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CLINICAL TRIALS (S HURVITZ, SECTION EDITOR)

Targeting the Immune System in Breast Cancer: Hype or Hope?: TILs and Newer Immune-Based Therapies Being Evaluated for HER2+ and TNBC

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Abstract Immunotherapy in breast cancer is currently an appealing topic of research. With the understanding of the complex mechanisms of the immune system and the interaction between this and the tumor, new potential targets have emerged. It is also becoming clear that some breast cancer subtypes, such as triple-negative and HER2+ breast cancer, can be considered immunologic tumors. Therefore, new therapeutic strategies can be investigated. In this review, we offer an overview on PD-1/PD-L1 and CTLA4 checkpoint inhibitors, the most studied immune target therapies in triple-negative breast cancer (TNBC) and HER2+ breast cancer. We will also focus our attention of tumor-infiltrating lymphocytes (TILs).

Keywords Immunotherapy · TNBC · HER2+ · PD-1 · CTLA-4 · Tumor-infiltrating lymphocyte (TILs) · Clinical trials · Breast cancer · Research · HER2+ breast cancer

Introduction

Cancer immunoediting is primarily responsible for the absence of an adequate immune response against cancer. During immune surveillance, those tumor cells recognized by the immune system are eradicated (elimination phase). However, under selective immune pressure, new cancer cell variants can arise. These defective cells may escape tumor surveillance, and their accumulation can lead to cancer growth. When inflammation shifts from acute to chronic (equilibrium phase), immune cell patterns change, leading to complete independence from immune surveillance (escape phase). During this process, the upregulation of inhibitory immune checkpoints is fundamental for the acquisition of cancer autonomy. Triple-negative breast cancer (TNBC) and HER2+ breast cancer (BC) are now recognized to be immunogenic tumors [1–5]. Therefore, understanding how tumors can evade the natural defenses of the organism and recognizing the role of the immune system in the battle against cancer is important to find new targets and develop new drugs that could improve the poor prognosis of TNBC and enrich the armamentarium in HER2+ BC.

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Tumor-Infiltrating Lymphocytes (TILs)

The role of TILs in the cancer microenvironment was under investigation for a long time [6–8]. In the older studies, both stromal and intratumoral TILs have been assessed. Given that stromal TILs have been recognized to be more reproducible, it is now recommended to evaluate stromal TILs as the principal parameter [9••]. If a tumor contains more TILs than tumor cells, it can be referred to as lymphocyte-predominant breast cancer (LPBC). However, simply counting the TILs does not take into account the type of lymphocytes. Differentiation between B and T lymphocytes can, e.g., be done by immunohistochemical staining [10]. LPBC varies among subgroups. Among TNBC, LPBC accounts for approximately 30 %, while in HR+/HER2−, the LPBC rate decreases to approximately 10 %. The presence of TILs correlates with a good prognosis, especially in TNBC [11••, 12] and HER2+ BC [13••]. In the FinHER trial, each 10 % increase in TILs was

significantly associated with decreased distant recurrence in TNBC and in HER2+ BC patients treated with trastuzumab [14••]. In the BIG02-98 trial, an increase in TILs was associated with a reduction in risk of relapse and death in TNBC, and with a better disease-free survival in HER2+ BC [13••].

TILs appear also to be a predictive biomarker for treatment response. TILs and CD3 and 20 positive cells indicate a higher pathological complete response (pCR), irrespective of the subtype [15, 16]. Lymphocyte-predominant breast cancer (LPBC) is usually defined as having a threshold of stromal lymphocytes around 50–60 % [9••]. In the GeparSixto study, LPBC defined as intratumoral or stromal TILs ≥60 % presented a higher pCR rate compared with non-LPBC (59.9 vs. 33.8 %; $p<0.001$) following anthracycline-taxane-based chemotherapy [16]. These findings were further validated in the GeparQuinto study [17•]. In the adjuvant setting, TILs were associated with good prognosis among patients with TNBC and with a higher response to anthracyclines in patients with HER2+ BC (BIG02-98 trial) [13••]. In the GeparSixto trial, when adding carboplatin to the antracycline-taxane-based chemotherapy backbone, pCR rates in the HER2+ group increased (pCR≥75 %; $p=0.002$) [18••]. This could be explained by a strong interaction of platinum agents with the immune system. Indeed, it has been shown that platinum agents could induce an immunogenic type of death [19•]. In treating naive HER2+ BC, an association between TILs and response to trastuzumab treatment was also identified [14••]. Several other studies confirmed the correlation between higher TIL levels and pCR in both HER2+ [20••, 21] and TNBC [11••, 22–24]. These results underline the importance of an interaction between the immune system and anticancer treatment in order to increase the chance of a tumor response.

