Clinics in Surgery

Review Article
Published: 25 Apr, 2017

Breast Cancer Immunotherapy: An Update

*Mohammad Atiq[1], Ahmed Alwbari[1], Thomas Kieber-Emmons[3] and Issam Makhoul[1]**

[1]Department of Internal Medicine, University of Arkansas for Medical Sciences, Little Rock, USA

[2]Department of Pathology, University of Arkansas for Medical Sciences, Little Rock, USA

Abstract

The immune system plays a major role in cancer surveillance. Harnessing its power to treat many cancers is now a reality that has led to cures in hopeless situations where no other solutions were available from traditional anticancer drugs. These spectacular achievements rekindled the oncology community's interest in extending the benefits to all cancers including breast cancer. The first section of this article reviews the biological foundations of the immune response to different subtypes of breast cancer and the ways cancer may overcome the immune attack leading to cancer-disease. The second section is dedicated to the actual immune treatments including breast cancer vaccines, checkpoint inhibitors, monoclonal antibodies and the "unconventional" immune role of chemotherapy.

Introduction

Breast cancer is the most common cancer in females with an estimated 249, 260 new cases in the United States in 2016 [1]. It is also the second leading cause of cancer death in women. Fortunately, with advances in detection and treatment, death rates from breast cancer are declining. More recent advancements in breast cancer therapy utilizing novel mechanisms involving actionable cancer mutations and the body's immune system have opened up new avenues for reducing the death rate further. Many of the obvious successes in immunotherapy have been in the field of melanoma, renal cancer, lung cancer and others that have traditionally been known to be immunogenic. However, these are not the only cancers in which strides in immunotherapy are being made. Breast cancer is one cancer that, while not originally thought to be immunogenic, has had many encouraging results in the past few years. We aim to provide a succinct overview of breast cancer immunotherapy as well as possible future directions.

The basis for immunotherapy in cancer has revolved around the concept of immunogenicity. For a long time, breast cancer has been considered non-immunogenic. However, the role of the immune system in the emergence of breast cancer has been firmly established [2,8]. Random or inherited genetic and epigenetic abnormalities confer proliferative and/or survival advantages on certain cells. These incipient cancer cells face internal and external control mechanisms including those from the immune system. By targeting the new antigens created by these genetic changes, the immune system plays a central role in cancer control that can be host-protective or tumor-promoting. A mutated gene leads to the production of a neo-antigen when it is transcribed then translated, highlighting the auto-antigenicity of self antigens as observed in model protein antigens [9].

Epitopes from the neo-antigen are presented after processing by the mammary epithelial cells in association with MHC class I (MHC-I) on their surface. When an Antigen-Presenting Cell (APC) encounters a neo-antigen released from debris of cancer cells or secreted in the environment, it internalizes it via several mechanisms including endocytosis. The antigen resurfaces again after processing on the MHC class II (MHC-II) receptors and can be recognized by T- Helper Cell Receptors (TCR). T-Helpers (Th) stimulate and drive cytotoxic T-Cells (Tc) and B-cells to further maturation. Tc maturation, proliferation, and survival require co-stimulatory signals from APCs that are antigen independent. If the co-stimulatory signal is lacking then the process of activation will be ineffective and may lead to Tc anergy. Once activated, Tc can attack the target cell by several mechanisms, including TCR-MHC-I recognition and binding. This leads to secretion of cytotoxic granules including perforin that result in cell lyses and demise [10]. Another mechanism by which Tc can attack target cells is via FAS receptors on Tc that bind FASL on the target cell leading to caspase 3 and 8 activation in the target cell and eventually apoptosis [11]. To ensure effective immune regulation, the very same APC that sends a co-stimulatory signal (B7 family receptors on APC bind the CD28 surface protein on T-cells) to intensify the activation of naïve T-cells also sends

OPEN ACCESS

Correspondence:
Issam Makhoul, Department of Internal Medicine, University of Arkansas for Medical Sciences, Little Rock, Arkansas 72205, USA,
E-mail: makhoulissam@uams.edu

Received Date: *16 Jan 2017*
Accepted Date: *18 Apr 2017*
Published Date: *25 Apr 2017*

Citation:
Atiq M, Alwbari A, Kieber-Emmons T, Makhoul I. Breast Cancer Immunotherapy: An Update. Clin Surg. 2017; 2: 1429.

inhibitory signals (B7 receptors bind CLTA4 on T-cell) to the already activated T-cell when the immune response has to wind down. The activated T-cell starts synthesizing CTLA4, which has higher affinity to B7 and competes with the stimulatory B7-CD28 binding [12]. This mechanism prevents overstimulation by transient T-cell activation.

The interaction between the immune system and incipient cancer cells, also called immunoediting, goes through three phases: *elimination*, *equilibrium* and *escape* [13-15]. *Elimination* is supported by a wealth of experimental evidence in animals and humans. The innate and adaptive arms of the immune system recognize incipient cancer cells by the new antigens (resulting from mutations or translocations) presented on their surface in association with MHC-I or by the distress signals usually expressed by transformed cells that have undergone chromosomal changes (aneuploidy or hyperploidy) [16,17] and eliminate them. *Equilibrium* is reached when the immune system fails to eliminate the transformed cells but stops them from progressing further. This can be conceived as the *dormancy phase* of cancer development. This phase is mediated by equilibrium between cells and cytokines that promote elimination (IL-12, IFNγ, TNFα, CD4 Th1, CD8+ T cells, NK cells, γδT cells) and those that promote persistence of the nascent tumor (IL-23, IL-6, IL10, TGFβ, NKT cells, CD4 Th2, Foxp3+ Treg cells, and MDSCs) [18-20]. Monocytes play an important role in this process. Under the influence of the tumor microenvironment they may differentiate into pro-inflammatory M1 or anti-inflammatory M2 types [21,22]. Immune *escape* of cancer cells occurs by different mechanisms. In HR positive breast cancer, the absence of strong tumor antigens and low expression of MHC-I allow the tumor to progress unnoticed by the immune system [23]. Estrogen plays an immunosuppressive role in the tumor microenvironment that promotes tolerance of the weakly immunogenic cancer. Most immune cells including macrophages, T- and B-lymphocytes and NK cells express ER [24]. In presence of estrogen, the immune response is polarized to Th2- rather than Th1-effector immune response [25]. In HER2 cancer cells, MHC-I presentation is inversely correlated with HER2 expression [26]. Triple Negative Breast Cancers (TNBC) exhibit a spectrum of MHC-I presentation and strong tumor antigen expression but immune escape in this subtype is mostly related to the development of the immunosuppressive tumor microenvironment (Tregs, MDSCs, PD1/PD-L1).

