Flow cytometry (tissue homogenates)	Cross-sectional study	105 treatment-naive BC patients	33 ER$^+$, 18 TNBC, 6 HER2$^+$	CTLA-4 was more highly expressed in TIL *Treg* cells when compared with normal tissue resident *Treg*
5	Kaewkangsadanet al. 2016 England	IHC Flow cytometry (blood T lymphocytes)	Cross-sectional study	16 BC patients undergoing NAC	Ø	There was a reduction of CTLA-4$^+$ *T cells* in the blood (% [$p=0.017$] and breast cancers (stromal [$p=0.029$]) following eight cycles of NAC. Immune changes induced by NAC suggests that immune-mediated cell death may be a crucial component of NAC-associated tumor cell destruction IHC techniques used, could be clinically useful to further define women that may benefit from NAC
6	Lu et al. 2017 USA	RNA-seq dataset	Cross-sectional study	1087 BC patients	ER: 77.0% positive, PR: 67.0% positive HER2 22.4% positive TNBC: 22.4% HER2-enriched: 7.1%	High T cell CTLA-4 levels was associated with a poor OS CTLA-4 profiles might reflect *T cell* activation score and might serve as a marker of personalized immunotherapy in BC patients

Table 1 (continued)

	References/location	CTLA-4 evaluation method	Design of the study	Number of patients	BC molecular Subtypes	Outcomes: CTLA-4 potential clinical significance in BC
7	Khaja et al. 2017 England	IHC Flow cytometry (tissue homogenates)	Case–control studies	11 BC patients 9 healthy	ER positive (8 patients) PR positive (7 patients) TNBC (2 patients)	*Tregs* are preferentially accumulated in tumor microenvironment when compared with normal tissue. These *Tregs* express high levels of CTLA-4 and PD-L1. This preferential accumulation of *Treg* subset with inhibitory molecules have a negative effect on BC prognosis
8	Ács et al. 2017 Hungary	IHC	Case–control study	42 BC patients	Luminal A (3 patients), Luminal B (19 patients), HER2 (5 patients), TNBC (15 patients)	CTLA-4 expression did not show significant association with any of the clinicopathologic factors
9	Solinas et al. 2017a, b Belgium	Flow cytometry (tissue homogenates) IHC	Cross-sectional study	95 BC patients	34% Luminal A ($n=32$), 33% Luminal B ($n=31$), 20% HER2$^+$ ($n=19$), 13% TNBC ($n=13$)	The presence of in situ carcinoma was associated with CTLA-4 low *CD4*$^+$ *TIL* CTLA-4 hi *CD4*$^+$ *TIL* most frequently identified in TNBC (71%) and HER2$^+$ (60%) BC
10	Gu-Trantien et al. 2017 Belgium	Flow cytometry (tissue homogenates)	Cross-sectional study	42 BC patients 5 healthy	Ø	Intracellular CTLA-4$^+$ *CD4*$^+$ *TIL* subpopulation are associated with extensive infiltration in BC High proliferation rates of specific lymphocytes subpopulations could reflect an active response to the tumor
11	Lan et al. 2018 China	IHC	Cross-sectional study	102 BC patients	Luminal B HER2-negative	DFS of the *tumor cell* CTLA-4 + group was significantly shorter when compared with patients with tumor *cell* CTLA-4- (mean, 89.070 vs. 39.022 months; $p<0.0001$) Additionally, the DFS of *interstitial* CTLA-4$^+$ group was shorter compared with the *interstitial* CTLA-4$^-$ group (mean, 85.526 vs. 46.574 months; $p<0.0001$)
12	Catacchio et al. 2019 Italy	IHC (on tissue microarrays)	Cross-sectional study	180 BC patients	ER positive status (82%) PR positive status (64%) HER2/positive (15.3%) TNBC 8.3%	Negative CTLA-4 expression was associated with the histological grade 2 ($p=0.0103$) No association between CTLA-4 expression and TIL location (stromal CD8$^+$ or intratumoral CD8$^+$)

BC breast cancer; *CTLA-4* cytotoxic T-lymphocyte antigen-4; *DFS* disease-free survival; *ER* estrogen receptors; *HER2* human epidermal growth factor receptor 2; *IHC* immunohistochemistry; *TIL* tumor infiltrating leukocytes; *mRNA* messenger ribonucleic acid; *NAC* neoadjuvant chemotherapy; *OS* overall survival; *PD-L1* programmed cell death ligand-1; *PR* progesterone receptors; *RT-qPCR* reverse transcription-quantitative polymerase chain reaction; *TNBC* triple negative breast cancer; *Treg* regulatory T lymphocytes

the intracellular expression was generally higher than the surface expression. Moreover, Mao et al. (2010) and other recent studies have also demonstrated that CTLA-4 is overexpressed in BC cells (Emens 2012; Plitas et al. 2016; Yu et al. 2015).

