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Checkpoint Inhibitors and Their Application in Breast Cancer

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Summary

Immune checkpoints are crucial for the maintenance of self-tolerance and for the modulation of immune responses in order to minimize tissue damage. Tumor cells take advantage of these mechanisms to evade immune recognition. A significant proportion of tumors, including breast cancers, can express co-inhibitory molecules that are important formediating the escape from T cell-mediated immune surveillance. The interaction of inhibitory receptors with their ligands can be blocked by specific molecules. Monoclonal antibodies (mAbs) directed against the cytotoxic T lymphocyte-associated antigen-4 (CTLA4) and, more recently, against the programmed cell death protein 1 (PD1), have been approved for the therapy of melanoma (anti-CTLA4 and anti-PD1 mAbs) and non-small cell lung cancer (anti-PD1 mAbs). Moreover, inhibition of PD1 signaling has shown extremely promising signs of activity in breast cancer. An increasing number of molecules directed against other immune checkpoints are currently under clinical development. In this review, we summarize the evidence supporting the implementation of checkpoint inhibition in breast cancer by reviewing in detail data on PD-L1 expression and its regulation. In addition, opportunities to boost anti-tumor immunity in breast cancer with checkpoint inhibitor-based immunotherapies alone and in combination with other treatment options will be discussed.

Introduction

Due to the recent advances in technologies for high-throughput analyses, molecular mechanisms responsible for T cell-mediated cancer elimination have been elucidated [1–3]. For their proper activation, T cells require 2 signals regulating T cell survival, proliferation, and/or responsiveness to antigens. The first signal is initiated by the T cell receptor (TCR) through antigen recognition, while the second one is mediated by an interaction between receptors and ligands of co-stimulatory and/or co-inhibitory signals, also known as immune checkpoints, which include in particular members of the B7 family [4, 5]. Under physiologic conditions, there exists a balance between co-inhibitory and co-stimulatory signals, which is crucial for the maintenance of self-tolerance and immune homeostasis, thereby protecting tissues from unnecessary damage when the immune system has efficiently cleared the pathogen [6]. In tumors, immune inhibitory molecules are expressed following oncogenic transformation resulting in the attenuation of excessive immune reactions and immune resistance. T cells are able to control diverse immune responses by integrating both adaptive and innate effector mechanisms. Therefore, agonists of co-stimulatory receptors or antagonists of inhibitory receptors might lead to an amplification of antigen-specific T cell response [7, 8]. A list of immune checkpoints and co-stimulatory molecules is provided in supplemental table 1 *(www.karger.com/?DOI=445335)*. Indeed, the blockade of immune checkpoints using respective monoclonal antibodies (mAbs) has been shown to trigger efficient anti-tumor responses not only in classical 'immunogenic' tumor types, such as melanoma and renal cell carcinoma [9–12], but also in many other solid tumors, including lung [13], colorectal [14], ovarian [15], esophageal [16], bladder [12], and more recently, breast cancer [17, 18]. In this review, the features of the best characterized checkpoints (cytotoxic T lymphocyte-associated an-

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tigen-4 (CTLA4) and programmed cell death protein 1 (PD1)) will be described. Data regarding the expression and regulation of the PD1 ligand PD-L1 in breast cancer will be discussed in detail. Finally, the state of the art of breast cancer checkpoint inhibition approaches and opportunities to increase their efficacy will be summarized.

Characteristics of the Cytotoxic T Lymphocyte-Associated Antigen-4

CTLA4 has been identified as the first immune checkpoint receptor, which counteracts the T cell co-stimulatory receptor CD28 [19]. After antigen recognition, CD28 interaction with its ligands CD80 and CD86 amplifies TCR signaling to activate T cells, which is followed by an upregulation of CTLA4 [20]. CTLA4 has a higher affinity for CD80 and CD86, thereby dampening the T cell activation and out-competing CD28 [21]. Although CTLA4 is expressed by activated CD8+ effector T cells, it is in particular important for CD4+ T cells where it modulates the T helper cell activity and enhances regulatory T cell (Treg)-mediated immune suppression [22, 23]. It is noteworthy that CTLA4 can also be expressed by cancer cells. In breast cancer, a recent study reported that the presence of cytoplasmic CTLA4 dots was associated with short survival [24]. Although the significance of this expression is unclear, studies in mouse models have shown that the soluble form of CTLA4 can exert a regulatory effect on T cells [25].

