HHS Public Access

Author manuscript

Curr Surg Rep. Author manuscript; available in PMC 2018 April 11.

Published in final edited form as:

Curr Surg Rep. 2017 December ; 5: .

Immunotherapy for Breast Cancer: Current and Future Strategies

Austin D. Williams[1], **Kyle K. Payne**[2], **Avery D. Posey Jr.**[4,5], **Christine Hill**[3], **Jose Conejo-Garcia**[3], **Carl H. June**[4,5], and **Julia Tchou**[1]

[1]Department of Surgery, Perelman School of Medicine, University of Pennsylvania, 3400 Civic Center Boulevard, 10th floor South, Philadelphia, PA 19104, USA

[2]The Wistar Institute, Philadelphia, PA, USA

[3]Department of Immunology, H. Lee Moffitt Cancer Center and Research Institute, Tampa, FL, USA

[4]Department of Pathology and Laboratory Medicine, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, USA

[5]Perelman School of Medicine, Center for Cellular Immunotherapies, University of Pennsylvania, Philadelphia, PA, USA

Abstract

Purpose of Review—The breast tumor microenvironment is immunosuppressive and is increasingly recognized to play a significant role in tumorigenesis. A deeper understanding of normal and aberrant interactions between malignant and immune cells has allowed researchers to harness the immune system with novel immunotherapy strategies, many of which have shown promise in breast cancer. This review discusses the application of immunotherapy to the treatment of breast cancer.

Recent Findings—Both basic science and clinical trial data are rapidly developing in the use of immunotherapy for breast cancer. The current clinical trial landscape includes therapeutic vaccines, immune checkpoint blockade, antibodies, cytokines, and adoptive cell therapy.

Summary—Despite early failures, the application of immunotherapeutic strategies to the treatment of breast cancer holds promise.

Keywords

Breast cancer; Immunotherapy; Cancer vaccines; Chimeric antigen receptor T cells (CAR T); Immune checkpoint; Oncolytic viruses

Correspondence to: Julia Tchou.

Conflict of interest Dr. June reports grants from Novartis, outside the submitted work; In addition, Dr. June has a patent 9464140, 9161971, 8975071, 8916381, and 8906682, with royalties paid to Novartis. Carl June is a scientific founder of Tmunity Therapeutics, a biotech dedicated to developing engineered T cells for therapy of cancer, infections and autoimmunity. Dr. June has founders stock from Tmunity. Ms. Hill, Drs. Williams, Posey, Conejo-Garcia, Tchou, and Payne declare no conflicts of interest relevant to this manuscript.

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.

Introduction

Cancer immunotherapy encompasses an array of strategies to harness a patient's own immune system to specifically attack and kill tumor cells. Pioneers such as surgeon William Coley had long been intrigued by the idea of activating the immune system to attack cancer cells by injecting tumors with combinations of pathogens and toxins more than 100 years ago [1]. Clinicians and researchers have long held hope that the patients' own immune systems can be employed to eradicate cancer. Unlike other treatment strategies, cell-based immunotherapy is a living drug, can adapt to stay ahead of evolving tumor cells and has the benefit of remaining effective even when conventional cytotoxic or targeted drug therapy fails. In addition to the direct attack on tumor cells, the immune system may elicit indirect effects by attacking the tumor stroma and "resetting" the immune system to an anti-tumor surveillance status that is protective against tumor outgrowth. Thus, cancer immunotherapy holds promise in the treatment and prevention of cancer.

Over the past several decades, Rosenberg and colleagues have focused their work on the use of patients' own tumor infiltrating lymphocytes as the ultimate targeted cancer therapy. However, the early successes in treating solid tumors were sporadic [2]. As a result of these mostly disappointing clinical results, the concept of immunotherapy as a novel cancer treatment strategy is often met with skepticism. In fact, the important role of the immune system in the pathogenesis of cancer, especially immune cells within the tumor stroma, was only recently highlighted as a next-generation hallmark of cancer [3••].

Despite the disappointing results in the past several decades, several key developments earned cancer immunotherapy the designation of breakthrough of the year among all fields of science and engineering by Science in 2013 [4]. Several landmark studies based on strategies against immune checkpoints and adoptive T cell therapy have propelled immunotherapy to the forefront. The concept is straight-forward: by blocking immune checkpoint receptors (ICR), which serve as natural inhibitors of cytotoxic T cells or by infusion of chimeric antigen receptor (CAR) T cells induced anti-tumor immunity [5••]. In 1996, Allison and colleagues described the effect of cytotoxic T-lymphocyte antigen (CTLA-4), an immune checkpoint receptor on T lymphocytes [6]. By manipulating the CTLA-4 receptor, they were able to demonstrate that CTLA-4 blockade restored immune function. Due to many difficulties in replicating murine discoveries in humans, it was not until 2010 that the results of the first phase III clinical trial demonstrated prolonged survival in patients with metastatic melanoma treated with ipilimumab, an anti-CTLA-4 antibody [7]. Multiple checkpoint inhibitors were subsequently investigated and approved over the next several years, including antibodies targeting programmed death (PD-1) receptor and its ligand, PD-L1, which have become important targets of this burgeoning immune-oncologic field [8].

Around the same time that the clinical effects of immune checkpoint blockade became apparent, a cell-based immunotherapy known as sipuleucel-T, an autologous cellular immunotherapy to treat metastatic prostate cancer, demonstrated a clinical benefit in patients with metastatic prostate cancer [9]. This therapy involves the isolation of a patient's

dendritic cells via leukapheresis and loading these dendritic cells with peptides derived from prostatic acid phosphatase (PAP), a prostate cancer-specific antigen, and further activating these dendritic cells with granulocyte-macrophage colony-stimulating factor (GM-CSF). These activated dendritic cells are then re-infused into the patient with the goal of stimulating the patient's immune cells to target prostate cancer cells that express PAP. Sipuleucel-T gained FDA approval in 2010 for use in prostate cancer. Two clinical trials to evaluate the effectiveness of sipuleucel-T demonstrated improved survival benefit while one trial did not [9-11]. Even though sipuleucel-T is FDA approved, the use of sipuleucel-T has never gained traction, due to a number of issues relating to logistics and unclear clinical benefit.

The infrastructure necessary to support the clinical use of sipuleucel-T, however, helped create the foundation for another cell-based immunotherapy known as CAR T cell therapy, which began to gain prominence after a landmark publication in 2011 [5]. CAR T cells are redirected effector immune cells genetically modified to deliver tumoricidal functions upon recognition of a target antigen. CAR T have demonstrated unprecedented effectiveness in the treatment of hematologic malignancies [5, 12, 13].

Whether these immunotherapy breakthroughs can be added to the current treatment repertoire for breast cancer is unclear. Barriers include the lack of a tumor-specific antigen that is uniquely expressed in breast cancer. In addition, much remains to be learned about the immuno-suppressive nature of the breast tumor microenvironment [14-17]. Generally, immune infiltrates (especially tumor infiltrating lymphocytes) in breast tumors have been associated with a good prognosis [18], but T-regulatory cells have also been shown to aid the tumor in evading effective immune surveillance and killing by blunting the immune response. Tumor infiltrates of predominately regulatory T cells are associated with a poorer prognosis [19]. Several other mechanisms employed by solid tumors, including breast cancer, to evade immune surveillance include downregulation of MHC class I molecules in order to reduce cytotoxic $CD8^+$ T cell activation [20•], and upregulation of cell surface molecules (such as PD-L1) that inhibit both helper and cytotoxic T cell function by binding to the immune checkpoint receptor [21]. Recently, myeloid suppressor cells within solid tumors, including breast cancers, have been shown to suppress cytotoxic $CD8^+$ T cell functions [22, 23], and have prognostic value, as they are associated with worse clinical outcomes [24, 25].

Despite the above-mentioned barriers, several immunotherapies are under development and show promise in the treatment of aggressive breast cancer. Here we review several of these immunotherapies, the challenges to their development and use, and their current clinical applications. A curated list of immunotherapy trials for breast cancer can be found at the following link: https://www.cancerresearch.org/scientists/science-of-immunotherapy/cancer-types/breast-cancer.

