

Immunotherapy for Breast Cancer is Finally at the Doorstep: Immunotherapy in Breast Cancer

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ABSTRACT

Background. Although immunotherapy is making rapid inroads as a major treatment method for melanoma, lung, bladder, and hereditary colon cancer, breast cancer (BC) remains one of the tumors yet to experience the cellular immunology explosion despite the fact that heavy lymphocyte responses in breast tumors improve response to therapy and can predict for long-term survival.

Results. Immunotherapies in the form of monoclonal antibodies such as trastuzumab and pertuzumab have had an impact on HER2-positive breast cancer (HER2+BC) treatment through antibody-dependent cellular cytotoxicity. Current evidence suggests that checkpoint inhibitors and other cellular therapies are at the doorstep of improving outcomes in triple-negative BC (TNBC) and HER2+BC, especially when combined with standard therapies.

Conclusions. Although this approach has benefitted small numbers of patients to date, numerous clinical trials are underway to define the relative role immunotherapy may play in the treatment of BC.

TUMOR-ASSOCIATED LYMPHOCYTES OFFER INSIGHTS INTO THE POTENTIAL OF IMMUNE-BASED THERAPIES

Tumor-infiltrating lymphocytes (TILs) are emerging as a strong prognostic factor for early-BC patients, especially in TNBC and HER2+BC. In 2014, the International TILs

Working Group published a guideline to standardize TIL evaluation.¹ Invasive BC tumors with more than 50% of lymphocytic infiltrate, called lymphocyte-predominant BC (LPBC), are known to have the greatest clinical benefit.² It is believed that TILs have a synergic effect with chemotherapy and immune checkpoint inhibitor therapy for improved clinical response in this patient population.

Several studies have found that most TILs in BC are CD8+ T cells indicative of type 1 immunity. In a systematic review investigating the variation of lymphocytic infiltration found that CD8+ T cell infiltrates were identified in 48% of all BCs, with similar levels observed for TNBC and HER2+BC and fewer observed for hormone receptor-positive BC (HR+BC).^{3–6}

The prognostic role of TILs in BC has been shown in the context of randomized adjuvant trials.^{7,8} For TNBC, Loi et al.⁸ showed a 10% increase in TILs correlated with a 17% decrease in the risk of recurrence and a 27% decrease in the risk of death. Similarly, with a 10% increase in TILs in HER2+BC, an overall survival (OS) rate of 18% was observed.⁹ However, HR+BC tumors with LPBC do not show the same survival benefit as the TNBC and HER2+BC subtypes.

The study of Denkert et al.¹⁰ and other studies^{11–13} have demonstrated that a high tumor infiltration by lymphocytes at diagnosis is associated with a higher likelihood of pathologic complete response (pCR) after neoadjuvant chemotherapy (NAC). In TNBC and HER2+BC, an incremental increase in TILs within and surrounding a tumor not only has been associated with improved survival, but also has been shown to correlate with response to treatment.^{7–9,14} Even among patients with residual disease after NAC for TNBC, those with elevated levels of TILs have had better outcomes with improved metastases-free and overall survival.^{15,16} The association between higher levels of TILs and increased trastuzumab benefit in HER2+BC also has been reported where the presence of

TILs correlates with improved survival and response to trastuzumab.⁸ Moreover, only a few studies have evaluated the value of TILs in predicting sensitivity to specific treatments. Dieci et al.⁹ investigated the clinical utility of TILs using archived samples from two large phase 3 randomized adjuvant trials and evaluated the prognostic and predictive role of TILs for BC patients treated with anthracyclines or no adjuvant chemotherapy. These authors confirmed the prognostic role of TILs in early-stage TNBC and suggested a prognostic impact for HER2+BC patients as well, although they did not see its use in the selection of patients for anthracyclines chemotherapy.⁹

High levels of TIL infiltrates in estrogen receptor-negative (ER-) BC predict pCR after NAC anthracycline-based chemotherapy, but not after treatment with cyclophosphamide, methotrexate, and fluorouracil (CMF).¹⁷ In addition, a greater reduction in FoxP3+regulatory T cells (Tregs) is seen in patients responding to the aromatase inhibitor letrozole, and the development of new TILs is associated with a response to NAC paclitaxel.^{18–21} Studies are underway to allow for a more detailed understanding of the variation in lymphocytic infiltration among BC subtypes and to help identify which tumors are more amenable to immunomodulation.