The anti-CTLA4 mAb ipilimumab has been approved by the Food and Drug Association (FDA) for the treatment of metastatic and high-risk resected melanoma (in 2011 and 2015, respectively). It is broadly accepted that the mechanism of action of ipilimumab relies in the enhancement of T cell response through the inhibition of CTLA4 signaling. However, it has also been shown that ipilimumab can mediate antibody-dependent cytotoxicity of CTLA4-positive melanoma cell lines [26].

Results from phase II trials in lung cancer, in which ipilimumab was tested in combination with chemotherapy, have been less exciting [27] but still intriguing, and phase III trials are currently ongoing.

Features of Programmed Cell Death Protein 1 and Its Ligand PD-L1

In addition to anti-CTLA4, mAbs directed against PD1 and PD-L1 are emerging as important therapeutic tools in the treatment of cancer patients. These drugs are characterized by a better safety profile and more pronounced anti-tumor activity. PD1 is an immune inhibitory receptor which is expressed on activated T cells, B cells, and monocytes, but also on Tregs. Following interaction with its ligands (i.e., PD-L1 and/or PD-L2), PD1 induces T cell anergy, therefore representing an important immune escape mechanism [28–30]. PD-L1 is the best characterized of the 2 known PD1 ligands. It can be expressed by tumor cells as well as by T and B cells, macrophages, and dendritic cells [23, 31]. The anti-PD1 mAbs nivolumab and pembrolizumab have already been approved by the FDA for the treatment of metastatic melanoma (in 2014) and non-small cell lung cancer (in 2015), while anti-PD-L1 have demonstrated similar anti-tumor activities and are currently in an effervescent stage of development [32–34].

Regulation of PD-L1 Expression in Breast Cancers

The regulation of PD-L1 is extremely complex. *PD-L1* is an interferon (IFN)-stimulated gene, and its modulation is tightly regulated by IFN-γ. This in part explains the observed strong correlation between the level of PD-L1 and the density of tumor-infiltrating lymphocytes (TILs) [35] which upon activation secrete large amounts of IFN-γ. In fact, in breast cancer, *PD-L1* transcript expression linearly correlates with that of *IFN-γ* and other inflammatory genes [36]. The intrinsic genetic program of tumor cells is also involved in the modulation of PD-L1, albeit with varying strength in distinct diseases. In non-Hodgkin's lymphoma, for example, amplification of chromosome 9p24 represents a recurrent genomic alteration accompanied by high expression levels of PD-L1 and PD-L2 [37]. In breast cancer, the same chromosomal amplification, which is associated with higher expression of PD1 ligands, was observed in 12 of 41 triple-negative breast cancer (TNBC) cases, but not in the estrogen receptor (ER)-positive or human epidermal growth factor receptor 2 (HER2)-positive mammary carcinoma tissues [38]. Another study has reported a higher frequency of *PD-L1* amplification or gain in basal-like tumors when compared to the other subtypes [39]. In general, in breast cancer, *PD-L1* transcripts correlate significantly but not heavily with copy number aberrations [40].

In a pan-cancer analysis of TCGA (The Cancer Genome Atlas) data, amplification 9p24 was associated with the degree of cytolytic activity determined as average expression of granzyme A (GZMA) and perforin (PRF1), which in turn correlated with PD-L1 expression [41]. In the tumor subtype analysis, however, the association between 9p24 amplification and cytolytic activity was only significant in stomach, head and neck, cervical, colorectal, and lung squamous tumors, but not in the other cancers such as lung adenocarcinoma, glioma, melanoma, and breast, kidney, ovarian, liver, uterine, prostate, kidney, and bladder cancer [41].