Interestingly, Denkert and colleagues showed a positive correlation of messenger RNA (mRNA) markers (CXCL9, CCL5, CD8A, CD80, CXCL13, IGKC, CD21, IDO1, PD-1, PD-L1, CTLA4, FOXP3) with proimmune markers, stromal TILs, and response to therapy [18••, 25]. In particular, tumors with a higher mutational burden were more likely to be LPBC and showed a higher probability of achieving a pCR [18••]. As stromal TILs are shown to be important in TNBC and HER2+ BC, both for prognosis and response to therapy, the investigation of new immune treatment in these subsets of the disease is fundamental. Moreover, TIL assessment in residual disease after neoadjuvant chemotherapy could help to identify patients at high risk who need additional therapy [26, 27••].

Immune Checkpoint Inhibitors

Immune response is initiated following an interaction between antigens and specific receptors present on the T cell surface (TCRs). T cells cannot recognize "free antigens." In order to initiate an immune response, antigen processing and presentation are necessary. A major role in this process is carried out by antigen-presenting cells (APCs), such as dendritic cells. After APCs have phagocytosed pathogens, they usually migrate to lymph nodes where T cells are present. Foreign antigens associated with the major histocompatibility complex (MHC) are then displayed to T cells. After the cross-talk between MHC and TCRs, the immune response can start. In order to maintain a balance between host defense, self-tolerance, and tissue protection against potential damage due to the response to pathogens itself, equilibrium between co-stimulatory and inhibitory signals (immune checkpoints) is necessary. The dysregulation of immune checkpoints is one of the most important mechanisms used by cancers to escape immune surveillance. As the inhibitory signals implicate a direct receptor-ligand interaction, the easiest way to overcome checkpoints is by using antibodies or recombinant forms of ligands or receptors. Therefore, the principal targets are receptors or their ligands on lymphocytes, instead of cancer cells themselves [28].

The currently most studied immune checkpoints in breast cancer are programmed cell death 1 (PD-1), its ligand programmed death ligand 1 (PD-L1), and cytotoxic T lymphocyte antigen 4 (CTLA-4). (Fig. 1)

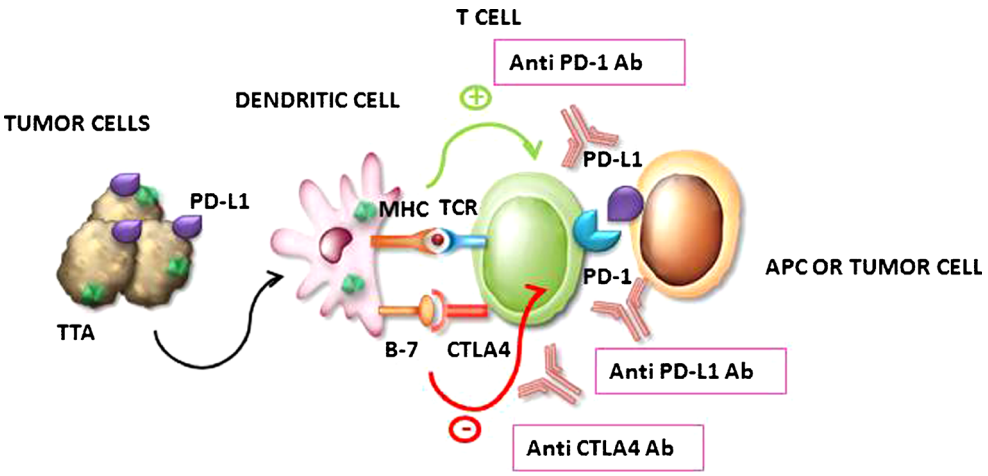

Fig. 1 Relevant immune checkpoints in breast cancer. *Ab* antibody, *APC* antigen-presenting cell, *CTLA4* cytotoxic T lymphocyte-associated antigen 4, *MHC* major histocompatibility complex, *PD-1* programmed death-1, *PD-L1* PD-1 ligand, *TCR* T cell receptor, *TTA* tumor-associated antigen