Breast Cancer Vaccines

The fundamental concept behind vaccination is exposure to a specific antigen such that, through active immunity, the immune system develops memory and is able to recognize and

swiftly respond to the antigen during future exposures. The vaccination paradigm, commonly used in the prevention of infectious diseases, has been applied to the development of vaccines for the prevention and treatment of cancer [26]. Preventive vaccines can target a virus known to be important in malignant transformation of human cells (e.g., human papilloma virus and cervical cancer) or antigens expressed early in the process of tumorigenesis. Therapeutic vaccines typically target known cancer antigens.

Intramuscular delivery of peptide antigens is the most basic technique of antigen exposure. Antigen-presenting cells (APC) phagocytize antigen which is processed intra-cellularly into peptide fragments (epitope) and then displayed (presented a) onto major histocompatibility complex (MHC) class I or II molecules, MHC-II or MHC-I. T cells that express T cell receptors that recognize and bind to the MHC-epitope complex will be activated to carry out their effector functions. There have been mixed results in the trials of peptide-based breast cancer vaccines, which have targeted human epidermal growth factor receptor 2 (HER2) [27•, 28] and mucin 1 (MUC1) [29]. While peptide vaccines are customizable, easy and relatively inexpensive to produce, injected peptides are readily degraded and have variable immunogenicity [30]. Additionally, limitations on interaction with the chosen peptide and the MHC haplotypes variants must be considered when designing a peptide vaccine [31]. Despite eliciting a measurable immune response, the impact on tumor growth has not been substantial, so combination therapies and alternative methods of antigen delivery have been developed [32].

One strategy to overcome the limited immunogenicity of specific peptide vaccines in established malignancies is to immunize patients with whole tumor cells derived from their own breast tumor or from breast cancer cell lines [33]. Once tumor cells are killed and phagocytized, their entire library of antigens, including antigens that have not yet been identified, can be presented to naïve T cells. The benefit of developing multiple tumor antigen targets simultaneously is that it decreases the likelihood of tumor evasion by down-regulating expression of one specific antigen [34]. Anti-tumor effects of whole-cell vaccines in animal models have been further improved with concurrent chemotherapy [33], highlighting the benefits of vaccination in combination therapies. Use of an autologous, tumor-derived cell line vaccine for breast cancer revealed that while not all patients mounted an anti-tumor immune response, those that did had increased survival [35]. Additional work with whole tumor cell lines has shown that modification of cell surface antigens can improve immunogenicity [36].

Another approach to antigen delivery is to transfect cells with bacterial DNA plasmids encoding the antigen of interest, or so-called DNA vaccines [37]. Both intradermal and intramuscular transfection methods have been utilized, which employ techniques to direct DNA plasmids into keratinocytes and Langerhans cells, by needle-free injections or electroporations [38]. Encoded antigen is subsequently translated from the intracellular plasmids and ultimately presented by dendritic cells to T cells to drive a tumor-specific immune response. The plasmid can be engineered to code for the antigen itself or a more complex single chain heterotrimer complex to include antigen along with MHC-I and β2-microglobulin molecules, allowing presentation of a specific peptide without reliance on intracellular processing [39]. Clinical trials of DNA vaccines against HER2 [40] and

mammaglobin [41], a secretory protein overexpressed in many breast cancers, have shown promise in stimulating immune responses against these tumor antigens. One issue seen in clinical trials of DNA vaccines, however, is the development of immune tolerance, or the failure to mount an immune response to the vaccine antigen due to immunoregulation. Novel DNA constructs that encode for peptides that have higher T cell binding efficiency and checkpoint blockade may help overcome these issues [38, 42].

Finally, many researchers have utilized techniques to manipulate patients' own dendritic cells ex vivo such that they present tumor antigens of interest, and subsequently administer to patients as a dendritic cell vaccine [43]. This strategy has been particularly attractive because dendritic cells are living vaccines as they are professional antigen-presenting cells which have the ability to activate both $CD4^+$ and $CD8^+$ T cell immune functions. Strategies to optimize priming of dendritic cells with tumor-specific antigens or to genetically engineered epitope expression by transduction using viral vectors are underway [44•]. Specifically, for breast cancer, HER2 has been one of the most studied antigens targeted by dendritic cell vaccines. Compelling data in clinical trials utilizing a HER2 dendritic cell vaccine have shown promise in early breast cancer, inducing tumor-specific T cell responses in vaccinated patients with $HER2^+$ breast cancer and eliminating breast cancer cells that express the antigen [45-48].

Immune Checkpoint Blockade

One reason why tumor-primed T cells have not been as effective as initially hoped may be due to the suppression of T cell responses as a result of ligation of their cell surface immune checkpoint receptors (ICRs) with their cognate ligand expressed by other leukocytes and tumor cells. Also known as co-inhibitory receptors, ICRs play important roles in inhibitory pathways that, in a healthy host, are necessary to maintain self-tolerance and protect against the development of autoimmunity [49]. In cancer, ligation of ICR with its ligand can blunt the tumor-primed T cells' response allowing the tumor to escape immune surveillance and elimination. In fact, the inflammatory cytokines produced within the local tumor microenvironment induce unrelenting expression of PD-1, thereby facilitating immune evasion [50]. Table 1 outlines the many ICRs and their ligands known to be important in human cancers.

The interruption of ICR/ligand interaction within the tumor microenvironment has become an important immunotherapeutic strategy. Blocking the inhibitory pathways has been shown to restore functional immune responses to tumor-associated antigens [51]. Monoclonal antibodies against ICRs and their ligands have shown promise in clinical trial for many cancer types. Trials have demonstrated modest responses in breast cancer patients to PD-1 and PD-L1 blockade as monotherapy or in combination with conventional chemotherapy [52–56]. Interestingly, the expression of PD-1/PD-L1 is more prevalent in TNBC, underscoring the modality's potential efficacy in treating this unfavorable subtype. PD-1 blockade in heavily pre-treated ER-positive and in triple-negative patients resulted in an overall response rate of > 14%, with long-lived responses and limited toxicity [52]. Comparable responses were observed upon blockade of PD-L1 [57].

To improve effectiveness, several approaches have been proposed and tested in preclinical models. For instance, combining class IIa histone deacetylase (HDAC) inhibition with T cell checkpoint blockade enhances the durability of tumor reduction by modulating macrophage phenotypes [58]. In addition, anti-angiogenic therapies appear to boost anti-tumor immunity, enhancing the effectiveness of PD-1 checkpoint blockade [59].

In breast cancer, PD-1 and its ligand, PD-L1, have been the most widely studied. The presence of both markers has been identified on tumor cells and tumor infiltrating lymphocytes, especially in triple-negative breast cancers. However, the clinical and prognostic predictive values of the PD-1 axis have been mixed [60-63]. The presence of other ICRs like TIGIT and LAG-3 noted in breast cancer (Table 1) may also play important roles in shaping the immune-tumor interface in breast cancer. In our preliminary studies using flow cytometry, CD4 expression of TIGIT and co-expression with TIM3 in the breast tumor microenvironment is prevalent and varies based on breast cancer subtype (unpublished data). These findings highlight differences in the tumor microenvironment between breast cancer subtypes and the need to evaluate individual immunotherapeutic strategies tailored for each breast subtype.

Immune checkpoint blockade is therefore a promising immunotherapeutic strategy that can synergize with conventional therapies in breast cancer [64]. Clinical trials, including those in the neoadjuvant setting, are still in progress and should clarify the effectiveness of checkpoint blockade in various breast cancer subtypes. Full characterization of the predominant ICRs in the breast tumor microenvironment and any differences between breast cancer subtypes will dictate which ICR/ligand axis will be the most effective target and may also serve as prognostic markers and biomarkers of immune activity.