However, it is still unclear how the balance established during the equilibrium phase gets tilted towards tumor progression. The answer to this question is very likely multifactorial. Aging is associated with reduced production of new B and T lymphocytes in the bone marrow and the thymus, respectively and with decreased function of the existing mature lymphocytes [27]. Systemic inflammation associated with aging and the local pro-inflammatory microenvironment in the breast are incriminated in promoting the cancerous transformation of mammary stem cells that have been primed by losing tumor suppressor genes [28,29]. Pro-inflammatory cytokines (TNFα and IL-6) are associated with overexpression of COX2 and the aromatase enzyme [30], which lead to increased local concentrations of estrogens. Estrogens induce the expansion of Tregs and MDSCs, as well as the inhibition of antigen presenting cells [31-34]. In addition to the gradual decline of the immune system, dietary, commensal microbiota, use of antibiotics, procreational and hormonal factors, all play some role of variable importance in tilting the balance from equilibrium to escape [35-38].

Assessment of Breast Cancer Immunogenicity

Traditional pathology and immunohistochemistry, gene expression profiling, RNA sequencing and combined scores have been used to assess the immunogenicity of breast cancer. Traditional pathology tools allow the assessment of breast cancer immunogenicity by studying the presence of tumor-Infiltrating Lymphocytes (TILs) and assessing their types and correlation with survival and recurrence. While tumor-Infiltrating Lymphocytes (TILs) were not found to have a prognostic value in the overall breast cancer population or estrogen receptor positive/human epidermal growth factor receptor 2 negative (ER+/HER2-) patients, TILs were found to have a prognostic value for Disease-Free Survival (DFS) and Overall Survival (OS) in TNBC [39]. In patients with TNBC who had residual disease after neoadjuvant chemotherapy, the presence of TILs was found to be associated with better OS as well as with metastasis-free survival [40]. In ER negative breast cancers, TILs, specifically CD8+ lymphocytes, were associated with better breast cancer specific survival [39,41]. The presence of CD8+ lymphocytes in patients with ER negative breast cancers was also related to longer DFS [41]. In general, the presence of TILs was positively correlated with MHC-I expression and inversely correlated with ER expression. The more immunogenic the breast cancer, the higher the concentration of TILs will be. Hence, it is not surprising that HR positive breast cancer is considered the least immunogenic.

Recent advances in genomics and proteomics allow the detection of neo-antigens that underlie immunogenicity in breast cancer and shed light on possible targets for therapy [42,43]. Immunogenicity of a tumor is evaluated by the assessment of its antigenicity and the latter is evaluated by assessing its mutagenicity. Mutational load, the average number of somatic mutations per cancer cell, is associated with antigenicity and is, in general, lower in breast cancer compared with other tumors such as melanoma or lung cancer. However, major differences exist between different subtypes of breast cancer; TNBC has the highest mutational load compared with HR positive breast cancers [44,45] and high mutational load is associated with better prognosis in TNBC and HER2+ compared with low mutational load in the same types of breast cancer (see below). Conversely, higher mutational load is associated with higher concentrations of TILs and with poor prognosis in HR positive breast cancer. Mutational load continues increasing in metastatic breast cancer but TILs, PD-1 and PDL-1 expression decreases, very likely as a result of immune exhaustion and not because of decreased immunogenicity in advanced disease as suggested by Luen "et al." [46]. Some specific mutations in DNA repair mechanisms such as those in the BRCA1/2 and MMR genes are associated with high mutational loads that can be localized (kataegis) or generalized [47,48]. High mutational load is associated with high rates of neo-antigens, which predict overall survival and response to check point inhibitors [42,43,49-51].

In assessing response to neoadjuvant treatment, the benefit of the presence of TILs can be seen here as well. Breast cancers with higher levels of TILs have better responses to neoadjuvant chemotherapy [7]. In patients with HER2+ or TNBC, those with >60% TILs treated with an anthracycline plus taxane combination were more likely to have a pathologic complete response and the rates of pathologic complete response were even higher when carboplatin was added to the treatment regimen [8]. ER negative breast cancers that are lymphocyte-rich have far greater pathologic complete response rates when treated with neoadjuvant anthracycline-based chemotherapy

compared to patients with lymphocyte-poor ER- breast cancers [52]. HER2+ breast cancers with TILs were associated with better disease-free survival as well as overall survival in response to treatment with anthracyclines [2]. There was a significantly associated decreasing risk of distant recurrence in patients being treated with adjuvant chemotherapy simultaneously with trastuzumab in HER2+ breast cancer for every 10% increase in TILs [3]. Moreover, irrespective of whether or not a patient received systemic adjuvant chemotherapy, TILs and immune signatures were associated with better prognosis in HER2+ breast cancer [53]. In patients with HER2 overexpression, a higher CD8+ infiltrate was seen after chemotherapy and this was associated with improved relapse-free survival [54].

Strategies to Harness the Power of the Immune System

Several strategies have been used to harness the power of the immune system and redirect it to eradicate breast cancer or to induce immune dormancy.

1. Breast cancer vaccines
2. Monoclonal antibodies
3. Checkpoint inhibitors
4. Enhance the immune-mediated effect of chemotherapy

Breast Cancer Vaccines

Breast cancer vaccines are used for primary or secondary prevention and some are therapeutic. Several strategies have been used including peptide vaccines, recombinant protein vaccines, dendritic cell vaccines, whole tumor cell vaccines, DNA vaccines, and recombinant viral vectors vaccines.

They are all designed to stimulate an intrinsic antitumor response targeting Tumor-Associated Antigens (TAAs). TAAs that are specifically recognized by T cells include HER2, mucin 1 (MUC-1), carcinoembryonic antigen (CEA), sialyl-Tn (STn), human telomerase reverse transcriptase (hTERT), Wilms' Tumor gene (WT1) and Tumor Associated Carbohydrate Antigens (TACAs) [55]. The antigens where current studies are primarily focused around include HER2, MUC-1, and TACAs.

As for the use of HER2 in vaccine developments, there have been a few attempts involving the E75, GP2, and AE37 peptides. Nelipepimut-S (Neu-Vax) is a combination of E75, a peptide from the extracellular domain of HER2 and GM-CSF; it stimulates cytotoxic T lymphocytes and CD8+ memory cells with high affinity for HLA-A2/A3. However, the immunity induced by the E75 vaccine waned after six months from initial vaccination requiring a booster given at six months from completion of the primary vaccination [56]. NeuVax was tested in a phase I/II trial and showed improvement of disease-free survival in HER2 positive breast cancer patients [57]. The study enrolled 187 early-stage breast cancer patients deemed at high risk for recurrence. Patients received six injections of NeuVax after tumor resection with standard of care (chemo or RT) as indicated. The 5-year DFS was 89.7% for the vaccinated group *vs.* 80.2% for the controls (P=0.08). When the optimally dosed cohort was considered, DFS was increased to 94.8% *vs.* 80.2% (P=0.05). Apparently, the induction of cytotoxic T lymphocytes was crucial for the response to NeuVax as only 1 recurrence was observed in 30 patients (3%) who achieved cytotoxic T lymphocytes above the mean, compared with 8 of 56 (14%) for patients with levels of cytotoxic T lymphocytes below the mean [58]. A phase III registration PRESENT trial is evaluating E75 in 758 early-stage, node-positive HLA-A2/A3 patients with low to intermediate HER2 expression with no evidence of disease after standard treatment. Patients are randomized to GM-CSF with E75 or GM-CSF with placebo, receiving six monthly injections, followed by a booster vaccination every 6 months for 3 years. The primary endpoint is disease-free survival at 3 years [59].