THE EMERGENCE OF CHECKPOINT THERAPY

Immune checkpoint transmembrane proteins, present in virtually all tissues, are part of the host immune response. Programmed cell death 1 (PD-1) or programmed death ligand 1 (PD-L1) are found on T lymphocytes or tumor cells modulating anti-tumor immunity. In 30–50% of BC, an upregulated PD-L1 receptor is found on the tumor cell surface (Fig. 1).^{22,23} Blocking with antibodies to PD-L1 results in the preferential activation of T cells with specificity for the cancer, restoring endogenous anti-tumor activity.^{24,25}

Initially, checkpoint inhibitors demonstrated promise in melanoma and later in non-small cell lung cancer. More recently, studies using single agents (monoclonal anti-PD-1 pembrolizumab and PD-L1 inhibitor atezolizumab) have shown promise in TNBC.^{26,27} In a phase 1 trial of pembrolizumab in TNBC, the KEYNOTE-012 trial (NCT01848834), the objective response rate (ORR) was 18.5%, the median progression-free survival time was 1.9 months, and the median overall survival (OS) time was 10.2 months. The responses to these agents were approximately 20% and appeared to be durable in metastatic TNBC, suggesting that these agents may have an impact on outcomes for responders.

For estrogen receptor-positive (ER+) patients, new therapies are in the pipeline, such as monoclonal antibody targeting cytotoxic T lymphocyte antigen-4 (CTLA-4) with an ORR (stable disease for 12 weeks) of 42%. The findings show an increase in peripheral CD4+ and CD8+ T cells expressing an inducible co-stimulator of T cell activation (ICOS), which may be a future biomarker of immune activation resulting from blockade of CTLA-4.²⁸

In addition, PD-1 may have an impact on prognosis. The ratio between effective Tregs and PD-1 expression may be used as a prognostic factor for operable BC patients. In a bivariate analysis, high tumor-infiltrating PD-1(+) cell counts correlated with significantly shorter patient survival, and PD-L1 expression in the tumor microenvironment has been correlated with high levels of TILs, translating into better outcomes in TNBC.^{25,29,30}

Recent study has investigated TILs and PD-L1 status in TNBCs before and after preoperative systemic therapy to evaluate the clinical significance of PD-L1 expression. These studies demonstrated that patients with TNBC who had combined low levels of TILs and high PD-L1 status before preoperative systemic therapy showed an unfavorable prognosis. This could represent a group of patients ideal as the therapeutic target for an immune checkpoint inhibitor.³¹

Despite great advances in checkpoint inhibitor therapy for BC, limitations do exist. This likely is related to the dynamic nature of the host immune response so that, ultimately, a single immunologic biomarker is unlikely to predict responses to any agent. For example, in a phase 1 trial, approximately 10% of the patients deemed to be PD-L1-negative did have clinical response to anti PD-L1 therapy.³²

CHECKPOINTS IN COMBINATION WITH CHEMOTHERAPY FOR NEOADJUVANT TRIPLE-NEGATIVE BREAST CANCER

Triple-negative BC, defined as no expression of any known BC tumor biomarkers, constitutes 15–20% of all BC.³³ In the past, atezolizumab as a single agent demonstrated a response in pretreated patients with PD-L1-positive TNBC.³⁴

Combination therapies currently are a major source of interest. In a phase 1 study, atezolizumab plus nab-paclitaxel was evaluated as a line of treatment regardless of PD-L1 status for patients with metastatic TNBC. For patients with PD-L1-positive TNBC, the ORR was higher (77.8%), with a stable disease rate of 22.2%. In the PD-L1-negative group, the ORR still remained high (57.1%), with a stable disease rate of 42.9%.³⁵ The I SPY 2 trial investigating 29 TNBC patients demonstrated that