Oncolytic Viruses

The development of oncolytic viruses as cancer therapies is a product of two disciplines, virology and immunology, specifically, and the identification of tumor-associated antigens. Viruses can be engineered to preferentially enter tumor cells by altering their target receptor to a known cell surface antigen or capitalizing on defective interferon pathways in tumor cells [65]. Alterations in viral nucleic acid and protein metabolism can be employed to harness the intrinsically higher expression of key enzymes in proliferating tumor cells compared to normal cells [66]. Once administered to the patient, viruses infect tumor cells, which are killed or lysed by intracellular replication of the virus, or through viral antigen processing onto MHC-I, activating virus-specific T cell clones to destroy tumor cells. Tumor cell lysis, much like a whole tumor cell vaccine, contributes to the overall anti-tumor immune response by releasing tumor-associated antigens, which are then processed by antigen-presenting cells – a phenomenon known as epitope spreading.

While there is currently no clinical trial to evaluate the effectiveness of oncolytic virus in breast cancer, preclinical models have rapidly advanced toward that goal. Some groups are focused on isolating the most potent virus (in terms of replication and progeny), while maintaining tumor specificity [67]. Other researchers are arming the viral vectors with

neutralizing soluble proteins, which interact with immunosuppressive molecules in the tumor microenvironment and increase tumor cell killing by immune cells [68].

Adoptive Chimeric Antigen Receptor T cell (CAR T) Therapy

As noted above, chimeric antigen receptors (CARs) are synthetic molecules that redirect T cells to mediate anti-tumor effects. However, in comparison to the robust activities in hematopoietic malignancies, the effectiveness of CAR T in the treatment of solid tumors remains modest. Barriers include on-target/off-tumor effects due to the presence of tumor-associated antigens in normal tissues. Preclinical development of novel CARs targeting abnormal O-glycosylation of MUC1 and aberrant expression of αvβ6 integrin have demonstrated promise for surmounting the barrier of normal tissue toxicities and should move toward the clinic [69, 70]. In addition, the tumor microenvironment of solid tumors is immunosuppressive, which may limit the potency of CAR T [6]. Therefore, the treatment of solid tumors using CAR T cells has been largely unsuccessful to date.

In preclinical studies, we found that the cell surface molecule c-Met was frequently expressed in breast cancer. We utilized a CAR that was specific for c-Met and demonstrated that the CAR T cells had potent anti-tumor activity in immune-incompetent mice with tumor xeno-grafts. Therefore, we conducted a phase 0 clinical trial (NCT01837602) to evaluate the safety and feasibility of treating metastatic breast cancer with intratumoral (IT) administration of RNA-transfected c-Met CAR T cells. RNA transfection was used to ensure safety by limiting on-target/off-tumor effects through transient expression of the CAR molecule. Patients with metastatic breast cancer presenting with accessible cutaneous or lymph node metastases received a single IT injection at one of two dose levels: 3×10^7 and 3×10^8 cells. Low-level CAR T mRNA was detectable in peripheral blood and in the injected tumor tissues shortly after IT injection in two and four patients, respectively. RNA CAR T c-Met injections were well tolerated in all patients, as none of the patients had cytokine release syndrome or an adverse event more severe than grade 1 related to the study drug. Tumors treated with IT RNA CAR T c-Met were excised and analyzed using immunohistochemistry. Examination of resected tumor revealed extensive tumor necrosis at the injection site, including abundant cellular debris with loss of c-Met immunoreactivity, surrounded by macrophages at the leading edge of necrotic zone (manuscript in preparation). We conclude that IT injections of RNA CAR T c-Met are well tolerated and that they evoke an inflammatory response within tumors.

The identification of other potential antigen targets will be important in the continued application of CAR T cell therapy to breast cancer with special attention given to CAR T cell strategies to tailor specificity and effectiveness against various breast cancer subtypes. The search for novel tumor antigens specific to breast cancer is crucial in the efforts to minimize on-target/off-tumor effects of CAR T therapy.

Antibody-Based Therapy

Trastuzumab, a monoclonal antibody against HER2/neu (HER2), is the most well-known antibody-based immunotherapy for breast cancer. Originally approved for metastatic HER2

positive breast cancer in 1998, this targeted therapy has become the mainstay of treatment regimens for all HER2-positive breast cancers [71-73]. Upon binding to HER2, trastuzumab induces antibody-dependent cellular cytotoxicity (ADCC) of tumor cells. Pertuzumab, another anti-HER2 antibody that was approved in 2013, works to prevent dimerization of HER2 and HER3 membrane receptor proteins and subsequent downstream signaling pathways. In combination with trastuzumab, pertuzumab has been shown to be synergistic when given in the neoadjuvant and, possibly, adjuvant chemotherapy settings [74, 75].

In addition to the immune checkpoint receptors described above, several other antibodies are currently being studied for the treatment of breast cancer (https://www.cancerresearch.org/scientists/science-of-immunotherapy/cancer-types/breast-cancer). Margetuximab, another anti-HER2 antibody, binds to the same epitope as trastuzumab but has been engineered with an Fc domain that promotes more potent ADCC activity. Margetuximab is under investigation in two clinical trials for metastatic and refractory HER2-positive cancer. In the first of these trials, reductions in tumor size were observed in 78% of response-evaluable breast cancer patients and ex vivo analysis of patient PBMCs confirmed enhanced ADCC activity of margetuximab compared to trastuzumab [76]. Other antibody-based therapies that target novel antigens including the cell surface molecule glycoprotein non-metastatic b (GPNMB) on triple-negative breast cancer cells and endoglin, a marker on endothelial cells essential for angiogenesis, are currently being investigated.

Strategies that Target Regulatory T Cells in Breast Cancer

In addition to endogenous suppressive mechanisms, such as ICRs, which have been shown to hamper the effectiveness of cytotoxic T cells, other cell-mediated suppressive mechanisms have been demonstrated to play significant roles in dampening anti-tumor immune effector responses. While the legion of lymphocytes that function to suppress immune function has broadened, including, most prominently, natural killer T cells [77], and gamma-delta T cells [78, 79], the study of suppressive lymphocytes in the context of cancer has been historically dominated by regulatory T cells (Tregs). Such focus on this suppressive lymphocyte subset is due to the accumulation of these cells in the solid tumor mass, which have been shown to correlate with a poor clinical outcome for patients with breast cancer [80, 81]. Tregs can be broadly characterized phenotypically as strongly expressing the high affinity IL-2 receptor α subunit, commonly referred to as CD25, along with the transcription factor FoxP3. These regulatory lymphocytes employ several mechanisms by which they may suppress tumor-reactive T cells, including the secretion of TGF-b and IL-10, the metabolism of ATP to adenosine, as well as the depletion of IL-2 from the local environment [82, 83].

As such, there exists a strong rationale to target these regulatory cells in breast cancer to rescue potential endogenous anti-tumor immune function as well as to support and promote the effectiveness of applied immunotherapies. Studies in animals have demonstrated the ability Treg depletion to synergize with immunotherapy in a murine model of mesothelioma [84]. Importantly, studies investigating the utility of targeting Tregs in cancer patients identified a novel mechanism by which the administration of FDA-approved daclizumab, a monoclonal antibody which specifically targets CD25, selectively reprogrammed Tregs to

produce IFN-gamma by driving the downregulation of FoxP3; this resulted in robust T cell responses against vaccine antigens without autoimmunity [85, 86].

Strategies that Target Immunosuppressive Macrophages or Myeloid Cells in Breast Cancer

Tumor-associated macrophages (TAMs) represent a large cellular component of murine and human breast cancer tumors [87, 88•], and acquire distinct phenotypes in response to signals present within the tumor microenvironment [89]. Within breast cancer, TAMs have been found to play an immunosuppressive role through release of cytokines and growth factors such as IL-10, IL-6, TGF-β, and EGF, which promotes tumor progression and metastasis [90, 91]. Immature myeloid cells, referred to as myeloid-derived suppressor cells (MDSCs), are also present in the tumor microenvironment, and have their own immunosuppressive capacity (including on $CD8^+$ T cells) and cytokine expression profile [22, 87]. MDSCs are phenotypically distinct from TAMs due to their increased relative expression of F4/80 [92]; however, MDSCs have been shown to contribute to TAM accumulation and maintenance in a mouse mammary model and can further differentiate into TAMs [87, 93]. Both macrophage and MDSC infiltration have been associated with poorer outcomes in breast cancer and have been found to modulate responses to chemotherapy [93, 94].