CTLA-4-Targeted Therapy Trials

CTLA-4 was the first immune checkpoint receptor to be targeted. Ipilimumab, an anti-CTLA-4 antibody was also the first immunotherapy to receive approval in solid tumors. CTLA-4 is a protein receptor located on the surface of activated CD8+ lymphocytes. After T cell activation, CTLA-4 is overexpressed and provides a downregulation of immune response by interacting with CD80 or CD86 on the surface of APCs. CTLA-4 is either weakly or not expressed in normal breast tissue in contrast to breast cancer. Among patients with TNBC, those without androgen receptor expression are more likely to be CTLA-4 positive [29]. Moreover, higher levels of CTLA-4 mRNA are associated with a higher risk of nodal metastases and higher stage. It has been shown that tumors with CTLA-4 expression can predict a shorter disease-free (DFS) (HR 2.17, p=0.029) and overall survival (OS) (HR 2.82, p=0.007), whereas interstitial CTLA-4+ lymphocytes are associated with longer DFS (HR 0.31, p=0.002) and OS (HR 0.31, p=0.005). Interestingly in xenograft models, the use of anti-CTLA-4 monoclonal antibodies can enhance the anti-tumor activity of trastuzumab; this might be an attractive strategy to overcome trastuzumab resistance.

The only trial designed for the TNBC subset using a CTLA-4 inhibitor has recently been withdrawn prior to enrollment (reason not specified) (NCT01936961), and no clinical trials are currently ongoing in the HER2+ BC subset (source: ClinicalTrials.gov), probably because of lack of conclusive preliminary data in this subset of disease (Table 1).

PD-1/PD-L1-Targeted Therapy Trials

PD-1 and its ligand PD-L1 are the best investigated immune targets in TNBC and HER2+ BC. PD-1 is expressed on activated T lymphocytes and acts by binding PD-L1. The ligand is constitutively expressed on immune cells or after induction by inflammatory cytokines [30]. Moreover, it can be overexpressed on tumor cells. Its major role is to limit the activity of T cells in peripheral tissues during inflammation and autoimmunity [31]. Interestingly, Mittendorf and colleagues showed that PD-L1 assessed on tissue microarrays is expressed in 20 % of TNBC. Even if further studies are needed to assess the real prevalence of PD-L1 in TNBC, PD-L1 is emerging as an appealing new target in this orphan disease [32]. Furthermore, the presence of PD-1-positive TILs is generally associated with an increased number of mutations in tumor cells. Particularly, phosphatase and tensin (PTEN) homolog loss seems to increase PD-L1 expression, leading to a decrease in T cell proliferation. In this scenario, the use of anti-PI3K pathway agents can increase the antitumor adaptive immune response [32]. The expression of PD-L1 is also more frequently observed in tumor protein p53 (*TP53*) or phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit alpha (*PIK3CA*)-mutated tumors [33].

Pembrolizumab (MK-3475) is a highly selective humanized monocolonal antibody of the IgG4/kappa isotype that acts by directly blocking the interaction between PD-1 and its ligands, PD-L1 and PD-L2. It can also modulate the level of interleukin-2 (IL-2), tumor necrosis factor alpha (TNFα), interferon gamma (IFNγ), and other cytokines. Pembrolizumab has recently gained accelerated approval by the Food and Drug Administration (FDA) for the treatment of patients with unresectable or metastatic melanoma and disease progression following ipilimumab, and in case of presence of a BRAF V600 mutation, a BRAF inhibitor has been given, based on tumor response rates and duration of response [34]. Moreover, pembrolizumab received breakthrough therapy designation for EGFR/ALK-negative NSCLC and disease progression following platinum-based chemotherapy, based on data derived from the Keynote-001 trial (overall response rate 47 % in patients with PD-L1 expression ≥50 %) [35].

In TNBC, preliminary data of two ongoing clinical trials are available. In the Keynote-012 trial (NCT01848834) [36], a phase Ib multi-cohort study, a total of 32 heavily pretreated PD-L1-positive patients (immunohistochemical expression in the stroma or in ≥1 % of tumor cells) received pembrolizumab every 2 weeks at a 10 mg/kg dose. Of those, efficacy data of 27 patients could be analyzed. A total of 5 patients presented an overall response (1 complete response and 4 partial responses), 7 had stable disease, and 12 patients progressed under treatment. The majority of patients with a response to

Table 1 Overview of ongoing trials with anti CTLA-4 and anti-PD-1/PD-L1 therapy in breast cancer (source: ClinicalTrials.gov)