Work with the GP2 peptide is currently ongoing in a phase II clinical trial where vaccines containing GP2, a class I epitope derived from the HER2 transmembrane domain, is combined with GM-CSF and then compared to treatment of patients with GM-CSF only. Interim analysis presented in 2009 was already showing a decreased recurrence rate at 17.9 months in a group of patients treated with GP2 and GM-CSF (VG) versus GM-CSF alone (CG), 7.4% (2/27) compared to 13% (3/23), respectively (p=0.65) [60]. At 34 (1-60) month median follow-up, DFS was compared in the intent to treat (ITT) (85% VG v 81% CG, p=0.57) and per-treatment (PT) (94% VG v 85% CG, p=0.17) populations. In patients with HER2 overexpression (51 VG and 50 CG) DFS was 94% VG v 89% CG, p=0.86 (ITT) and 100% VG v 89% CG, p = 0.08 (PT) [61].

The premise behind the AE37 vaccine is that it stimulates a CD4+ T lymphocyte response that could potentially result in a more sustained immune response. The current data from clinical trials does suggest that this vaccine has an effect on the risk of recurrence [62]. The trial enrolled 298 patients; 153 received AE37+GM-CSF and 145 received GM-CSF alone. At the time of the primary analysis, the recurrence rate in the vaccinated group was 12.4% versus 13.8% in the control group [relative risk reduction 12%, HR 0.885, 95% Confidence Interval (CI) 0.472–1.659, P=0.70]. The Kaplan–Meier estimated 5-year DFS rate was 80.8% in vaccinated versus 79.5% in control patients. In planned subset analyses of patients with IHC 1+/2+ HER2-expressing tumors, 5-year DFS was 77.2% in vaccinated patients (n=76) versus 65.7% in control patients (n=78) (P=0.21). In patients with triple-negative breast cancer (HER2 IHC 1+/2+ and hormone receptor negative) DFS was 77.7% in vaccinated patients (n=25) versus 49.0% in control patients (n=25) (P=0.12) [63]. Although the trial was negative for the whole population, the results in the triple negative subset of patients were encouraging and warrant further investigation.

The presence of high levels of antibodies to specific glycoforms of the MUC-1 antigen has been shown to be associated with reduced rates and delay to metastasis in patients who have early stage breast cancer [64]. One of these particular glycoforms, STnMUC1, has already been used in a phase III trial in the form of the vaccine Theratope (STnMUC1, keyhole limpet hemocyanin, and the adjuvant Detox B). Given as a single agent, Theratope did not show any improvement in survival. However, when given along with endocrine therapy, there was a demonstrated improvement in time to progression and overall survival [65]. The reactivity of antibodies to MUC1 glycoforms might still be deceptive and can be related to an artifact rather than a true immune response to MUC1. The example of anti-Gal alpha (1,3) Gal antibodies is instructive. These antibodies are observed to react with mucin 1 (MUC1) found on the surface of human breast cancer cells [66]. Natural occurring anti-Gal alpha (1,3) Gal antibodies found in all human serum can react with self peptides (MUC1) expressed in large amounts on the surface of tumor cells, but not on normal cells. These findings are of interest and serve to explain reported findings that human cells can, at times, express Gal alpha (1,3) Gal; in reality,

such expression is suggested as an artifact in that anti-Gal alpha (1,3) Gal antibodies react with mucin peptides [66]. However, some antibodies display exquisite specificity, like those directed toward the Thomsen-Friedenreich (TF) antigen [67]. TF antibodies may arise in the postpartum period against carbohydrate structures expressed on the cell walls of the gastrointestinal flora and, presumably, may provide an early barrier against TF-carrying tumor cells.

The widely used regimen of neoadjuvant chemotherapy is demonstrated to stimulate the immune response to Tumor Associated Carbohydrate Antigens (TACA) in some patients [68]. Small retrospective studies have suggested that post-chemotherapy lymphocyte infiltrates could be associated with better outcomes in patients who did not reach pathologic complete response [68]. The high levels of anti-TF antibody before surgery is another example in which antibody targeting is associated with a better survival of stage II breast cancer patients [69]. This may indicate that the selection of immunopotentiating regimens of neoadjuvant chemotherapy might be beneficial for the host in conjunction with the functional activity of natural anti-cancer antibodies.

Since tumor tissue rejection is the goal of cancer immunotherapies, broad-spectrum tumor associated antigens, like TACAs, are plausible targets once the problem of their low immunogenicity is solved [70]. The fact that multiple proteins and lipids on the cancer cell are modified with the same carbohydrate structure creates a powerful advantage for TACAs as cancer targets in immunotherapy strategies. Thus, targeting TACAs has the potential to broaden the spectrum of target pathways recognized by the immune response, thereby lowering the risk of developing escape variants due to the loss of a given protein or carbohydrate antigen. While TACAs are poor immunogens, certain investigators succeeded in eliciting cytotoxic antibodies reactive with naturally occurring forms of TACA using molecular mimicry to generate peptide mimotopes of TACA (carbohydrate mimetic peptides - CMPs). Vaccination of mice with TACA peptide mimotopes reduced tumor growth and prolonged host survival in a murine tumor model [71]. The first reports of this strategy in humans are promising and trials exploring their role in different types of breast cancer are underway [72].

Multivalent vaccines comprised of two or more candidate proteins are considered to substantially enhance the efficacy of vaccination against breast tumors. The enhancement in anti-tumor effect by using a multivalent vaccination approach would be achieved on two levels: 1) by increasing the strength of immune response against arising tumor due to activation of a larger T cell repertoire comprised of multiple T cell lineages reactive to more than one tumor specific target; 2) by covering a broader range of tumors, including those that do not express the target protein by a univalent vaccination approach such as HER2 or MUC1. In addition, a multivalent vaccine will have the potential to target tumors that have lost or down-regulated expression of one or more proteins or acquired expression of alternate proteins due to transcriptional dysregulation during their evolution from normal to dysplastic, to carcinoma in situ, to invasive, and to metastatic stages of breast tumor evolution. In other words, a multivalent vaccine approach could apply greater multi-target immunological pressure both on early and evolving tumors. It will thereby cover a larger tumor variety and increase efficacy of prevention as well as provide more effective therapy by lowering the probability of tumor escape and generation of resistance to the vaccine. Such approaches are heading to the clinic.