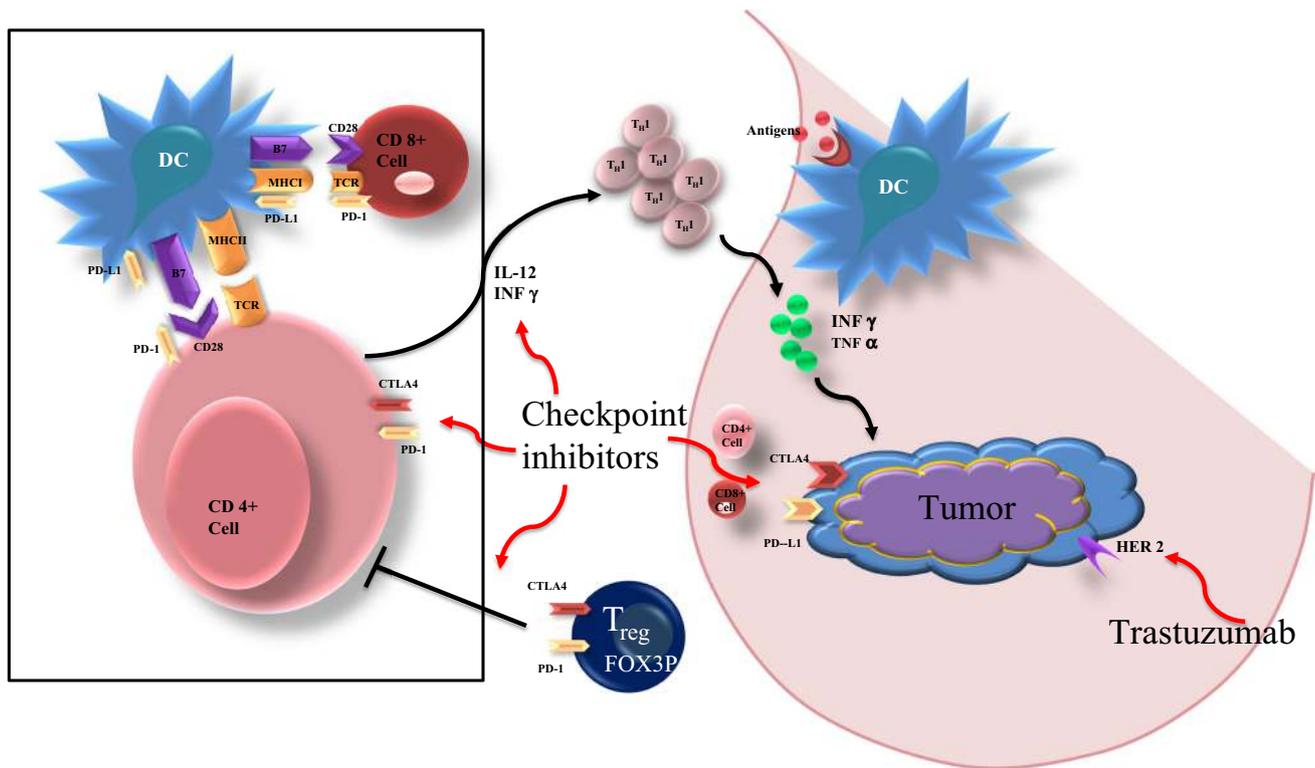


FIG. 1 Immunotherapy pathways in breast cancer treatment

pembrolizumab increased the raw pCR rate by more than 50% and the estimated pCR rate by 40%. The respective rates for 40 HR+/HER– patients were 13 and 21%.³⁶ This trial has graduated to phase 3.

Currently, multiple clinical trials, such as the SWOG-21418 (NCT02954874) and A-BRAVE trials (NCT02926196), are underway evaluating virtually all presentations of TNBC and examining the effect of adjuvant pembrolizumab and avelumab correspondingly on TNBC patients without a pCR after NAC. For patients with locally advanced TNBC, the KEYNOTE-522 trial (NCT03036488) is randomizing patients to pembrolizumab or placebo combined with paclitaxel and carboplatin in the NAC setting, and the NeoTRIPaPDL1 trial (NCT02620280) phase 3 trial is randomizing patients with locally advanced TNBC to NAC nab-paclitaxel and carboplatin with or without atezolizumab.

For metastatic TNBC, pembrolizumab also is being evaluated in a phase 3 trial, the KEYNOTE-355 trial (NCT02819518). It also is being evaluated as a first-line agent in a phase 3 trial, the KEYNOTE-119 trial (NCT02555657), comparing capecitabine, eribulin, gemcitabine, and vinorelbine beyond a first-line treatment with anthracycline, a taxane, or both.

Finally, the IMpassion130 trial is randomizing nab-paclitaxel plus placebo or atezolizumab as first-line therapy for untreated TNBC. Investigation of other checkpoint

inhibitors such as avelumab and nivolumab in monotherapies or combinations are currently underway in phase 1 and 2 trials. Treatment of TNBC is reaching a new frontier with the maturation of many of these studies, which may change the way we see TNBC outcomes and treatment pathways.

OPPORTUNITIES TO INCREASE TUMOR-ASSOCIATED LYMPHOCYTES TO IMPROVE CHECKPOINT RESPONSES

Checkpoint inhibitor therapies act by enhancing the body's immunologic activity against tumors. Despite considerable success of checkpoint inhibitors, several challenges exist. Many patients still do not respond to PD-1 blockade, with some patients experiencing treatment resistance or toxicity. Tumor-intrinsic and microenvironmental-extrinsic factors may contribute to this varied treatment response and resistance.