Trial description	Phase	NCT number
Nivolumab (anti-PD-1 inhibitor) and nab-paclitaxel in recurrent mBC	Phase I	NCT02309177
Pembrolizumab (anti-PD-L1 inhibitor) in solid tumors including advanced TNBC	Phase Ib	NCT01848834
MEDI4736 (anti-PD-L1 inhibitor)+wPaclitaxel and ddAC in stage I–III TNBC	Phase I/II	NCT02489448
MEDI4736 in advanced solid tumors including TNBC	Phase I/II	NCT01693562
Entinostat, nivolumab, and ipilimumab (anti–CTLA-4) in solid tumors including locally advanced or metastatic HER2− BC	Phase I	NCT02453620

ddAC dose-dense doxorubicin/cyclophosphamide, *mBC* metastatic breast cancer, *PD-1* programmed death-1, *PD-L1* PD-1 ligand, *TNBC* triple-negative breast cancer, *w* weekly

pembrolizumab remained on treatment for at least 48 weeks, with a response duration ranging among 15 to more than 40 weeks (median duration of response was not reached). Those results underline that even if the response to immune agents is not frequently obtained, responder patients could benefit from long-lasting disease control. As expected, the trial showed a late onset of tumor response (median time to response was 18 weeks). The safety and tolerability profile was acceptable. The most common adverse events were arthralgia, fatigue, myalgia, and nausea, occurring in at least 15 % of patients [36] Interestingly in Keynote-001 trials, PD-L1 positivity showed a correlation with pembrolizumab response [37••], whereas a recent study showed that mismatch-repair status can predicted a clinical benefit with pembrolizumab [38••], indicating a potential role as biomarkers for treatment response.

MPDL3280A, a monoclonal antibody against PD-L1 is currently under investigation in a phase I study including highly pretreated TNBC patients (92 % had two or more prior systemic therapies) regardless of PD-L1 status (PD-L1+ ≥5 % or PD-L1− <5 %). Overall, three out of nine evaluable patients (median age 55 years) presented a tumor response, including one complete response. The median duration of response has not yet been reached [39]. The anti PD-1 antibody MK-3475 is under investigation in patients with advanced trastuzumab-resistant HER2+ BC (PANACEA trial; NCT02129556), in order to define if blockade of the PD-1 pathway can be exploited to reverse trastuzumab resistance in patients that have previously progressed on trastuzumab (Table 1).

Cancer Vaccines

The history of breast cancer vaccines has not been enthusiastic so far. The main reason could be that this class of drugs was tested in the metastatic setting, where normally a higher disease burden is present. To test vaccines, the presence of a low cancer burden (prevention or adjuvant setting) or minimal residual disease (e.g., after neoadjuvant chemotherapy), especially in less pretreated patients, are the preferred scenarios. The main reason is that each cancer vaccine can activate a T cell-specific response directed only against a small subset of specific antigens. A lower cancer burden allows time to create an adequate immune response and to better deal with the immunosuppressive environment created by the tumor itself [40]. Moreover, in the advanced setting, a rapid tumor response is often required but the vaccination strategy needs time to show its effects. In those patients, vaccines can be used to modulate the immune system in order to render it more susceptible to other therapeutic approaches such as chemotherapy or radiotherapy [41]. A good patient selection is important in order to show an advantage in terms of less disease recurrence. Choosing a population at sufficient early recurrence risk may maximize the probability to show a potential clinical benefit of vaccines use [42]. Therefore, TNBC and HER2+ tumors could be the optimal target cohort to test vaccines.