However, it should be mentioned that the interpretation of correlative results between copy number and PD-L1 is challenging. In fact, the absence of PD-L1 could make a tumor more permissive to T cell invasion. Then, IFN-γ secreted by activated lymphocytes (or natural killer cells) can induce tumor cells to express PD-L1 resulting in a lack of linearity between PD-L1 constitutional activation and ex vivo expression. Vice versa, a constitutive expression of PD-L1 (e.g., following amplification of the corresponding gene) can counteract T cell infiltration, with consequent decreased release of IFN-γ and less sustained PD-L1 expression. Therefore, even though copy number variations can influence the expression of PD-L1, their correlation with PD-L1 expression ex vivo will never be perfect. As an example, the different relationship between

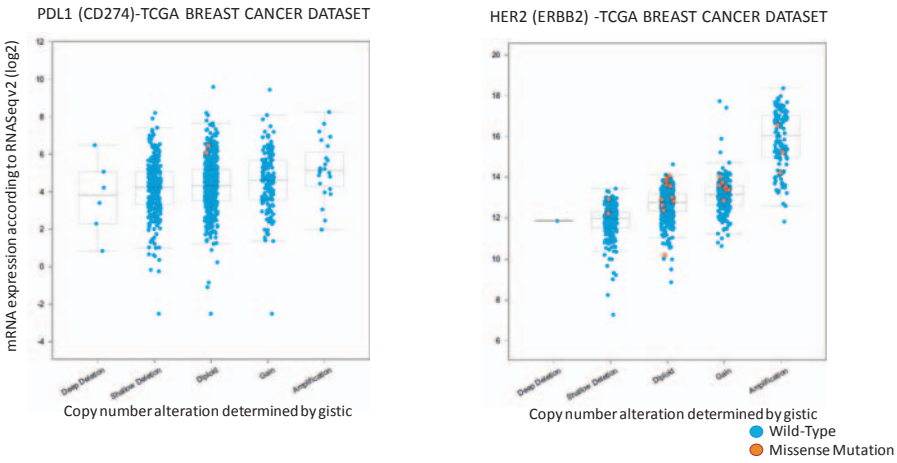

Fig. 1. Copy number variation and transcript levels: ERBB2 (HER2) vs. CD274 (PD-L1). The relationship between copy number variations and transcript levels for ERB2 and CD274 in the TCGA (The Cancer Genome Atlas) breast cancer datasets is shown. Plots are generated with cBioportal *(www.cbioportal.org/)* [103].

copy number variations and transcript levels for HER2 whose expression is largely controlled by DNA amplification, and for PD-L1 whose expression is significantly modified by micro-environmental variables, is shown in figure 1.

Oncogenic pathways can also modulate the expression of PD-L1, adding complexity to the interpretation of correlative *ex vivo* studies. In lung cancer, oncogenic activation of the mTOR-AKT induces expression of PD-L1 [42]. In breast cancer, deletion of PTEN, a negative modulator of the PI3K pathway, triggers the expression of PD-L1, which is followed by diminished T cell proliferation and increased apoptosis [43].

Moreover, it has been shown that PD-L1 expression could be induced in breast cancer cells by chemotherapeutics such as paclitaxel, etoposide, and 5-fluorouracil. This promoted PD-L1-mediated T cell apoptosis, thereby demonstrating a potential link between chemotherapy and cancer immune resistance [44]. In contrast, doxorubicin treatment caused a downregulation of PD-L1 surface expression in vitro, which was also confirmed in a xenograft mouse model. Interestingly, the doxorubicin-mediated downregulation of PD-L1 surface expression was accompanied by an upregulation of PD-L1 in the nucleus. This cellular re-distribution of PD-L1 into the nuclei of breast cancer cells suggests a function of PD-L1 beyond inhibition of T cells [45].

PD-L1 Expression in Breast Cancer

PD-L1 expression has been shown in different cancers, such as kidney, lung, pancreas, esophagus, ovarian, colorectal, head and neck squamous cell carcinoma, melanoma, and glioma [35, 46–48].