In contrast to a multivalent approach, a pan-immunogen that elicits responses to several antigens but as a univalent vaccine can achieve the same end as a multivalent vaccine. TACAs are among the most challenging of clinical targets for cancer immunotherapy, but this difficulty can be overcome by CMPs. CMPs are sufficiently potent to activate broad-spectrum anti-tumor reactivity. However, the activation of immune responses against terminal mono- and disaccharide constituents of TACA raises concerns regarding the balance between "tumor destruction" and "tissue damage", as mono- and disaccharides are also expressed on normal tissue. To support the development of CMPs for clinical trial testing, we have demonstrated in preclinical safety assessment studies in mice that vaccination with CMPs can enhance responses to TACAs without mediating tissue damage to normal cells expressing TACA [73] and are pursuing such an approach in multiple Phase II trials. Particularly important is that these CMP-induced antibodies can overcome resistance to anoikis and drug resistance against breast cancer and enhance the efficacy of taxanes. This aspect might suggest that immunization with such CMPs can change the clinical paradigm in the neoadjuvant/adjuvant setting.

Monoclonal Antibodies

Monoclonal antibodies are an integral part of our armamentarium in the fight against cancer. They can be divided into those that target the immune system (check point inhibitors) and those that target oncogenic membrane receptors (HER2) or other surface molecules of unknown function (CD20). Trastuzumab is a standard component of the treatment of HER2-positive breast cancer. Its development in the 1990's was considered a landmark achievement in the field of targeted therapy. When combined with chemotherapy it improves progression free survival and OS in metastatic HER2-positive breast cancer and DFS and OS in early stage HER2-positive breast cancer.

Trastuzumab's mechanism of action remains elusive. It targets HER2 and leads to its internalization and degradation. It inhibits downstream signaling pathways leading to decreased proliferation and increased apoptosis of cancer cells. Recently, its role in activating the immune system against tumor cells emerged as the main mechanism of action. The FinHer investigators found that every 10% increase in TILs was associated with decreased distant recurrence [89] and other studies found that TILs had a prognostic and predictive value as their presence predicted for higher pCR to trastuzumab-containing chemotherapy and better DFS [19,90]. A meta-analysis of neoadjuvant RCTs showed that the pCR rate was significantly higher in patients with lymphocyte predominant breast cancer (LPBC) in HER2-positive BC settings, with an absolute difference of 33.3% (95% CI, 23.6%–42.7%) [91].

The nature of tumor infiltrating immune cells is more important than the mere presence or absence of TILs. Using CIBERSORT (leukocyte gene matrix LM22) to characterize immune cell composition of 7270 unrelated breast cancer samples from their gene expression profiles, Bense "et al." [92] showed that the composition of the immune cell types differed per breast cancer subtype and interacted with the treatment. Increased fraction of regulatory T-cells in HER2–positive tumors was associated with a lower pCR rate (OR=0.15) as well as shorter DFS (HR=3.13) and OS (HR=7.69). Increased fraction of γδT-cells in all breast cancer patients was associated with a higher pCR rate (OR=1.55), prolonged DFS (HR=0.68), and, in HER2-positive tumors, with prolonged OS (HR=0.27). A higher fraction of activated mast cells was associated with worse DFS (HR=5.85) and

OS (HR=5.33) in HER2-positive tumors. Furthermore, a high CD8+ T-cell exhaustion signature score was associated with shortened DFS in patients with ER-positive tumors regardless of HER2 status (HR=1.80) [92].

The implications of these findings are substantial. Sorting out the anti-oncogenic from the immune stimulating roles of trastuzumab may be very difficult. However, the available data from the ALTTO study suggest that interrupting HER2 downstream signaling using lapatinib does not add any benefit in early stage breast cancer [93]. It is not clear whether all TKIs will behave like lapatinib but if this observation is confirmed other TKIs may not add more benefit either. The challenge for future development of novel drugs is to capitalize on the immune mechanism.

Checkpoint Inhibitors

Targeting programmed death-1 and programmed death-ligand 1 (PD-1/PD-L1) in breast cancer appears increasingly appealing after the success of such an approach in other cancers. The PD-1 receptor inhibits innate and adaptive immunity when upregulated on immune cells and engaged by its ligand, PD-L1 [94]. Cancers take advantage of this mechanism to induce a local immunosuppression by overexpressing PD-L1. The prognostic significance of PD-L1 is still unclear, as some studies have described its value as a positive and other as negative prognostic factor [75,76]. Regardless, the concept of inhibiting the PD-1/PD-L1 pathway is based on the idea of "inhibiting the inhibition" of the immune system. The agents being tried in breast cancer draw from those already being used in melanoma and other malignancies including Nivolumab and Pembrolizumab (anti-PD-1 antibodies). Currently, results from a phase I study in heavily pretreated TNBC patients who received Pembrolizumab demonstrated an acceptable toxicity and good safety profile and it is now in a phase II study [77]. More trials using PD-1/PD-L1 inhibitors are being planned in TNBC as this is the breast cancer subtype in which PD-1+ TILs and PD-L1+ cancer cells are more commonly seen [78]. A randomized, phase III trial to evaluate the efficacy and safety of Pembrolizumab as adjuvant therapy for triple negative breast cancer with ≥1 cm residual invasive cancer or positive lymph nodes (ypN+) after neoadjuvant chemotherapy started accruing patients in November 2016 [79].

CTLA-4 is another immune checkpoint that is being targeted in breast cancer. Similar to the PD-1/PD-L1 inhibitors, most ongoing clinical trials involving CTLA-4 generally revolve around melanoma. Ipilimumab is a CTLA-4 monoclonal antibody FDA-approved for the treatment of unresectable melanoma [80]. It is currently being used in a phase I study examining its safety in combination with a new anti-B7-H3 mAb, Enoblituzumab, to patients with multiple refractory cancers, including triple-negative breast cancer [81]. Ipilimumab is also being combined with Entinostat and Nivolumab in a phase I study for metastatic HER2-negative breast cancer as well as with just Nivolumab in a phase II study for patients with recurrent Stage IV HER2-negative breast cancer [82]. There are other ongoing trials evaluating the combination of a CTLA-4 inhibitor, with additional treatments. There is a phase II study of tremelimumab (CTLA-4 inhibitor) with a PD-L1 inhibitor, MEDI4736, in patients with HER2-negative breast cancer to look for the safety and efficacy of this regimen [83]. A phase I study has already been completed with the combination of tremelimumab and exemestane in patients with hormone-responsive advanced breast cancer [84]. Besides demonstrating that this treatment regimen is tolerable, the study showed that there was an associated increase in T cells with inducible costiumulators (ICOS) and that more of the patients with stable disease tended to express higher levels of ICOS+ T cells versus the patients with progressive disease [84]. CTLA-4 inhibitors have been evaluated in combination with other interventions as well. A phase I trial evaluating preoperative intervention in the form of ipilimumab and/or cryoablation in early stage breast cancer showed these treatments to be safe and tolerable and plans are being made for a phase II trial with this regimen [85].