On the other hand, except for the case–control comparisons and cell type, Kaewkangsadan et al. (2016) and Catacchio et al. (2019) analyzed CTLA-4 expression according to lymphocytes tissue location, whether stromal or intratumoral TIL. Catacchio et al. (2019) found no association between T cells CTLA-4 expression and T cells location. Nonetheless, Kaewkangsadan et al. (2016) reported that high levels of CTLA-4$^+$ (stromal) T cells were associated with a pathological complete response (pCR) ($p = 0.041$), but no association was seen with CTLA-4$^+$ levels (intratumoral) T cells and pCR ($p = 0.060$). Moreover, Mao et al. (2010) reported that elevated expression of CTLA-4 in BC tissues was related to obvious axillary lymph nodes metastases and higher clinical stage.

Regarding BC molecular subtypes, Gu-Trantien et al. (2017) found that CTLA-4 expression, principally on CD4$^+$ T cells, was heterogeneous among BC subtypes, with high expression CTLA-4high CD4$^+$ TIL most frequently identified in TNBC (71%) and HER2$^+$ (60%) BC. Furthermore, the presence of in situ carcinoma was associated with iCTLA-4 low/intCD4$^+$ TIL. However, Mao et al. (2010) did not identify a correlation between CTLA-4 levels and BC molecular subtypes. Plitas et al. (2016), Lu et al. (2017), Khaja et al. (2017), Ács et al. (2017), Solinas et al. (2017a, b) and Catacchio et al. (2019) did not identify the association between tissue expression of CTLA-4 and the BC molecular subtypes. Finally, a recent literature review on TNBC also did not identify distinct CTLA-4 expressions for this molecular subtype of BC (Stovgaard et al. 2018). Yu et al. (2015) also found no differences in CTLA-4 expression among subtypes of BC. Nevertheless, CTLA-4 is an independent predictor of shorter DFS (hazard ratio (HR) 2.176, and OS (HR 2.820) in 130 BC patients) (Stovgaard et al. 2018). Therefore, the small number of studies found in the literature reinforce the need to discuss the meaning of CTLA-4 expression in breast cancer.

Discussion

Understanding the interaction between the immune system and BC microenvironment is essential to identify patients most likely to respond to treatment and to optimize immunotherapy-based treatment strategies (Santa-Maria and Nanda 2018). The immune system requires the CTLA-4 checkpoint function to avoid uncontrolled immune responses and also prevent autoimmune reactions (Pardoll 2012). The CTLA-4:B7 interactions inhibit the early activation of T lymphocytes in secondary lymphoid organs (Buchbinder and Desai 2016; Buhlmann et al. 2003). However, tumors may co-opt these immunoregulatory pathways to decrease T-cell function in antitumor immune surveillance (Emens and Middleton 2015; Makkouk and Weiner 2015). In this sense, determining CTLA-4 expression on BC patients may be critical to investigate its role in initiating and maintaining the neoplastic pathogenesis.

Besides the 12 studies reported in our systematic review, other studies collaborate the BC immune evasion process. Previous studies implicated CTLA-4 in immune dysregulation of BC and found CTLA-4 to be highly expressed in breast tumors. Contardi et al. (2005) found CTLA-4 to be expressed on certain tumor cell lines at various degrees of intensity and it can cause apoptosis of CTLA-4-expressing tumor cells. Drake and Jaffee (2006) reported tolerance of tumor-specific T cells overexpressing inhibitor receptors, including CTLA-4 and PD-1, is responsible for the immunosuppressive environment, which prevents elimination of cancer cells. Furthermore, Huurman et al. (2007) described soluble CTLA-4 can inhibits T-cell immunity, and CTLA-4 blockade could reverse this process.

Subsequent studies have demonstrated that CTLA-4 signaling pathways in BC were more upregulated in invasive ductal carcinomas than in ductal carcinoma in situ (Gil Del Alcazar et al. 2017). It supports the notion of a suppressive immune microenvironment during invasive progression. Finally, a recent in vitro study has shown that mononuclear blood cells increase CTLA-4 expression when exposed to a TNBC cell culture. (Saleh et al. 2019).

Concerning methodological approaches, eight studies applied immunohistochemistry (IHC) to evaluate CTLA-4 expression in BC tissue and three studies performed RT-qPCR. Flow cytometry was concomitantly used in tissue homogenates and plasma in seven studies. These studies disclose the exhaustion or anergy in antigen-specific T cells in BC patients. Hence, tumor biology research remains essential for understanding the interaction between the immune system and BC microenvironment. Studies often describe and discuss immune checkpoints by IHC (Muenst et al. 2014). However, although the IHC technique is well described, study results are controversial due to the use of different primary antibodies, different staining platforms, and/or varied cut-off values for positivity (Ilie et al. 2016; Patel and Kurzrock 2015). On the other hand, assessment of tumor samples by flow cytometry is very difficult to conduct, mainly due to the limitations of sample volume. Flow cytometry, however, may prove to be most informative, since it can provide functional data on specific immune cell types rather than on the tumor as a whole, and allow more accurate quantification than IHC and gene expression profiling. Besides, technological developments using multicolor tissue immunofluorescence can be incorporated to maximize the information obtained from BC biopsy specimens. Further

technical methods of TIL characterization remain important in the research conception to elucidate discrepancies in experimental findings, particularly of those controversial.