The first study on the prevalence of PD-L1 expression in breast cancer dates back to 2006 when Ghebeh et al. [49] reported that expression of this molecule, evaluated by immunohistochemistry (IHC) on either tumor cells or TILs, was present in 22/44 (50%) of the analyzed primary breast cancer samples.

Investigations into this matter have intensified in the last couple of years largely due to the enthusiasm generated by the results of PD1 blockade in multiple tumors. These studies are difficult to compare due to different cut-offs used for PD-L1 positivity (e.g., cut-off at 1 or 5%), staining evaluated on cancer cells or immune infiltrates, the kind of antibodies employed, as well as the use of different assays (IHC, gene expression profiling, or in situ RNA hybridization); however, some common findings have emerged from these reports. First, PD-L1 expression positively associates with the presence of immune-infiltrates [39, 40, 43, 48–54]. Second, TNBC (or basal-like) tumors express PD-L1 more frequently than other subtypes [39, 40, 51, 55]. The main finding of studies that assessed PD-L1 expression in breast cancer are summarized in supplemental table 2 *(www.karger.com/?DOI=445335)* [39, 40, 43, 48–57].

The largest IHC evaluation assessing almost 4,000 breast cancer samples detected PD-L1 expression (cut-off at 1%) in 1.7 % of all tumors and in 19% of the 302 TNBC samples [40]. However, PD-L1 expression of TILs was present in 6% overall and in 39% of TNBCs. Similarly, Mittendorf et al. [43] reported a PD-L1 positivity rate of 19% by assessing 105 TNBCs (cut-off at 5% on tumor cell membranous staining).

In another study with 161 TNBCs, PD-L1 positivity using a 1% cut-off was even higher: 64% for tumor cell membranous staining, 80% for cytoplasmic staining, and 93% for stromal staining [52]. Even with a 5% cut-off, the proportion of PD-L1-positive tumors remains high: 60, 77, and 93%, for tumor cell membranous, cytoplasmic, and stromal staining, respectively [52]. It is possible that these discrepancies are at least in part influenced by the kind of antibody used, therefore highlighting the urgent need for harmonization procedures [58, 59]. Luminal subtypes (e.g., luminal A and B) are the most prevalent breast cancer tumors. Although PD-L1 expression is not that frequent in luminal subtypes given their high prevalence, they still represent a considerable proportion of PD-L1-positive tumors (i.e., 44% of all PD-L1-positive tumors in the study by Ali et al. [40]). This subgroup of luminal PD-L1-positive patients might benefit from immunotherapy.

A transcriptomic meta-analysis of 5,454 breast cancer lesions demonstrated a highly variable frequency of PD-L1 mRNA expression [39]. Expression was most prominent in basal tumors, followed by HER2, and then luminal subtypes (supplemental table 2, *www.karger.com/?DOI=445335)*. High PD-L1 expression levels were associated with negative prognostic features such as large tumor size, high grade, lack of ER, progesterone receptor and

HER2, and high proliferative index [39]. Another study confirmed that high proliferative capacity in breast cancer cell lines is associated with higher PD-L1 expression [60]. It is noteworthy that the frequency of PD-L1 is higher in inflammatory breast cancer (IBC) when compared to non-IBC [54]. Quite interestingly, PD-L1 expression is higher in breast cancer cell lines bearing a basal-like phenotype as compared with luminal subtype [39]. In the study by Gatalica et al. [48], PD-L1expression in solid tumors was correlated with high mutational load of the tumor suppressor gene TP53, while no association between TP53 and PD-L1 expression was observed in breast cancer by Ali et al. [40].

Recently, PD-L1 expression was detected in metastatic tumor cells circulating in the blood of hormone receptor-positive, HER2-negative breast cancer patients [61]. Thus, PD-L1 expression of circulating tumors cells might be used for stratification and monitoring of tumor patients undergoing immune checkpoint blockade using liquid biopsy.