Future development of these treatments should balance their benefit with their potential toxicity. CTLA-4 mAbs have been shown to have immune-related adverse events mostly affecting the skin and gastrointestinal tract [80]. Other toxicities include hepatitis, thyroiditis, colitis, and hypophysitis [86]. Compared to treatments targeting CTLA-4, therapy targeting PD-1/PD-L1 appears to have a lower frequency of immune-related adverse events [87]. The combinations of anti-PD-1/PD-L1 mAbs and anti-CTLA-4 mAbs are more effective than single agents but they may be associated with increased incidence of pneumonitis that responds to holding the drug and/or using immunosuppressive agents; the rate of pneumonitis was 5% in one study [88].

The Immune-mediated Effect of Chemotherapy

Traditionally, the effect of chemotherapy has been explained by the induction of apoptosis of cancer cells after interrupting their cell cycle apparatus. However, alternative mechanisms involving the immune system have been recently invoked [94,95]. Taxanes, doxorubicin, and cyclophosphamide, which are standard chemotherapeutic agents in the treatment of breast cancer, are known to have major effects on the immune system in animals and human experiments [95-100]. For example, taxanes, as a class, increase serum IFN-gamma, IL-2, IL-6, and GM-CSF levels as well as reducing the levels of IL-1 and TNF-alpha [101]. Paclitaxel given neoadjuvantly increases the levels of tumor-infiltrating lymphocytes within the tumor itself [102].

The immune effects of chemotherapy may be summarized by: 1) rendering dying cancer cells more visible to the immune system by exposing their TAAs; 2) stimulating the innate immune system; 3) stimulating T cell differentiation; 4) promoting a cytokine profile that increases the likelihood of Th1 polarization; 5) inhibition of myeloid-derived suppressor cells and M2 macrophages and 6) suppression of FOXP3+ regulatory T cells [99]. Acknowledging these mechanisms is of major importance to optimize their benefit and minimize toxicity to the immune system that becomes an important executioner of chemotherapy effect. Furthermore, integrating chemotherapy with vaccines or checkpoint inhibitors is promising [103,104].

Conclusion and Future Directions

Immunogenicity of breast cancer is subtype-dependent with a spectrum that spans from the most immunogenic to the non-immunogenic subtypes. On one end, TNBC is the most immunogenic with high mutation and neo-antigen load and high MHC-I expression. The immune system is already activated against the cancer as attested by the high TILs, but the cancer is counterattacking by creating an immune suppressive environment (Tregs, MDSCs) or expressing checkpoint immune inhibitory molecules (CTLA4, PD-1/PD-L1). On the other end, Luminal A is the least immunogenic with the lowest mutation and neo-antigen load and the loss or down regulation of the expression of TAAs. MHC-I expression is significantly reduced

or absent. Hence, infiltration with TILs is minimal if any. High local concentrations of estrogen stimulate growth and maintain a local immune suppression by attracting Tregs and MDSCs. The other breast cancer subtypes fall in between these two extremes.

The overall goal of cancer immunotherapy is the activation of the immune system against the cancer. Vaccination has traditionally been to boost the latent immune response to tumor-specific antigens. Approaches have included cell-based protocols involving immunization with whole autologous or allogeneic tumors, as well as antigen-based strategies involving immunization with proteins or peptides overexpressed in tumors and under expressed in normal tissues. HER2 and MUC1 are the predominant antigens used in human breast cancer vaccine trials. Although vaccination using these antigens may demonstrate tumor-reducing effects, neither antigen provides any tissue or tumor specificity since both are expressed in a variety of normal tissues and tumors raising concerns about the possibility of off target-damage if a robust immune response is developed. However, despite the lack of inherent tissue specificity of HER2 and MUC1, these concerns about systemic autoimmune sequelae have not been substantiated so far. TACAs are pan-immunogens that elicit responses to several antigens, thus achieving the same goal as a multivalent vaccine. To overcome their low immunogenicity, investigators have used CMPs that seem to elicit a broad-spectrum anti-tumor reactivity. Here again, the activation of immune responses against TACAs raises concerns regarding the balance between "tumor destruction" and "tissue damage", as TACAs are also expressed on normal tissues. The evidence gleaned from phase I and II trials is reassuring. It is not clear which subtype of breast cancer would benefit from this approach.

Monoclonal antibodies are an integral part of our armamentarium in the fight against cancer. They can be divided into those that target the immune system and those that target oncogenic membrane receptors (HER2) or other surface molecules of unknown function (CD20). Anti-HER2 antibodies have changed the outlook of this disease. The failures of small molecules that inhibit the oncogenic stimulation of HER2 and the lack or minimal response to these antibodies in tumors that lack TILs suggest that their action is more immune-mediated than oncogenic-mediated.

Monoclonal antibodies that inhibit checkpoints (checkpoint inhibitors) are changing the paradigm of care in many solid tumors. The first results of their use in breast cancer suggest that they are the most effective in TNBC. Their use is being investigated in the other subtypes. Due to the low immunogenicity of luminal A and B breast cancers, a combination strategy using vaccines to stimulate the immune response followed by checkpoint inhibitors is rational but its clinical usefulness remains to be proven.

Finally, the immune mechanism of chemotherapy is being increasingly recognized. Its contribution in the total effect of chemotherapy relative to the direct cytotoxic effect is not known. Any further development of chemotherapy in the future should take this aspect into consideration to maximize the immune stimulatory effect and minimize the immune suppressive effect of chemotherapy.

Funding

This work was partially supported by funds from the Laura F. Hutchins, M.D. Distinguished Chair for Hematology and Oncology.