The use of checkpoint blockade as monotherapy, such as PD-L1 inhibitors, seems to have limited, or only temporary, therapeutic response due to exhaustion of immune cells or due to development of resistance by the tumor. Moreover, recent data defines a novel immune signature in PD-L1-negative estrogen receptors-positive BC patients that are more likely to benefit from immune-checkpoint (Terranova-Barberio et al. 2020). Considering that, dual checkpoint blockade therapy is moving forward in BC (Liu et al. 2018; Santa-Maria et al. 2018). It seems intuitive: CTLA-4 carries out its function at the sites of priming, whereas PD-1 is responsible for maintaining tolerance by dampening the activation of T lymphocytes in the periphery (Wei et al. 2018). Thus, anti-CTLA-4 combined with anti-PD-1 could be the ideal approach (De Silva et al. 2020). However, combinatory approaches have shown improved efficacy at the cost of increased toxicity (Chrétien et al. 2019). Therefore, understanding the underlying structure and molecular pathways of these drugs is essential to lead successful clinical trials and overcome the biological hurdles found along the way (Abdou et al. 2020). Moreover, the revolution of cancer immunotherapy has brought attention to its financial costs, and the combination of nivolumab and ipilimumab, although efficient, is not cost-effective (Oh et al. 2017).

Identifying patients most likely to respond to treatments is essential because some patients may not derive any benefit from immunotherapies (Luen et al. 2017). This highlights the importance of the development of predictive biomarkers. Yu et al. (2015) and Lu et al. (2017) propose CTLA-4 expression profiles may be an additional biomarker of response to immunotherapies. In this perspective, for melanoma, Van Allen et al. (2015) reported CTLA-4 greater expression in good-response patients to checkpoint inhibitors treatments ($p = 0.033$). Results of a pan-cancer analysis indicate that CTLA-4 is a useful prognostic biomarker in some cancer types (Liu et al. 2020); however, the literature is controversial on BC. Differently, Fang et al. (2020) found correlations between immune checkpoint CTLA-4 gene expression, better OS, and relapse-free survival in BC patients. Currently, the main biomarkers to predict response to checkpoint inhibitors under investigation are TILs (Adams et al. 2014), PD-L1 tissue expression (Emens et al. 2019) and tumor mutational burden (Thomas et al. 2018).

Thus, the studies found in our review support the feasibility of clinical trials on dual checkpoint blockade therapy or anti-CTLA-4 as second-line therapy for BC. Clinical trials are now investigating the dual checkpoint blockade in BC (NCT03546686, NCT03818685, NCT02536794). Finally, Sun et al. (2020) translational research recently emphasized that the combined immunotherapy with these immune checkpoint inhibitors can inhibit breast tumor growth, prevent postsurgical cancer relapse and increase survival of experimental breast tumor.

Conclusions

Despite the increasing number of clinical trials in immunotherapy, relatively few studies have assessed CTLA-4 expression on TILs and tumor cells in large series of BC tissue biopsies or surgical excision specimens. Abnormal expression and deregulation of CTLA-4 could partly explain the mechanism of evasion of anti-tumor immune responses in BC patients. Some studies have reported its increased levels being associated with advanced disease clinical stage. It emphasizes CTLA-4 importance in the development and progression of breast cancer. Thus, CTLA-4 expression in BC is a potential prognostic biomarker and a logic therapeutic target in the emerging field of BC immunotherapy.

BC inflammatory milieu is characterized by the different phenotype of lymphocytes, so the expression of this additional immune checkpoint should be considered for the development of new therapeutic approaches. Useful synergies between existing therapies for BC and new immunotherapies remain to be discovered. In this sense, novel immunotherapy combinations may have the potential to reduce conventional chemotherapy and provide long-term immunity, which could reflect on increased cure rates.

Finally, the evaluation of immunogenic status in BC setting must be considered to support novel treatments with checkpoint inhibitors. Thus, oncologists may predict which patients would best respond to which checkpoint blockade therapy. This is the reason why accurate predictive biomarkers are needed and CTLA-4 study and seems appropriate to be further explored in the emerging field of breast cancer immunotherapy.

Author Contributions The authors equally contributed to the study design, written and discussion.

Availability of Data and Material All data are provided in the manuscript.

Declarations

Conflict of Interest The authors have no conflict to declare.

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