PD-L1 Expression in Breast Cancer and Clinical Relevance

A multitude of studies across solid tumors has shown that tumors displaying a T helper 1 polarization respond better to immunotherapy and are associated with a better prognosis [2, 62–65]. These tumors are characterized by the activation of specific molecular pathways that are also found in other forms of immune-mediated rejection, such as allograft rejection or flares of autoimmunity [66, 67]. We refer to them as the Immunologic Constant of Rejection (ICR) [2, 68–71]. These pathways comprise the IFN-stimulated gene pathway (centered on IRF1 and STAT1), CXCR3 and CCR5 ligand pathways (e.g., CXCL9–11 and CCL3–5), and immune effector function genes (e.g., perforin, granulysin) [2, 69]. As discussed in detail elsewhere [36], various predictive and prognostic immune-related signatures described in breast cancer are centered on the ICR pathways. In breast cancer, the activation of the ICR pathways has been associated with prolonged survival and response to neoadjuvant chemotherapy [72–76] or adjuvant anti-HER2 therapy [77]. However, the prognostic value of immune gene signatures is influenced by intrinsic molecular subtypes and proliferative capacity [36, 78, 79].

It is tempting to speculate that the prognostic role of the ICR pathways resides in their ability to describe an intra-tumor immune response that can slow down tumor growth or counteract metastatic processes [2]. Their predictive role in the context of immunotherapy could rely on the ability to capture a more permissive cancer phenotype in which immune manipulations might more easily trigger the development of an acute anti-tumor inflammatory process. As for the prediction of response to conventional therapy, it has been proposed that the presence of a subacute inflammatory status can facilitate tumor clearance following the induction of chemotherapy-mediated immunologic cell death [80] or, as for anti-HER2 therapy, the enhancement of an antibody-dependent mediated cytotoxicity [81].

However, the presence of such a molecular inflammatory status is accompanied by the activation of immune-regulatory mechanisms, and a strong correlation exists between pro-inflammatory (e.g., CXCL9–11, CCL5, IRF1, and STAT1) and regulatory (e.g., CTLA4, PD1, PDL1, FOXP3, and IDO1) transcripts [36, 63, 64, 74].

In anti-PD1 trials of melanoma and lung cancer, PD-L1 expression [9, 35, 82, 83] as well as a high mutational load [13, 84] have been invariably associated with response to checkpoint inhibitors.

In primary basal-like breast cancers or TNBCs, most of the studies, and in particular the largest ones [39, 40, 53], have reported a positive correlation between PD-L1 expression and favorable prognosis. However, in overall populations, a reverse correlation between PD-L1 and prognosis has been noted by some investigators, including a large study in the Chinese population [55]. As expression of PD-L1 is associated with negative prognostic features, it is possible that the prognostic role of those unfavorable variables can prevail when the analysis is not stratified according to clinicopathologic groups. Nevertheless, one relatively large study showed a reverse association between PD-L1 expression and survival in all but the luminal A subtypes [51], and the reasons for this discrepancy are not immediately clear (supplemental table 2, *www.karger.com/?DOI=445335)*. Prospective validations of these findings are lacking although it is unlikely that PD-L1 expression alone will retain significance as a prognostic factor when confronted with other variables.

The influence of confounding variables is less strong in the therapeutic setting where expression of PD-L1, which is in turn associated with the expression of ICR genes, is correlated with responsiveness to neoadjuvant breast cancer chemotherapy [54, 74]. The predictive role of PD-L1 in the metastatic setting is completely unknown.

Checkpoint Inhibitors as Novel Strategies for Breast Cancer and Opportunities to Further Boost Anti-Tumor Immune Response

Breast cancer was considered non-immunogenic for a long time, and therefore patients had limited access to immunotherapies. In the metastatic setting, vaccination strategies have shown some signs of activity [85, 86], but results have been overall disappointing with low objective response (OR) rates. Adoptive therapy with TILs is extremely active in melanoma patients [87, 88]. However, this approach has not yet been implemented in breast cancer due to the difficulty to generate TIL cultures with specificity against the tumor from which they are generated [89]. A phase I/IIa study in metastatic breast cancer by Domschke et al. [90] and Stefanovic et al. [91] demonstrated encouraging results in terms of immunological response, disease control, and survival by using bone marrow-derived tumor-reactive memory T cells. The investigators obtained an intriguing median overall survival (OS) of 34 months, with 3 (20%) patients alive at last follow-up and more than 7 years after treatment. Interestingly,

the survival rate correlates with the immunological response in the peripheral blood. The same group is now testing this approach in combination with cyclophosphamide to counteract the response to Tregs in a phase II study (Schuetz F., personal communication; Breast Cancer Immunotherapy Symposium, Doha, Qatar, 2015).