Therapies targeting myeloid cells have primarily been aimed at inhibiting localization of immunosuppressive myeloid cells at tumor sites. However, since immunosuppressive myeloid cells are recruited to the tumor microenvironment by an array of chemokines, production of effective anti-macrophage immunotherapies has been difficult. In a mouse model of breast cancer, administration of a macrophage colony-stimulating factor (CSF-1) receptor antagonist in combination with paclitaxel-based chemotherapy enhanced the efficacy of paclitaxel, inhibited metastasis, and increased cytotoxic $CD8^+$ T cell infiltration in tumors [94]. Currently, one trial of CSF-1R inhibitors in breast cancer is ongoing (NCT0104239). In one murine breast cancer model, anti-chemokine ligand 2 (CCL2) antibodies were shown to reduce tumor growth but upon withdrawal of the anti-CCL2 treatment a rebound effect was observed [88•]. Several phase I and II clinical trials have tested anti-CCL2 antibodies but found limited effect and none have been performed specifically in breast cancer. Additionally, several vaccines are under development aimed at modulating T regulatory cells and MDSCs (NCT02780401 and NCT02157051). Taken together, targeting immunosuppressive myeloid cells represents a promising immunotherapy approach for breast cancer treatment.

Current Clinical Trial Landscape

In addition to the evaluations of novel immunotherapies in breast cancer described above, there is a broad array of ongoing breast cancer immunotherapy clinical trials. A search for trials of immunotherapies on BreastCancerTrials.org and the Cancer Research Institute (cancerresearch.org) yielded 80 clinical trials that are currently enrolling breast cancer patients with disease statuses ranging from remission to failure of traditional therapies. Half of the trials ($n = 42$) are testing drugs related to immune checkpoint blockade, and almost all of these trials are focused on suppression of the PD-1/PD-L1 axis. The immune checkpoint

inhibitors are being tested in combination with one or multiple conventional therapies, including radiation therapy.

The next most prevalent immunotherapy strategy currently being tested in the treatment of breast cancer is vaccine-based therapy. Of the 17 vaccine trials, 6 are evaluating the use of peptide vaccines, 4 are evaluating cell-based vaccines (including dendritic cells), and 4 are testing DNA vaccines. The HER2 antigen is the most common target of these therapies, and several trials are testing vaccination as a prevention strategy against cancer recurrence.

Other therapies represent a minority of the current breast cancer immunotherapy clinical trial landscape. Several CAR T cell trials are currently enrolling, including trials targeting CEA, HER2, mesothelin, MUC1, NKG2D ligands, ROR1, CD70, and CD133. Other trials are utilizing cytokines and other small molecules to engage the immune system.

Conclusion

Immunotherapy for breast cancer has lagged behind the field of oncology in tumors such as melanoma and lung cancer. However, the emergence of immune oncology has offered a new toolbox to researchers and clinicians in breast cancer treatment, especially for breast cancer subtypes that lack targeted therapy such as triple-negative breast cancer. In addition to continuing efforts to identify novel breast cancer-associated antigens, there is still much to learn about how to most effectively modulate the immune response before, during and after the administration of these novel immunotherapies to optimize safety and efficacy. Trials of combination therapies featuring novel immunotherapy and conventional therapy, and even combinations of immunotherapeutic strategies (such as immune checkpoint blockade synergy with CAR T) are needed, as these may improve outcome, while mitigating adverse effects. Future strategies will include the development of therapeutic strategies to address the immunosuppressive tumor microenvironment to enhance the safety and efficacy of immunotherapy.

References

Recently published papers of particular interest have been highlighted as:

- • Of importance
- •• Of major importance

1. McCarthy EF. The toxins of William B. Coley and the treatment of bone and soft-tissue sarcomas. Iowa Orthop J. 2006; 26:154–8. [PubMed: 16789469]

2. Rosenberg SA, Spiess P, Lafreniere R. A new approach to the adoptive immunotherapy of cancer with tumor-infiltrating lymphocytes. Science. 1986; 233(4770):1318–21. [PubMed: 3489291]

3••. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell. 2011; 144(5):646–674. This review highlights the normal and aberrant cellular processes that lead to the development of human cancer from initial genetic changes to metastatic potential. DOI: 10.1016/j.cell.2011.02.013 [PubMed: 21376230]

4. Couzin-Frankel J. Breakthrough of the year 2013. Cancer immunother Sci. 2013; 342(6165):1432–3. DOI: 10.1126/science.342.6165.1432

5••. Porter DL, Levine BL, Kalos M, Bagg A, June CH. Chimeric antigen receptor-modified T cells in chronic lymphoid leukemia. N Engl J Med. 2011; 365(8):725–733. This is the initial descripton

of the use of CAR-T cells in a clinical application for CLL, and describes the first-of-its-kind immunotherapy to be FDA approved. DOI: 10.1056/NEJMoa1103849 [PubMed: 21830940]

6. Leach DR, Krummel MF, Allison JP. Enhancement of antitumor immunity by CTLA-4 blockade. Science. 1996; 271(5256):1734–6. [PubMed: 8596936]
7. Hodi FS, O'Day SJ, McDermott DF, Weber RW, Sosman JA, Haanen JB, Gonzalez R, Robert C, Schadendorf D, Hassel JC, Akerley W, van den Eertwegh AJ, Lutzky J, Lorigan P, Vaubel JM, Linette GP, Hogg D, Ottensmeier CH, Lebbe C, Peschel C, Quirt I, Clark JI, Wolchok JD, Weber JS, Tian J, Yellin MJ, Nichol GM, Hoos A, Urba WJ. Improved survival with ipilimumab in patients with metastatic melanoma. N Engl J Med. 2010; 363(8):711–23. DOI: 10.1056/NEJMoa1003466 [PubMed: 20525992]
8. Robert C, Ribas A, Wolchok JD, Hodi FS, Hamid O, Kefford R, Weber JS, Joshua AM, Hwu WJ, Gangadhar TC, Patnaik A, Dronca R, Zarour H, Joseph RW, Boasberg P, Chmielowski B, Mateus C, Postow MA, Gergich K, Elassaiss-Schaap J, Li XN, Iannone R, Ebbinghaus SW, Kang SP, Daud A. Anti-programmed-death-receptor-1 treatment with pembrolizumab in ipilimumab-refractory advanced melanoma: a randomised dose-comparison cohort of a phase 1 trial. Lancet. 2014; 384(9948):1109–17. DOI: 10.1016/S0140-6736(14)60958-2 [PubMed: 25034862]
9. Kantoff PW, Higano CS, Shore ND, Berger ER, Small EJ, Penson DF, Redfern CH, Ferrari AC, Dreicer R, Sims RB, Xu Y, Froh-lich MW, Schellhammer PF. Investigators IS. Sipuleucel-T immunotherapy for castration-resistant prostate cancer. N Engl J Med. 2010; 363(5):411–22. DOI: 10.1056/NEJMoa1001294 [PubMed: 20818862]
10. Small EJ, Schellhammer PF, Higano CS, Redfern CH, Nemunaitis JJ, Valone FH, Verjee SS, Jones LA, Hershberg RM. Placebo-controlled phase III trial of immunologic therapy with sipuleucel-T (APC8015) in patients with metastatic, asymptomatic hormone refractory prostate cancer. J Clin Oncol. 2006; 24(19):3089–94. DOI: 10.1200/JCO.2005.04.5252 [PubMed: 16809734]
11. Higano CS, Schellhammer PF, Small EJ, Burch PA, Nemunaitis J, Yuh L, Provost N, Frohlich MW. Integrated data from 2 randomized, double-blind, placebo-controlled, phase 3 trials of active cellular immunotherapy with sipuleucel-T in advanced prostate cancer. Cancer. 2009; 115(16): 3670–9. DOI: 10.1002/cncr.24429 [PubMed: 19536890]
12. Porter DL, Kalos M, Zheng Z, Levine B, June C. Chimeric Antigen Receptor Therapy for B-cell Malignancies. J Cancer. 2011; 2:331–2. [PubMed: 21716851]
13. Kalos M, Levine BL, Porter DL, Katz S, Grupp SA, Bagg A, June CH. T cells with chimeric antigen receptors have potent antitumor effects and can establish memory in patients with advanced leukemia. Sci Transl Med. 2011; 3(95):95ra73.doi: 10.1126/scitranslmed.3002842
14. DeNardo DG, Coussens LM. Inflammation and breast cancer. Balancing immune response: crosstalk between adaptive and innate immune cells during breast cancer progression. Breast Cancer Res. 2007; 9(4):212.doi: 10.1186/bcr1746 [PubMed: 17705880]
15. Jiang X, Shapiro DJ. The immune system and inflammation in breast cancer. Mol Cell Endocrinol. 2014; 382(1):673–82. DOI: 10.1016/j.mce.2013.06.003 [PubMed: 23791814]
16. Denkert C, Loibl S, Noske A, Roller M, Müller BM, Komor M, Budczies J, Darb-Esfahani S, Kronenwett R, Hanusch C. Tumor-associated lymphocytes as an independent predictor of response to neoadjuvant chemotherapy in breast cancer. J Clin Oncol. 2009; 28(1):105–13. [PubMed: 19917869]
17. Denkert C, Loibl S, Noske A, Roller M, Müller BM, Komor M, Budczies J, Darb-Esfahani S, Kronenwett R, Hanusch C, Törne Cv, Weichert W, Engels K, Solbach C, Schrader I, Dietel M, Minckwitz Gv. Tumor-Associated Lymphocytes As an Independent Predictor of Response to Neoadjuvant Chemotherapy in Breast Cancer. J Clin Oncol. 2010; 28(1):105–13. DOI: 10.1200/jco.2009.23.7370 [PubMed: 19917869]
18. Bindea G, Mlecnik B, Fridman WH, Galon J. The prognostic impact of anti-cancer immune response: a novel classification of cancer patients. Semin Immunopathol. 2011; 33(4):335–40. DOI: 10.1007/s00281-011-0264-x [PubMed: 21461991]
19. Nizar S, Copier J, Meyer B, Bodman-Smith M, Galustian C, Kumar D, Dalgleish A. T-regulatory cell modulation: the future of cancer immunotherapy? Br J Cancer. 2009; 100(11):1697–703. DOI: 10.1038/sj.bjc.6605040 [PubMed: 19384299]
20•. Comber JD, Philip R. MHC class I antigen presentation and implications for developing a new generation of therapeutic vaccines. Ther Adv Vaccines. 2014; 2(3):77–89. This paper highlights