References

1. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2016. CA: a cancer journal for clinicians. 2016;66(1):7-30.
2. Loi S, Sirtaine N, Piette F, Salgado R, Viale G, Van Eenoo F, et al. Prognostic and predictive value of tumor-infiltrating lymphocytes in a phase III randomized adjuvant breast cancer trial in node-positive breast cancer comparing the addition of docetaxel to doxorubicin with doxorubicin-based chemotherapy: BIG 02-98. J Clin Oncol. 2013;31(7):860-67.
3. Loi S, Michiels S, Salgado R, Sirtaine N, Jose V, Fumagalli D, et al. Tumor infiltrating lymphocytes are prognostic in triple negative breast cancer and predictive for trastuzumab benefit in early breast cancer: Results from the FinHER trial. Ann Oncol. 2014;25(8):1544-50.
4. Liu S, Lachapelle J, Leung S, Gao D, Foulkes WD, Nielsen TO. CD8 lymphocyte infiltration is an independent favorable prognostic indicator in basal-like breast cancer. Breast Cancer Research. 2012;14(2):1.
5. Adams S, Gray RJ, Demaria S, Goldstein L, Perez EA, Shulman LN, et al. Prognostic value of tumor-infiltrating lymphocytes in triple-negative breast cancers from two phase III randomized adjuvant breast cancer trials: ECOG 2197 and ECOG 1199. J Clin Oncol. 2014;32(27):2959-66.
6. Dieci MV, Criscitiello C, Goubar A, Viale G, Conte P, Guarneri V, et al. Prognostic value of tumor-infiltrating lymphocytes on residual disease after primary chemotherapy for triple-negative breast cancer: A retrospective multicenter study. Ann Oncol. 2014;25(3):611-8.
7. Denkert C, Loibl S, Noske A, Roller M, Müller BM, Komor M, et al. Tumor-associated lymphocytes as an independent predictor of response to neoadjuvant chemotherapy in breast cancer. J Clin Oncol. 2010;28(1):105-13.
8. Denkert C, von Minckwitz G, Brase JC, Sinn BV, Gade S, Kronenwett R, et al. Tumor-infiltrating lymphocytes and response to neoadjuvant chemotherapy with or without carboplatin in human epidermal growth factor receptor 2-positive and triple-negative primary breast cancers. J Clin Oncol. 2015;33(9):983-91.
9. Kieber-Emmons T, Kohler H. Evolutionary origin of autoreactive determinants (autogens). Proc Natl Acad Sci U S A. 1986;83(8):2521-5.
10. Milstein O, Hagin D, Lask A, Reich-Zeliger S, Shezen E, Ophir E, et al. CTLs respond with activation and granule secretion when serving as targets for T-cell recognition. Blood. 2011;117(3):1042-52.
11. Andersen MH, Schrama D, Thor Straten P, Becker JC. Cytotoxic T cells. J Invest Dermatol. 2006;126(1):32-41.
12. Tai X, Cowan M, Feigenbaum L, Singer A. CD28 costimulation of developing thymocytes induces Foxp3 expression and regulatory T cell differentiation independently of interleukin 2. Nat Immunol. 2005;6(2):152-62.
13. Mittal D, Gubin MM, Schreiber RD, Smyth MJ. New insights into cancer immunoediting and its three component phases—elimination, equilibrium and escape. Curr Opin Immunol. 2014;27:16-25.
14. Schreiber RD, Old LJ, Smyth MJ. Cancer immunoediting: Integrating immunity's roles in cancer suppression and promotion. Science. 2011;331(6024):1565-70.
15. Dunn GP, Bruce AT, Ikeda H, Old LJ, Schreiber RD. Cancer immunoediting: from immunosurveillance to tumor escape. Nat Immunol. 2002;3(11):991-8.
16. Croxford JL, Tang ML, Pan MF, Huang CW, Kamran N, Phua CM, et al. ATM-dependent spontaneous regression of early emu-myc-induced murine B-cell leukemia depends on natural killer and T cells. Blood. 2013;121(13):2512-21.
17. Senovilla L, Vitale I, Martins I, Tailler M, Pailleret C, Michaud M, et al. An immunosurveillance mechanism controls cancer cell ploidy. Science. 2012;337(6102):1678-84.

18. Wu X, Peng M, Huang B, Zhang H, Wang H, Huang B, et al. Immune microenvironment profiles of tumor immune equilibrium and immune escape states of mouse sarcoma. Cancer Lett. 2013;340(1):124-33.

19. Salgado R, Denkert C, Demaria S, Sirtaine N, Klauschen F, Pruneri G, et al. The evaluation of tumor-infiltrating lymphocytes (TILs) in breast cancer: Recommendations by an international TILs working group 2014. Ann Oncol. 2015;26(2):259-71.

20. Dieci MV, Griguolo G, Miglietta F, Guarneri V. The immune system and hormone-receptor positive breast cancer: Is it really a dead end? Cancer Treat Rev. 2016;46:9-19.

21. Baumgarten SC, Frasor J. Minireview: Inflammation: An instigator of more aggressive estrogen receptor (ER) positive breast cancers. Molecular Endocrinology. 2012;26(3):360-71.

22. Jinushi M, Komohara Y. Tumor-associated macrophages as an emerging target against tumors: Creating a new path from bench to bedside. Biochimica et Biophysica Acta (BBA)-Reviews on Cancer. 2015;1855(2):123-30.

23. Lee HJ, Song IH, Park IA, Heo SH, Kim YA, Ahn JH, et al. Differential expression of major histocompatibility complex class I in subtypes of breast cancer is associated with estrogen receptor and interferon signaling. Oncotarget. 2016;7(21):30119-32.

24. Pierdominici M, Maselli A, Colasanti T, Giammarioli AM, Delunardo F, Vacirca D, et al. Estrogen receptor profiles in human peripheral blood lymphocytes. Immunol Lett. 2010;132(1):79-85.

25. Salem ML. Estrogen, a double-edged sword: Modulation of TH1-and TH2-mediated inflammations by differential regulation of TH1/TH2 cytokine production. Current Drug Targets-Inflammation & Allergy. 2004;3(1):97-104.

26. Inoue M, Mimura K, Izawa S, Shiraishi K, Inoue A, Shiba S, et al. Expression of MHC Class I on breast cancer cells correlates inversely with HER2 expression. Oncoimmunology. 2012;1(7):1104-10.

27. Montecino-Rodriguez E, Berent-Maoz B, Dorshkind K. Causes, consequences, and reversal of immune system aging. J Clin Invest. 2013;123(3):958-65.

28. Bonafè M, Storci G, Franceschi C. Inflamm-aging of the stem cell niche: breast cancer as a paradigmatic example: breakdown of the multi-shell cytokine network fuels cancer in aged people. Bioessays. 2012;34(1):40-9.

29. Dieci MV, Griguolo G, Miglietta F, Guarneri V. The immune system and hormone-receptor positive breast cancer: Is it really a dead end? Cancer Treat Rev. 2016;46:9-19.

30. Irahara N, Miyoshi Y, Taguchi T, Tamaki Y, Noguchi S. Quantitative analysis of aromatase mRNA expression derived from various promoters (I. 4, I. 3, PII and I. 7) and its association with expression of TNF-a, IL-6 and COX-2 mRNAs in human breast cancer. Int J Cancer. 2006;118(8):1915-21.

31. Prieto GA, Rosenstein Y. Oestradiol potentiates the suppressive function of human CD4 CD25 regulatory T cells by promoting their proliferation. Immunology. 2006;118(1):58-65.