The first study employing checkpoint inhibitors tested the anti-CTLA4 mAb tremelimumab in combination with endocrine therapy (examestane) in metastatic ER-positive patients. Unfortunately, no OR was induced by treatment although 42% of patients achieved stable disease for more than 3 months [92].

The anti-CTLA4 mAb ipilimumab is now being tested in patients with lower tumor burden. In early breast cancer, addition of ipilimumab to preoperative cryotherapy was able to induce a stronger expansion of clonal TILs as compared with either approach alone [93]. Investigators are now planning to evaluate in a follow-up randomized trial whether this expansion correlates with clinical outcome.

Based on the predictive and/or prognostic role of TILs [94, 95] and immune signatures [36] in breast cancers, and in view of the striking activity of PD1 blockade across multiple tumors, this strategy has been recently investigated in mammary carcinoma.

Because TNBCs have, in general, a higher density of TILs, and considering that the prognostic role of TILs is more prominent in TNBC than in other subtypes, the efficacy of PD1 inhibition has so far been evaluated in this setting. Results from 2 studies assessing the anti-PD1 mAb pembrolizumab and the anti-PDL1 atezolizumab were recently presented.

The pembrolizumab phase Ib KEYNOTE-012 trial recruited 32 metastatic TNBC patients, most of whom had previously received at least 3 lines of chemotherapy for metastatic disease [17]. Only patients with PD-L1 staining in the stroma or in ≥ 1 % of tumor cells (evaluated by IHC) in archived samples were eligible. An extremely promising OR rate of 19 % was detected, including 1 complete and 4 partial responders.

The atezolizumab phase Ia expansion trial enrolled 54 TNBC patients [18]. Even in this case, patients were heavily pretreated (85% had received 4 or more lines of chemotherapy). In the 21 PD-L1 patients in whom efficacy was evaluable, a similar OR rate of 24% was reported, including 3 partial and 2 complete responses. In this case, tumors were considered PD-L1-positive if PD-L1 was expressed in 5% or more of the infiltrating immune cells.

Although the OR rate in breast cancer was lower than the rates obtained in PD-L1-positive melanoma (55–60%) [9, 96] or lung cancer (45%) [83], this is the first time that a single immunotherapeutic agent induced tumor shrinkage (and in some cases disappearance of the tumor) in a considerable proportion of breast cancer patients. Similarly to what was observed in other tumor types, responses tend to be long-lasting, with some ongoing at the time of this report [17, 18].

Importantly, both molecules were extremely well tolerated, with toxicities similar to those in other disease settings. Phase III trials are currently ongoing testing pembrolizumab alone vs. chemotherapy or atezolizumab in combination with abraxane (a new generation taxane) (supplemental table 3, *www.karger.com/?DOI=445335)* [85].

The combination of the anti-PD1 mAb nivolumab and ipilimumab has been demonstrated to be more effective than either strategy alone [96] in melanoma and has been recently approved by the FDA in this setting. A flurry of early combinatorial trials has been initiated to assess the activity of these and other anti-PD1/PD-L1 mAbs in multiple tumors, including breast cancer. These trials include combinations with co-stimulatory molecules (e.g., anti-OX-40 and anti-CD-27), other checkpoint inhibitors (IDO inhibitors, anti-CTLA4), p53 vaccine, anti-HER2 mAb (trastuzumab and trastuzumab emtansine (TDM1)), histone deacetylase inhibitors (etinostat, vorinostat), eribulin (a novel microtubule synamic inhibitor), PLX3397 (a novel tyrosine kinase inhibitor), poly I:C (a toll-like receptor agonist), bevacizumab (an anti-angiogenic mAb), and radiotherapy, as summarized in supplemental table 3 *(www.karger.com/?DOI=445335)*.