the importance of harnessing the immune system's intrinsic processes, such as intracellular antigen processing, when developing immunotherapies that target a certain action of the immune system. DOI: 10.1177/2051013614525375 [PubMed: 24790732]

21. Muenst S, Schaerli AR, Gao F, Däster S, Trella E, Droeser RA, Muraro MG, Zajac P, Zanetti R, Gillanders WE, Weber WP, Soysal SD. Expression of programmed death ligand 1 (PD-L1) is associated with poor prognosis in human breast cancer. Breast Cancer Res Treat. 2014; 146(1):15–24. DOI: 10.1007/s10549-014-2988-5 [PubMed: 24842267]
22. Gabrilovich DI, Nagaraj S. Myeloid-derived suppressor cells as regulators of the immune system. Nat Rev Immunol. 2009; 9(3):162–74. DOI: 10.1038/nri2506 [PubMed: 19197294]
23. Marvel D, Gabrilovich DI. Myeloid-derived suppressor cells in the tumor microenvironment: expect the unexpected. J Clin Invest. 2015; 125(9):3356–64. DOI: 10.1172/JCI80005 [PubMed: 26168215]
24. Diaz-Montero CM, Salem ML, Nishimura MI, Garrett-Mayer E, Cole DJ, Montero AJ. Increased circulating myeloid-derived suppressor cells correlate with clinical cancer stage, metastatic tumor burden, and doxorubicin-cyclophosphamide chemotherapy. Cancer Immunol Immunother. 2009; 58(1):49–59. DOI: 10.1007/s00262-008-0523-4 [PubMed: 18446337]
25. Zhang S, Ma X, Zhu C, Liu L, Wang G, Yuan X. The role of myeloid-derived suppressor cells in patients with solid tumors: a meta-analysis. PLoS ONE. 2016; 11(10):e0164514.doi: 10.1371/journal.pone.0164514 [PubMed: 27780254]
26. Finn OJ. Vaccines for cancer prevention: a practical and feasible approach to the cancer epidemic. Cancer Immunol Res. 2014; 2(8):708–13. DOI: 10.1158/2326-6066.CIR-14-0110 [PubMed: 25092812]
27•. Mittendorf EA, Clifton GT, Holmes JP, Schneble E, van Echo D, Ponniah S, Peoples GE. Final report of the phase I/II clinical trial of the E75 (nelipepimut-S) vaccine with booster inoculations to prevent disease recurrence in high-risk breast cancer patients. Ann Oncol 25. 2014; 25(9): 1735–1742. An important report demonstrating safety and clinical efficacy of immunotherapy, specifically a breast cancer vaccine, targeting recurrence in high risk patient. DOI: 10.1093/annonc/mdu211
28. Limentani SA, Campone M, Dorval T, Curigliano G, de Boer R, Vogel C, White S, Bachelot T, Canon JL, Disis M, Awada A, Berliere M, Amant F, Levine E, Burny W, Callegaro A, de Sousa Alves PM, Louahed J, Brichard V, Lehmann FF. A non-randomized dose-escalation Phase I trial of a protein-based immunotherapeutic for the treatment of breast cancer patients with HER2-overexpressing tumors. Breast Cancer Res Treat. 2016; 156(2):319–30. DOI: 10.1007/s10549-016-3751-x [PubMed: 26993131]
29. Apostolopoulos V, Pietersz GA, Tsibanis A, Tsikkinis A, Drakaki H, Loveland BE, Piddlesden SJ, Plebanski M, Pouniotis DS, Alexis MN. Pilot phase III immunotherapy study in early-stage breast cancer patients using oxidized mannan-MUC1 [ISRCTN71711835]. Breast Cancer Res. 2006; 8(3):R27. [PubMed: 16776849]
30. Peres Lde P, da Luz FA, Pultz Bdos A, Brigido PC, de Araujo RA, Goulart LR, Silva MJ. Peptide vaccines in breast cancer: The immunological basis for clinical response. Biotechnol Adv. 2015; 33(8):1868–77. DOI: 10.1016/j.biotechadv.2015.10.013 [PubMed: 26523780]
31. Castilleja A, Carter D, Efferson CL, Ward NE, Kawano K, Fisk B, Kudelka AP, Gershenson DM, Murray JL, O'Brian CA, Ioannides CG. Induction of tumor-reactive CTL by C-side chain variants of the CTL epitope HER-2/neu protooncogene (369-377) selected by molecular modeling of the peptide: HLA-A2 complex. J Immunol. 2002; 169(7):3545–54. [PubMed: 12244144]
32. Milani A, Sangiolo D, Aglietta M, Valabrega G. Recent advances in the development of breast cancer vaccines. Breast Cancer. 2014; 6:159–68. DOI: 10.2147/BCTT.S38428 [PubMed: 25339848]
33. Machiels JP, Reilly RT, Emens LA, Ercolini AM, Lei RY, Weintraub D, Okoye FI, Jaffee EM. Cyclophosphamide, dox-orubicin, and paclitaxel enhance the antitumor immune response of granulocyte/macrophage-colony stimulating factor-secreting whole-cell vaccines in HER-2/neu tolerized mice. Cancer Res. 2001; 61(9):3689–97. [PubMed: 11325840]
34. Brusic A, Hainz U, Wadleigh M, Neuberg D, Su M, Canning CM, Deangelo DJ, Stone RM, Lee JS, Mulligan RC, Ritz J, Dranoff G, Sasada T, Wu CJ. Detecting T-cell reactivity to whole cell

vaccines: Proof of concept analysis of T-cell response to K562 cell antigens in CML patients. Oncoimmunology. 2012; 1(7):1095–103. DOI: 10.4161/onci.20954 [PubMed: 23170257]