Effector T cells are potent mediators of anti-tumor immunity, and BC tumors with high levels of TILs are associated with a good prognosis.³⁷ Tumors with pre-existing TILs may have fewer immunologic barriers against entry to the tumor microenvironment or may be more immunogenic, thus resulting in a better response to treatment and a better outcome as noted previously. Therefore, tumors without inducing TILs may be more difficult, and a

potential solution involves finding ways to increase recruitment of TILs to the tumor site. Effective anti-tumor T cells can be expanded *ex vivo* from natural TILs, from endogenous high-avidity T cell clones recognizing tumor-associated antigens, from T cells transduced with exogenous cloned T cell receptors (TCRs), or from chimeric antigen receptors (CARs), whereas endogenous T cells also can be effectively activated by pharmacologic checkpoint inhibitors.³⁸ For HER2+BC, vascular endothelial growth factor (VEGF) blockade in a transgenic mouse model induced a *de novo* TILs infiltrate, which enabled therapeutic efficacy of VEGF blockade, as CD8⁺ T cell depletion abrogated the therapeutic efficacy of the anti-VEGFR-2 antibody, DC101.³⁹

TRASTUZUMAB AND CHECKPOINT NOT YET THE PANACEA FOR HER2-EXPRESSING BREAST CANCER

In some studies, HER2+BC has been associated with higher levels of proliferation and higher TILs compared with HER2-negative tumors. The PANACEA phase 1b and 2 study investigated the safety and efficacy of using immunotherapy with pembrolizumab, an anti-PD-1 monoclonal antibody targeting the T cell checkpoint PD-1, in combination with the standard therapy of trastuzumab for patients with HER2+BC. The authors hypothesized that HER2 may be able to evade destruction from the immune system, which may be an important mechanism contributing to tumor growth and progression in this patient population. Checkpoint inhibitors may play an important role in the tumor immune-evasion mechanism and may potentially be a therapeutic intervention for patients with trastuzumab-resistant HER2+BC.

In the PD-L1-positive cohort, the trial met its primary objective, showing an ORR of 15% and a disease control rate of 25%. In a subgroup analysis of the PD-L1-positive cohort with 5% or more TILs present in the metastatic tumor, the ORR reached 39%, and the disease control rate was 47%, suggesting that quantification of TILs may help to identify patients who will benefit most from this treatment. Unfortunately, no objective responses were observed in the PD-L1-negative cohort. Limitations exist, because not all HER2+BC patients are PD-L1-positive, which limits the patient population that would benefit from this additional therapy. Larger randomized trials are proposed, but for those patients with low TILs, checkpoint inhibitor therapies also may be needed to increase TILs or PDL1 expression.

RESTORING TH1 CYTOKINES IN HER2 BREAST CANCER AS AN APPROACH TO ENHANCE RESPONSES TO STANDARD THERAPY

The CD4⁺T helper cells exhibit a profound effect in initiating and maintaining anti-tumor immunity, with secretion of Th1 cytokines such as interleukin 2 (IL-2) and interferon gamma (IFN- γ), which promote CD8⁺ cytotoxic and natural killer cell function and induce MHC class 2 molecule expression on tumor cells (Fig. 1).^{40,41} Healthy donors and patients with HER2-negative BC are noted to have preserved HER2-specific Th1 response compared with a deficient Th1 response in patients with HER2-positive ductal carcinoma in situ (DCIS), and a nearly absent response in women with HER2+BC.⁴² Zhu et al.⁴³ identified that advanced BC patients with lymph node involvement experience further reduction in the Th1 response. This also has been seen in TNBC and ER+BC, suggesting that a loss of anti-oncogene Th1 responses may be found across all tumor profiles and not only that occurring in HER-2 tumorigenesis.⁴⁴

Administration of DCs to HER2-positive DCIS and stage 1 BC patients has shown induction of anti-HER2 Th1 immunity, with pCR rates approaching 25%.^{45,46} In the neoadjuvant setting, anti-HER2 Th1 immunity can be restored, and this restoration of Th1 immunity is not seen with neoadjuvant trastuzumab and/or chemotherapy.⁴⁶ Therefore, the ability to restore and improve anti-HER2 Th1 immunity may be an additional tool in the arsenal to improve both current standard and checkpoint therapy effects and may have an impact on patient outcomes.

CONCLUSION

The promise of immunotherapy currently has been realized as the fourth major method in cancer therapy for several solid tumors including lung, bladder, colon cancer, and melanoma. Breast cancer currently is poised to become a member of this community. Early clinical trials in BC suggest that TNBC and HER2+BC are about to join the list of cancers for which immune-based therapies have an impact on outcomes. Immunotherapy finally has landed at the doorstep and is about to cross the threshold a century after Cooley's toxin was first introduced and join the list of therapies that put the Halstead hypothesis and mastectomy further into the rear view mirror.

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