32. Polanczyk MJ, Hopke C, Vandenbark AA, Offner H. Estrogen-mediated immunomodulation involves reduced activation of effector T cells, potentiation of treg cells, and enhanced expression of the PD-1 costimulatory pathway. J Neurosci Res. 2006;84(2):370-8.

33. Nadkarni S, McArthur S. Oestrogen and immunomodulation: new mechanisms that impact on peripheral and central immunity. Curr Opin Pharmacol. 2013;13(4):576-81.

34. Svoronos N, Perales-Puchalt A, Allegrezza MJ, Rutkowski MR, Payne KK, Tesone AJ, et al. Tumor cell-independent estrogen signaling drives disease progression through mobilization of myeloid-derived suppressor cells. Cancer Discov. 2016.

35. Rossini A, Rumio C, Sfondrini L, Tagliabue E, Morelli D, Miceli R, et al. Influence of antibiotic treatment on breast carcinoma development in proto-neu transgenic mice. Cancer Res. 2006;66(12):6219-24.

36. Kassayova M, Bobrov N, Strojny L, Kisková T, Mikeš J, Demečková V, et al. Preventive effects of probiotic bacteria lactobacillus plantarum and dietary fiber in chemically-induced mammary carcinogenesis. Anticancer Res. 2014;34(9):4969-75.

37. Velicer CM, Heckbert SR, Lampe JW, Potter JD, Robertson CA, Taplin SH. Antibiotic use in relation to the risk of breast cancer. JAMA. 2004;291(7):827-35.

38. Rutkowski MR, Stephen TL, Svoronos N, Allegrezza MJ, Tesone AJ, Perales-Puchalt A, et al. Microbially driven TLR5-dependent signaling governs distal malignant progression through tumor-promoting inflammation. Cancer cell. 2015;27(1):27-40.

39. Mao Y, Qu Q, Chen X, Huang O, Wu J. The Prognostic Value of Tumor-Infiltrating Lymphocytes in Breast Cancer: A Systematic Review and Meta-Analysis. PLoS One. 2016;11(4):e0152500.

40. Dieci MV, Criscitiello C, Goubar A. Prognostic value of tumor-infiltrating lymphocytes on residual disease after primary chemotherapy for triple-negative breast cancer: A retrospective multicenter study. Ann Oncol. 2014.

41. Mahmoud SM, Paish EC, Powe DG, Macmillan RD, Grainge MJ, Lee AH, et al. Tumor-infiltrating CD8+ lymphocytes predict clinical outcome in breast cancer. J Clin Oncol. 2011;29(15):1949-55.

42. Robbins PF, Lu Y, El-Gamil M, Li YF, Gross C, Gartner J, et al. Mining exomic sequencing data to identify mutated antigens recognized by adoptively transferred tumor-reactive T cells. Nat Med. 2013;19(6):747-52.

43. van Rooij N, van Buuren MM, Philips D, Velds A, Toebes M, Heemskerk B, et al. Tumor exome analysis reveals neoantigen-specific T-cell reactivity in an ipilimumab-responsive melanoma. J Clin Oncol. 2013;31(32):e439-42.

44. Vogelstein B, Papadopoulos N, Velculescu VE, Zhou S, Diaz LA Jr, Kinzler KW. Cancer genome landscapes. Science. 2013;339(6127):1546-58.

45. Cancer Genome Atlas Network. Comprehensive molecular portraits of human breast tumours. Nature. 2012;490(7418):61-70.

46. Luen S, Virassamy B, Savas P, Salgado R, Loi S. The genomic landscape of breast cancer and its interaction with host immunity. Breast. 2016;29:241-50.

47. Nik-Zainal S, Alexandrov LB, Wedge DC, Van Loo P, Greenman CD, Raine K, et al. Mutational processes molding the genomes of 21 breast cancers. Cell. 2012;149(5):979-93.

48. Haricharan S, Bainbridge MN, Scheet P, Brown PH. Somatic mutation load of estrogen receptor-positive breast tumors predicts overall survival: An analysis of genome sequence data. Breast Cancer Res Treat. 2014;146(1):211-20.

49. Snyder A, Makarov V, Merghoub T, Yuan J, Zaretsky JS, Desrichard A, et al. Genetic basis for clinical response to CTLA-4 blockade in melanoma. N Engl J Med. 2014;371(23):2189-99.

50. Rizvi NA, Hellmann MD, Snyder A, Kvistborg P, Makarov V, Havel JJ, et al. Cancer immunology. mutational landscape determines sensitivity to PD-1 blockade in non-small cell lung cancer. Science. 2015;348(6230):124-8.

51. Brown SD, Warren RL, Gibb EA, Martin SD, Spinelli JJ, Nelson BH, et al. Neo-antigens predicted by tumor genome meta-analysis correlate with increased patient survival. Genome Res. 2014;24(5):743-50.

52. West NR, Milne K, Truong PT, Macpherson N, Nelson BH, Watson PH. Tumor-infiltrating lymphocytes predict response to anthracycline-based chemotherapy in estrogen receptor-negative breast cancer. Breast Cancer Research. 2011;13(6):R126.

53. Perez EA, Thompson EA, Ballman KV, Anderson SK, Asmann YW, Kalari KR, et al. Genomic analysis reveals that immune function genes are strongly linked to clinical outcome in the north central cancer treatment group n9831 adjuvant trastuzumab trial. J Clin Oncol. 2015;33(7):701-8.

54. Ladoire S, Mignot G, Dabakuyo S. In situ immune response after neoadjuvant chemotherapy for breast cancer predicts survival. J Pathol. 2011;224(3):389-400.

55. Criscitiello C. Tumor-associated antigens in breast cancer. Breast Care (Basel). 2012;7(4):262-6.

56. Downs-Holmes C, Silverman P. Breast cancer: overview & updates. Nurse Pract. 2011;36(12):20-6.

57. Mittendorf EA, Clifton GT, Holmes JP, Schneble E, van Echo D, Ponniah S, et al. 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. 2014;25(9):1735-42.

58. Schneble EJ, Berry JS, Trappey FA, Clifton GT, Ponniah S, Mittendorf E, et al. The HER2 peptide nelipepimut-S (E75) vaccine (NeuVax™) in breast cancer patients at risk for recurrence: Correlation of immunologic data with clinical response. Immunotherapy. 2014;6(5):519-31.

59. Mittendorf EA. Efficacy and safety study of NeuVax™ (nelipepimut-S or E75) vaccine to prevent breast cancer recurrence (PRESENT). 2017.

60. Clifton G, Holmes J, Perez S, Lorentz D, Georgakopoulou K, Benavides L, et al. Interim analysis of a randomized phase II study of the novel HER2/neu peptide (GP2) vaccine to prevent breast cancer recurrence: United states military cancer institute clinical trials group study I-05. Cancer Res. 2009;69(24):5110.