35. Dillman RO, Beutel LD, Barth NM, de Leon C, O'Connor AA, DePriest C, Nayak SK. Irradiated cells from autologous tumor cell lines as patient-specific vaccine therapy in 125 patients with metastatic cancer: induction of delayed-type hypersensitivity to autologous tumor is associated with improved survival. Cancer Biother Radiopharma. 2002; 17(1):51–66.
36. Xue D, Liang Y, Duan S, He J, Su J, Zhu J, Hu N, Liu J, Zhao Y, Lu X. Enhanced anti-tumor immunity against breast cancer induced by whole tumor cell vaccines genetically modified expressing alpha-Gal epitopes. Oncol Rep. 2016; 36(5):2843–51. DOI: 10.3892/or.2016.5128 [PubMed: 27666541]
37. Yang B, Jeang J, Yang A, Wu TC, Hung CF. DNA vaccine for cancer immunotherapy. Hum Vaccin Immunother. 2014; 10(11):3153–64. DOI: 10.4161/21645515.2014.980686 [PubMed: 25625927]
38. Saade F, Petrovsky N. Technologies for enhanced efficacy of DNA vaccines. Expert Rev Vaccines. 2012; 11(2):189–209. DOI: 10.1586/erv.11.188 [PubMed: 22309668]
39. Li L, Herndon JM, Truscott SM, Hansen TH, Fleming TP, Goedegebuure P, Gillanders WE. Engineering superior DNA vaccines: MHC class I single chain trimers bypass antigen processing and enhance the immune response to low affinity antigens. Vaccine. 2010; 28(8):1911–8. DOI: 10.1016/j.vaccine.2009.10.096 [PubMed: 20188246]
40. Norell H, Poschke I, Charo J, Wei WZ, Erskine C, Piechocki MP, Knutson KL, Bergh J, Lidbrink E, Kiessling R. Vaccination with a plasmid DNA encoding HER-2/neu together with low doses of GM-CSF and IL-2 in patients with metastatic breast carcinoma: a pilot clinical trial. J Transl Med. 2010; 8:53.doi: 10.1186/1479-5876-8-53 [PubMed: 20529245]
41. Tiriveedhi V, Fleming TP, Goedegebuure PS, Naughton M, Ma C, Lockhart C, Gao F, Gillanders WE, Mohanakumar T. Mammaglobin-A cDNA vaccination of breast cancer patients induces antigen-specific cytotoxic CD4 + ICOShi T cells. Breast Cancer Res Treat. 2013; 138(1):109–18. DOI: 10.1007/s10549-012-2110-9 [PubMed: 22678162]
42. Kang TH, Mao CP, La V, Chen A, Hung CF, Wu TC. Innovative DNA vaccine to break immune tolerance against tumor self-antigen. Hum Gene Ther. 2013; 24(2):181–8. DOI: 10.1089/hum.2012.141 [PubMed: 23268564]
43. Palucka K, Banchereau J. Cancer immunotherapy via dendritic cells. Nat Rev Cancer. 2012; 12(4):265–77. DOI: 10.1038/nrc3258 [PubMed: 22437871]
44•. Datta J, Terhune JH, Lowenfeld L, Cintolo JA, Xu S, Roses RE, Czerniecki BJ. Optimizing dendritic cell-based approaches for cancer immunotherapy. Yale J Biol Med 87. 2014; 87(4):491–18. An important immunotherapeutic focus is on tumor associated antigens, and this paper carefully outlines the importance of dendritic cells for presentation of of tumor associated antigens and their use in immuotherapy.
45. Koski GK, Koldovsky U, Xu S, Mick R, Sharma A, Fitzpatrick E, Weinstein S, Nisenbaum H, Levine BL, Fox K, Zhang P, Czerniecki BJ. A novel dendritic cell-based immunization approach for the induction of durable Th1-polarized anti-HER-2/ neu responses in women with early breast cancer. J Immunother. 2012; 35(1):54–65. DOI: 10.1097/CJI.0b013e318235f512 [PubMed: 22130160]
46. Sharma A, Koldovsky U, Xu S, Mick R, Roses R, Fitzpatrick E, Weinstein S, Nisenbaum H, Levine BL, Fox K, Zhang P, Koski G, Czerniecki BJ. HER-2 pulsed dendritic cell vaccine can eliminate HER-2 expression and impact ductal carcinoma in situ. Cancer. 2012; 118(17):4354–62. DOI: 10.1002/cncr.26734 [PubMed: 22252842]
47. Lowenfeld L, Mick R, Datta J, Xu S, Fitzpatrick E, Fisher CS, Fox KR, DeMichele A, Zhang PJ, Weinstein SP, Roses RE, Czerniecki BJ. Dendritic Cell Vaccination Enhances Immune Responses and Induces Regression of HER2pos DCIS Independent of Route: Results of Randomized Selection Design Trial. Clin Cancer Res. 2016; doi: 10.1158/1078-0432.CCR-16-1924
48. De La Cruz LM, Nocera NF, Czerniecki BJ. Restoring anti-on-codriver Th1 responses with dendritic cell vaccines in HER2/neu-positive breast cancer: progress and potential. Immunotherapy. 2016; 8(10):1219–32. DOI: 10.2217/imt-2016-0052 [PubMed: 27605070]
49. Nishimura H, Nose M, Hiai H, Minato N, Honjo T. Development of lupus-like autoimmune diseases by disruption of the PD-1 gene encoding an ITIM motif-carrying immunoreceptor. Immunity. 1999; 11(2):141–51. [PubMed: 10485649]