Cancer vaccines are mostly investigated in HER2+ breast carcinoma. Park et al. tested lapuleucel-T (APC8024) in patients with metastatic BC with HER2 overexpression or amplification (phase I trial) [43]. The vaccine is built of in vitro activated mononuclear cells with the use of an antigen construct consisting of sequences from intracellular and extracellular domains of HER2 linked to granulocyte-macrophage colony-stimulating factor (GM-CSF). The vaccine induced a significant immune response, with some patients experiencing a long-lasting response. It was well tolerated and showed a good safety profile. An allogenic GM-CSF secreting breast cancer cell vaccine [44] was tested in metastatic HER2+ BC in combination with low-dose cyclophosphamide and trastuzumab. Median PFS was 7 months, with a median OS of 42 months. Murray et al. showed that the use of vaccination with E75+ granulocyte macrophage colony-stimulating factor (GM-CSF) can induce both peptide-specific IFNγ and epitope-specific CTLs, which lyse HER2+ tumors in stage IV patients. The antigen E75 was chosen because it is a dominant CTL epitope, whereas GM-CSF is one of the most effective cytokines for the activation of dendritic cells [45]. von Minckwitz and colleagues conducted a dose-finding phase I trial in patients with solid tumors including BC, using ScFv(FRP5)-ETA, a recombinant antibody toxin binding specifically to HER2. It consists of an N- terminal single-chain antibody fragment (scFv), linked to truncated pseudomonas exotoxin A (ETA). Overall, 18 patients were recruited; of whom 2 experienced stable disease, 3 clinical benefit, and 11 disease progression [46]. Another phase I/II study evaluated the use of a HER2/neu T-helper peptide-based vaccine in HER2+ metastatic BC and showed a good safety and immunogenicity profile [47, 48]. The results of a phase I/II trial with E75 (nelipepimut-S) has recently been published [49•]. Nelipepimut-S is a human leukocyte antigen (HLA)-A2/A3-restricted immunogenic peptide derived from the HER2 protein. Its use was investigated in association with GM-CSF in the adjuvant setting in order to prevent disease recurrence. The study population included patients with any degree of HER2 expression with the assumption that HER2 expression is not as important for generating an immune response to an HER2 vaccine as it is in order to obtain a response with monoclonal antibodies. Mittendorf et al. reported a 5-year DFS of 89.7 % in the vaccinated group versus 80.2 % in the control group (p= 0.08). A significant difference between those patients that received full dose, and those not optimally dosed was found (5-year DFS 94.6 vs. 87.1 %; p=0.05). The toxicity profile appeared to be acceptable. A phase III trial evaluating the optimal dose and including booster inoculations is currently ongoing.

To the best of our knowledge, there are no available results on vaccination in patients with TNBC. Studies currently ongoing in HER2+ and TNBC are shown in Table 2.

Table 2 Ongoing vaccine trials in HER2+ and TNBC patients (only active ongoing trials are shown) (source: ClinicalTrials.gov)

Trial description	Phase	NCT number
HER2+ breast cancer		
HER-2 pulsed dendritic cell vaccine in pt. with residual disease after NACT	Phase I	NCT02061423
HER-2 pulsed dendritic cell vaccine in pt after CT+/−trastuzumab or NED after disease recurrence	Phase I	NCT02063724
Adenoviral transduced autologous dendritic cell vaccine	Phase I	NCT01730118
ICD HER2 vaccine w/wo polysaccharide-K in metastatic pt. receiving trastuzumab	Phase I/II	NCT01922921
Trastuzumab+nelipepimut-S/GM-CSF (NeuVax) versus trastuzumab+GM-CSF alone	Phase II	NCT02297698
An alphaviral vector encoding HER2 extracellular and transmembrane region (DUKE-002-VRP) in pt. with locally advanced or metastatic disease	Phase I	NCT01526473
HER2-pulsed dendritic cell in pt. with metastatic disease	Phase I	NCT02473653
Triple-negative breast cancer		
Anakinra+CT+dendritic cell vaccine in pt with locally advanced disease	Phase I/II	NCT02018458

CT chemotherapy, *DCIS* ductal in situ, *GM-CSF* granulocyte-macrophage colony-stimulating factor, *ICD* intracellular domain, *NACT* neoadjuvant chemotherapy, *NED* no evidence of disease, *pt.* patient

Conclusion

The interaction between immune system and breast cancer is becoming an appealing topic to discover new targets and to develop new drugs. In the past, breast cancer has been considered a non-immunological tumor, but when looking inside the different subtypes, this paradigm seems to be no longer valid. However, efficacy data derived from large phase III trials are still lacking. In TNBC and HER2+ breast cancer, the immune system seems to play a role that may be as important as traditional anticancer treatment in eradicating the tumor. Therefore, every effort should be made to improve the host immune response and to escape the immunosuppression created by the tumor itself. Promising results are arising from checkpoint inhibitors and vaccination strategies. Moreover, combinatorial strategies of multiple immune modulator inhibitors represent a valid approach to further enhance tumor response. Another approach that needs further investigation is the probable synergistic effect between chemotherapy and immunotherapy, given the evidence that certain chemotherapy can in part overcome the immune suppression created by the tumor [50]. In conclusion, immune therapy in breast cancer has the potential to generate new hope in a disease like TNBC and to increase the treatment modalities in HER2+ breast cancer.

Compliance with Ethics Guidelines

Conflict of Interest Sibylle Loibl and Jenny Furlanetto declare that they have no conflict of interest

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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