An emerging approach to increase the activity of checkpoint inhibition is represented by targeting oncogenic pathways associated with immune suppression and T cell exclusion [97]. It has been recently reported that suppression of TIL recruitment or retention in breast cancer is associated with genomic alterations of Ras/MAPK [98]. This was further confirmed by i) in vitro data demonstrating that MEK inhibition upregulated MHC class I surface antigens and reduced immunosuppressive markers, and ii) a combinatorial treatment of a syngeneic mouse model of breast cancer with MEK inhibitors and anti-PD1 antibodies demonstrating synergistic effects. Based on these results, a combination of MEK inhibition and anti-PD-L1 expression might be a promising novel therapeutic approach for the treatment of this disease. In addition, we described specific MAPK mutations associated with the absence of ICR pathway activation [99].

As PTEN deletion influences the expression of PD-L1, another intriguing approach could be represented by the combination of checkpoint inhibitors with PI3K inhibitors. Studies in melanoma animal models have recently demonstrated the therapeutic efficacy of this approach [43].

Although some clinical studies are dissecting the effects of combination therapies on breast cancer immunity and therapy response, experimental models mimicking the human disease are urgently required. This has recently become possible by the modification of the well characterized transgenic BALB/c WAP-T mouse model for breast cancer with strong similarities to the corresponding human disease by an additional transgene coding for an immune-dominant T cell epitope of the nucleoprotein (WAP-TNP), which allows the monitoring of T cell responses. Using the WAP-TNP model, it could be shown that the impaired T cell response was due to PD1 expression, which could be overcome by treatment with anti-PD1 antibodies, suggesting the WAP-TNP mice to be a suitable tool to analyze parameters to overcome the blockade of immune checkpoints in breast cancer patients including combination therapies [100].

Conclusion

Until now, immune checkpoint inhibitor agents have shown promising results in the treatment of solid tumors, including breast cancer. However, further investigations are required to determine whether these co-inhibitors could be used in combination with each other or may require the use of chemotherapy or radiation [101, 102], and whether the outcome would be synergistic. Additionally, the safety profile of such combinations needs to be carefully assessed. Furthermore, other immune checkpoint blockade agents should be developed to optimize the anti-tumor qualities of the immune system to enhance the immune response and abrogate the immunosuppressive microenvironment in breast cancers.

The heterogeneity of PD-L1 expression in breast cancer subtypes postulates that anti-PD1/PD-L1 agents may only be part of the solution, suggesting that additional agents are required to complement tumor cell killing. The ability to distinguish between 'super'-responders and non-responders using biomarkers might be the key to future combinatorial regimens. The selection criteria for enrolling breast cancer patients for immune checkpoint inhibitors would not only depend on the heterogeneity of the primary tumor and subtype regarding the expression of PD-L1, but also on the putative distinct immune signature of tumor-initiating stem cells, as well as on the individual profile of tumor-infiltrating and peripheral immune cells. Therefore, the molecular characterization of breast tumors may further identify other phenotypes that could benefit from immunotherapy in addition to TNBCs.

We believe that the intensification of genomic studies assessing the genetic determinants of spontaneous or treatment-induced anti-tumor immunity combined with the optimization of pre-clinical breast models and the implementation of combinatorial strategies will lead to a significant increase in the therapeutic efficacy of immune manipulations in the near future.

Online Supplemental Tables

Supplemental Table 1. Immune checkpoints and co-stimulatory molecules and their targeting agents

Supplemental Table 2. Expression of PD-L1 in breast cancer lesions

Supplemental Table 3. Immune checkpoint agents under clinical development in breast cancer

To access the online supplemental tables, please refer to *www.karger.com/?DOI=445335*.

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Disclosure Statement

The authors have declared that no conflict of interest exists.

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