50. Stephen TL, Payne KK, Chaurio RA, Allegrezza MJ, Zhu H, Perez-Sanz J, Perales-Puchalt A, Nguyen JM, Vara-Ailor AE, Eruslanov EB, Borowsky ME, Zhang R, Laufer TM, Conejo-Garcia JR. SATB1 Expression Governs Epigenetic Repression of PD-1 in Tumor-Reactive T Cells. Immunity. 2017; 46(1):51–64. DOI: 10.1016/j.immuni.2016.12.015 [PubMed: 28099864]
51. Spranger S, Bao R, Gajewski T. Melanoma-intrinsic b-catenin signaling prevents T cell infiltration and anti-tumor immunity. J immunother cancer. 2014; 2(3):O15.
52. Nanda R, Chow LQ, Dees EC, Berger R, Gupta S, Geva R, Pusztai L, Pathiraja K, Aktan G, Cheng JD, Karantza V, Buisseret L. Pembrolizumab in Patients With Advanced Triple-Negative Breast Cancer: Phase Ib KEYNOTE-012 Study. J Clin Oncol. 2016; 34(21):2460–7. DOI: 10.1200/JCO.2015.64.8931 [PubMed: 27138582]
53. Emens LA, Braiteh FS, Cassier P, Delord J-P, Eder JP, Fasso M, Xiao Y, Wang Y, Molinero L, Chen DS. Inhibition of PD-L1 by MPDL3280A leads to clinical activity in patients with metastatic triple-negative breast cancer (TNBC). 2015 2859.
54. Adams S, Diamond J, Hamilton E, Pohlmann P, Tolaney S, Molinero L. Phase Ib trial of atezolizumab in combination with nab-paclitaxel in patients with metastatic triple-negative breast cancer (mTNBC). J Clin Oncol. 2016; 34(suppl) abstr: 1009.
55. Rugo H, Delord J, Im S, Ott P, Piha-Paul S, Bedard P, Sachdev J, Le Tourneau C, Van Brummelen E, Varga A. Abstract S5-07: Preliminary efficacy and safety of pembrolizumab (MK-3475) in patients with PD-L1–positive, estrogen receptor-positive (ER ?)/ HER2-negative advanced breast cancer enrolled in KEYNOTE-028. AACR. 2016
56. Sharma P, Allison JP. Immune checkpoint targeting in cancer therapy: toward combination strategies with curative potential. Cell. 2015; 161(2):205–14. [PubMed: 25860605]
57. Voutsadakis IA. Immune Blockade Inhibition in Breast Cancer. Anticancer Res. 2016; 36(11): 5607–22. DOI: 10.21873/anticanres.11145 [PubMed: 27793883]
58. Guerriero JL, Sotayo A, Ponichtera HE, Castrillon JA, Pourzia AL, Schad S, Johnson SF, Carrasco RD, Lazo S, Bronson RT, Davis SP, Lobera M, Nolan MA, Letai A. Class IIa HDAC inhibition reduces breast tumours and metastases through anti-tumour macrophages. Nature. 2017; 543(7645):428–32. DOI: 10.1038/nature21409 [PubMed: 28273064]
59. Schmittnaegel M, Rigamonti N, Kadioglu E, Cassara A, Wyser Rmili C, Kiialainen A, Kienast Y, Mueller HJ, Ooi CH, Laoui D, De Palma M. Dual angiopoietin-2 and VEGFA inhibition elicits antitumor immunity that is enhanced by PD-1 checkpoint blockade. Sci Transl Med. 2017; doi: 10.1126/scitranslmed.aak9670
60. Schalper KA, Velcheti V, Carvajal D, Wimberly H, Brown J, Pusztai L, Rimm DL. In situ tumor PD-L1 mRNA expression is associated with increased TILs and better outcome in breast carcinomas. Clin Cancer Res. 2014; 20(10):2773–82. [PubMed: 24647569]
61. Cimino-Mathews A, Thompson E, Taube JM, Ye X, Lu Y, Meeker A, Xu H, Sharma R, Lecksell K, Cornish TC, Cuka N, Argani P, Emens LA. PD-L1 (B7-H1) expression and the immune tumor microenvironment in primary and metastatic breast carcinomas. Hum Pathol. 2016; 47(1):52–63. DOI: 10.1016/j.humpath.2015.09.003 [PubMed: 26527522]
62. Ghebeh H, Mohammed S, Al-Omair A, Qattan A, Lehe C, Al-Qudaihi G, Elkum N, Alshabanah M, Bin Amer S, Tulbah A, Ajarim D, Al-Tweigeri T, Dermime S. The B7-H1 (PD-L1) T lymphocyte-inhibitory molecule is expressed in breast cancer patients with infiltrating ductal carcinoma: correlation with important high-risk prognostic factors. Neoplasia. 2006; 8(3):190–8. DOI: 10.1593/neo.05733 [PubMed: 16611412]
63. Sun WY, Lee YK, Koo JS. Expression of PD-L1 in triple-negative breast cancer based on different immunohistochemical antibodies. J Transl Med. 2016; 14(1):173.doi: 10.1186/s12967-016-0925-6 [PubMed: 27286842]
64. Brignone C, Gutierrez M, Mefti F, Brain E, Jarcau R, Cvitkovic F, Bousetta N, Medioni J, Gligorov J, Grygar C, Marcu M, Triebel F. First-line chemoimmunotherapy in metastatic breast carcinoma: combination of paclitaxel and IMP321 (LAG-3Ig) enhances immune responses and antitumor activity. J Transl Med. 2010; 8:71.doi: 10.1186/1479-5876-8-71 [PubMed: 20653948]
65. Wong HH, Lemoine NR, Wang Y. Oncolytic Viruses for Cancer Therapy: Overcoming the Obstacles. Viruses. 2010; 2(1):78–106. DOI: 10.3390/v2010078 [PubMed: 20543907]

66. Fukuhara H, Ino Y, Todo T. Oncolytic virus therapy: A new era of cancer treatment at dawn. Cancer Sci. 2016; 107(10):1373–9. DOI: 10.1111/cas.13027 [PubMed: 27486853]

67. Conner J, Braidwood L. Expression of inhibitor of growth 4 by HSV1716 improves oncolytic potency and enhances efficacy. Cancer Gene Ther. 2012; 19(7):499–507. DOI: 10.1038/cgt.2012.24 [PubMed: 22595793]

68. Seth P, Wang Z-G, Pister A, Zafar MB, Kim S, Guise T, Wakefield L. Development of oncolytic adenovirus armed with a fusion of soluble transforming growth factor-b receptor II and human immunoglobulin Fc for breast cancer therapy. Hum Gene Ther. 2006; 17(11):1152–61. [PubMed: 17032151]

69. Posey AD, Jr, Schwab, RD., Boesteanu, AC., Steentoft, C., Mandel, U., Engels, B., Stone, JD., Madsen, TD., Schreiber, K., Haines, KM., Cogdill, AP., Chen, TJ., Song, D., Scholler, J., Kranz, DM., Feldman, MD., Young, R., Keith, B., Schreiber, H., Clausen, H., Johnson, LA., June, CH. Engineered CAR T Cells Targeting the Cancer-Associated Tn-Glycoform of the Membrane Mucin MUC1 Control Adenocarcinoma. Immunity. 2016; 44(6):1444–54. DOI: 10.1016/j.immuni.2016.05.014 [PubMed: 27332733]

70. Whilding LM, Parente-Pereira AC, Zabinski T, Davies DM, Petrovic RMG, Kao YV, Saxena SA, Romain A, Costa-Guerra JA, Violette S, Itamochi H, Ghaem-Maghami S, Vallath S, Marshall JF, Maher J. Targeting of Aberrant alphavbeta6 Integrin Expression in Solid Tumors Using Chimeric Antigen Receptor-Engineered T Cells. Mol Ther. 2017; 25(1):259–73. DOI: 10.1016/j.ymthe.2016.10.012 [PubMed: 28129120]

71. Vogel CL, Cobleigh MA, Tripathy D, Gutheil JC, Harris LN, Fehrenbacher L, Slamon DJ, Murphy M, Novotny WF, Burch-more M, Shak S, Stewart SJ, Press M. Efficacy and safety of trastuzumab as a single agent in first-line treatment of HER2-overexpressing metastatic breast cancer. J Clin Oncol. 2002; 20(3):719–26. DOI: 10.1200/JCO.2002.20.3.719.123 [PubMed: 11821453]

72. Spector NL, Blackwell KL. Understanding the mechanisms behind trastuzumab therapy for human epidermal growth factor receptor 2-positive breast cancer. J Clin Oncol. 2009; 27(34):5838–47. DOI: 10.1200/JCO.2009.22.1507 [PubMed: 19884552]

73. Hudis CA. Trastuzumab–mechanism of action and use in clinical practice. N Engl J Med. 2007; 357(1):39–51. DOI: 10.1056/NEJMra043186 [PubMed: 17611206]

74. Nahta R, Hung MC, Esteva FJ. The HER-2-targeting antibodies trastuzumab and pertuzumab synergistically inhibit the survival of breast cancer cells. Cancer Res. 2004; 64(7):2343–6. [PubMed: 15059883]

75. Gianni L, Pienkowski T, Im YH, Roman L, Tseng LM, Liu MC, Lluch A, Staroslawska E, de la Haba-Rodriguez J, Im SA, Pedrini JL, Poirier B, Morandi P, Semiglazov V, Srimuninnimit V, Bianchi G, Szado T, Ratnayake J, Ross G, Valagussa P. Efficacy and safety of neoadjuvant pertuzumab and trastuzumab in women with locally advanced, inflammatory, or early HER2-positive breast cancer (NeoSphere): a randomised multicentre, open-label, phase 2 trial. Lancet Oncol. 2012; 13(1):25–32. DOI: 10.1016/S1470-2045(11)70336-9 [PubMed: 22153890]

76. Bang YJ, Giaccone G, Im SA, Oh DY, Bauer TM, Nordstrom JL, Li H, Chichili GR, Moore PA, Hong S, Stewart SJ, Baughman JE, Lechleider RJ, Burris HA. First-in-human phase 1 study of margetuximab (MGAH22), an Fc-modified chimeric monoclonal antibody, in patients with HER2-positive advanced solid tumors. Ann Oncol. 2017; 28(4):855–61. DOI: 10.1093/annonc/mdx002 [PubMed: 28119295]

77. Dasgupta S, Kumar V. Type II NKT cells: a distinct CD1d-restricted immune regulatory NKT cell subset. Immunogenetics. 2016; 68(8):665–76. DOI: 10.1007/s00251-016-0930-1 [PubMed: 27405300]

78. Rutkowski MR, Stephen TL, Svoronos N, Allegrezza MJ, Tesone AJ, Perales-Puchalt A, Brencicova E, Escovar-Fadul X, Nguyen JM, Cadungog MG, Zhang R, Salatino M, Tchou J, Rabinovich GA, Conejo-Garcia JR. Microbially driven TLR5-dependent signaling governs distal malignant progression through tumor-promoting inflammation. Cancer Cell. 2015; 27(1):27–40. DOI: 10.1016/j.ccell.2014.11.009 [PubMed: 25533336]

79. Payne KK. Lymphocyte-mediated Immune Regulation in Health and Disease: The Treg and gammadelta T Cell Co-conspiracy. Immunol Invest. 2016; 45(8):767–75. DOI: 10.1080/08820139.2016.1213278 [PubMed: 27617588]

80. Gobert M, Treilleux I, Bendriss-Vermare N, Bachelot T, Goddard-Leon S, Arfi V, Biota C, Doffin AC, Durand I, Olive D, Perez S, Pasqual N, Faure C, Ray-Coquard I, Puisieux A, Caux C, Blay JY, Menetrier-Caux C. Regulatory T cells recruited through CCL22/CCR4 are selectively activated in lymphoid infiltrates surrounding primary breast tumors and lead to an adverse clinical outcome. Cancer Res. 2009; 69(5):2000–9. DOI: 10.1158/0008-5472.CAN-08-2360 [PubMed: 19244125]

81. Yan M, Jene N, Byrne D, Millar EK, O'Toole SA, McNeil CM, Bates GJ, Harris AL, Banham AH, Sutherland RL, Fox SB. Recruitment of regulatory T cells is correlated with hypoxia-induced CXCR4 expression, and is associated with poor prognosis in basal-like breast cancers. Breast Cancer Res. 2011; 13(2):R47.doi: 10.1186/bcr2869 [PubMed: 21521526]

82. Mougiakakos D, Choudhury A, Lladser A, Kiessling R, Johansson CC. Regulatory T cells in cancer. Adv Cancer Res. 2010; 107:57–117. DOI: 10.1016/S0065-230X(10)07003-X [PubMed: 20399961]

83. Schmidt A, Oberle N, Krammer PH. Molecular mechanisms of treg-mediated T cell suppression. Front Immunol. 2012; 3:51.doi: 10.3389/fimmu.2012.00051 [PubMed: 22566933]

84. Fisher SA, Aston WJ, Chee J, Khong A, Cleaver AL, Solin JN, Ma S, Lesterhuis WJ, Dick I, Holt RA, Creaney J, Boon L, Robinson B, Lake RA. Transient Treg depletion enhances therapeutic anti-cancer vaccination. Immun Inflamm Dis. 2017; 5(1):16–28. DOI: 10.1002/iid3.136 [PubMed: 28250921]

85. Rech AJ, Mick R, Martin S, Recio A, Aqui NA, Powell DJ Jr, Colligon TA, Trosko JA, Leinbach LI, Pletcher CH, Tweed CK, DeMichele A, Fox KR, Domchek SM, Riley JL, Vonderheide RH. CD25 blockade depletes and selectively reprograms regulatory T cells in concert with immunotherapy in cancer patients. Sci Transl Med. 2012; 4(134):134ra162.doi: 10.1126/scitranslmed.3003330

86. Rech AJ, Vonderheide RH. Clinical use of anti-CD25 antibody daclizumab to enhance immune responses to tumor antigen vaccination by targeting regulatory T cells. Ann N Y Acad Sci. 2009; 1174:99–106. DOI: 10.1111/j.1749-6632.2009.04939.x [PubMed: 19769742]

87. Franklin RA, Liao W, Sarkar A, Kim MV, Bivona MR, Liu K, Pamer EG, Li MO. The cellular and molecular origin of tumor-associated macrophages. Science. 2014; 344(6186):921–5. DOI: 10.1126/science.1252510 [PubMed: 24812208]

88•. Mantovani A, Marchesi F, Malesci A, Laghi L, Allavena P. Tumour-associated macrophages as treatment targets in oncology. Nat Rev Clin Oncol. 2016; Macrophages have become a recent focus in understanding the tumor microenvironment, and this paper discusses the dual proand anti-inflammatory behavior exhibited by macrophages and the ultimate impact on tumor progression and response to therapy. doi: 10.1038/nrclinonc.2016.217

89. Martinez FO, Gordon S. The M1 and M2 paradigm of macrophage activation: time for reassessment. F1000Prime Rep. 2014; 6:13.doi: 10.12703/P6-13 [PubMed: 24669294]

90. Biswas SK, Mantovani A. Macrophage plasticity and interaction with lymphocyte subsets: cancer as a paradigm. Nat Immunol. 2010; 11(10):889–96. DOI: 10.1038/ni.1937 [PubMed: 20856220]

91. Mantovani A, Sozzani S, Locati M, Allavena P, Sica A. Macrophage polarization: tumor-associated macrophages as a paradigm for polarized M2 mononuclear phagocytes. Trends Immunol. 2002; 23(11):549–55. [PubMed: 12401408]

92. Bronte V, Brandau S, Chen SH, Colombo MP, Frey AB, Greten TF, Mandruzzato S, Murray PJ, Ochoa A, Ostrand-Rosenberg S, Rodriguez PC, Sica A, Umansky V, Vonderheide RH, Gabrilovich DI. Recommendations for myeloid-derived suppressor cell nomenclature and characterization standards. Nat Commun. 2016; 7:12150.doi: 10.1038/ncomms12150 [PubMed: 27381735]

93. De Palma M, Lewis CE. Macrophage regulation of tumor responses to anticancer therapies. Cancer Cell. 2013; 23(3):277–86. DOI: 10.1016/j.ccr.2013.02.013 [PubMed: 23518347]

94. DeNardo DG, Brennan DJ, Rexhepaj E, Ruffell B, Shiao SL, Madden SF, Gallagher WM, Wadhwani N, Keil SD, Junaid SA, Rugo HS, Hwang ES, Jirstrom K, West BL, Coussens LM. Leukocyte complexity predicts breast cancer survival and functionally regulates response to chemotherapy. Cancer Discov. 2011; 1(1):54–67. DOI: 10.1158/2159-8274.CD-10-0028 [PubMed: 22039576]

Table 1

Immune checkpoint receptors important in human cancers

Immune checkpoint receptor	Ligand(s)	Reported in breast cancer	FDA-approved immune checkpoint inhibitors
B- and T-lymphocyte attenuator (BTLA)	Herpes virus entry mediator (HVEM)		
Cytotoxic T-lymphocyte–associated antigen-4 (CTLA-4)	CD80, CD86	Yes	*Ipilimumab* (melanoma)
Killer immunoglobulin receptor (KIR)	HLA-C		
Lymphocyte activation gene-3 (LAG3)	MHC-II, LSECtin	Yes	
Programmed death-1 (PD-1)	PD-L1, PD-L2	Yes	*Pembrolizumab* (melanoma, non-small cell lung cancer (NSCLC), head and neck cancer)
			Nivolumab (melanoma, NSCLC, renal cancer, Hodgkin's lymphoma, head and neck cancer)
			Atezolizumab (bladder cancer, NSCLC)
T cell immunoglobulin and ITM domain (TIGIT)	CD155, CD112		
T cell immunoglobulin-3 (TIM3)	GAL-9, HMGB-1, Ceacam-1, PtdSer	Yes	