61. Schneble EJ, Perez SA, Murray JL, Berry JS, Trappey AF, Vreeland TJ, et al. Primary analysis of the prospective, randomized, phase II trial of GP2+GM-CSF vaccine versus GM-CSF alone administered in the adjuvant setting to high-risk breast cancer patients. J Clin Oncol. 2014;32(26):abstr 134.

62. Sears AK, Perez SA, Clifton GT, Benavides LC, Gates JD, Clive KS, et al. AE37: a novel T-cell-eliciting vaccine for breast cancer. Expert Opin Biol Ther. 2011;11(11):1543-50.

63. Mittendorf EA, Ardavanis A, Symanowski J, Murray JL, Shumway NM, Litton JK, et al. Primary analysis of a prospective, randomized, single-blinded phase II trial evaluating the HER2 peptide AE37 vaccine in breast cancer patients to prevent recurrence. Ann Oncol. 2016;27(7):1241-8.

64. Blixt O, Bueti D, Burford B, Allen D, Julien S, Hollingsworth M, et al. Autoantibodies to aberrantly glycosylated MUC1 in early stage breast cancer are associated with a better prognosis. Breast Cancer Research. 2011;13(2):1.

65. Ibrahim NK, Murray JL, Zhou D, Mittendorf EA, Sample D, Tautchin M, et al. Survival advantage in patients with metastatic breast cancer receiving endocrine therapy plus sialyl tn-KLH vaccine: Post hoc analysis of a large randomized trial. J Cancer. 2013;4(7):577-84.

66. Sandrin MS, Vaughan HA, Xing PX, McKenzie IF. Natural human anti-Gal alpha(1,3)Gal antibodies react with human mucin peptides. Glycoconj J. 1997;14(1):97-105.

67. Butschak G, Karsten U. Isolation and characterization of thomsen-friedenreich-specific antibodies from human serum. Tumour biology: the journal of the International Society for Oncodevelopmental Biology and Medicine. JID - 8409922. 2002.

68. Andre F, Dieci MV, Dubsky P, Sotiriou C, Curigliano G, Denkert C, et al. Molecular pathways: Involvement of immune pathways in the therapeutic response and outcome in breast cancer. Clin Cancer Res. 2013;19(1):28-33.

69. Kurtenkov O, Klaamas K, Mensdorff-Pouilly S, Miljukhina L, Shljapnikova L, Chužmarov V. Humoral immune response to MUC1 and to the thomsen-friedenreich (TF) glycotope in patients with gastric cancer: Relation to survival. Acta Oncol. 2007;46(3):316-23.

70. Monzavi-Karbassi B, Pashov A, Kieber-Emmons T. Tumor-Associated Glycans and Immune Surveillance. Vaccines (Basel). 2013;1(2):174-203.

71. Kieber-Emmons T, Saha S, Pashov A, Monzavi-Karbassi B, Murali R. Carbohydrate-mimetic peptides for pan anti-tumor responses. Structural and Computational Glycobiology: Immunity and Infection. 2015:23.

72. Makhoul I, Hutchins L, Emanuel PD, Pennisi A, Siegel E, Jousheghany F, et al. Moving a carbohydrate mimetic peptide into the clinic: Clinical response of a breast cancer patient after mimotope-based immunotherapy. Human vaccines & immunotherapeutics. 2015;11(1):37-44.

73. Hennings L, Artaud C, Jousheghany F, Monzavi-Karbassi B, Pashov A, Kieber-Emmons T. Carbohydrate mimetic peptides augment carbohydrate-reactive immune responses in the absence of immune pathology. Cancers. 2011;3(4):4151-69.

74. Yao S, Zhu Y, Chen L. Advances in targeting cell surface signalling molecules for immune modulation. Nat Rev Drug Discov. 2013;12(2):130-46.

75. Baptista MZ, Sarian LO, Derchain SF, Pinto GA, Vassallo J. Prognostic significance of PD-L1 and PD-L2 in breast cancer. Hum Pathol. 2016;47(1):78-84.

76. Muenst S, Schaerli AR, Gao F, Däster S, Trella E, Droeser RA, et al. 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.

77. Nanda R, Chow LQ, Dees EC, Berger R, Gupta S, Geva R, et al. Pembrolizumab in patients with advanced triple-negative breast cancer: Phase ib KEYNOTE-012 study. J Clin Oncol. 2016:JCO648931.

78. Gatalica Z, Snyder C, Maney T, Ghazalpour A, Holterman DA, Xiao N, et al. Programmed cell death 1 (PD-1) and its ligand (PD-L1) in common cancers and their correlation with molecular cancer type. Cancer Epidemiol Biomarkers Prev. 2014;23(12):2965-70.

79. Pusztai L. Pembrolizumab in treating patients with triple-negative breast cancer. 2017.

80. Peggs KS, Quezada SA. Ipilimumab: attenuation of an inhibitory immune checkpoint improves survival in metastatic melanoma. Expert Rev Anticancer Ther. 2010;10(11):1697-701.

81. Urba WJ. Safety study of enoblituzumab (MGA271) in combination with ipilimumab in refractory cancer. 2016.

82. Connolly R. Entinostat, nivolumab, and ipilimumab in treating patients with solid tumors that are metastatic or cannot be removed by surgery or locally advanced or metastatic HER2-negative breast cancer. 2016.

83. Santa-Maria C. MEDI4736 and tremelimumab in treating patients with metastatic HER2 negative breast cancer. 2016.

84. Vonderheide RH, LoRusso PM, Khalil M, Gartner EM, Khaira D, Soulieres D, et al. Tremelimumab in combination with exemestane in patients with advanced breast cancer and treatment-associated modulation of inducible costimulator expression on patient T cells. Clin Cancer Res. 2010;16(13):3485-94.

85. Page DB, Diab A, Yuan J. Pre-operative immunotherapy with tumor cryoablation (cryo) plus ipilimumab (ipi) induces potentially favorable systemic and intratumoral immune effects in early stage breast cancer (ESBC) patients. J Immunother Cancer. 2015;3(1):1.

86. Cimino-Mathews A, Foote JB, Emens LA. Immune targeting in breast cancer. Oncology (Williston Park). 2015;29(5):375-85.

87. Chen DS, Irving BA, Hodi FS. Molecular pathways: Next-generation immunotherapy--inhibiting programmed death-ligand 1 and programmed death-1. Clin Cancer Res. 2012;18(24):6580-7.

88. Naidoo J, Wang X, Woo KM, Iyriboz T, Halpenny D, Cunningham J, et

al. Pneumonitis in Patients Treated With Anti-Programmed Death-1/Programmed Death Ligand 1 Therapy. J Clin Oncol. 2017;35(7):709-17.

89. Loi S, Sirtaine N, Piette F. Prognostic and predictive value of tumor-infiltrating lymphocytes in a phase III randomized adjuvant breast cancer trial in node-positive breast cancer comparing the addition of docetaxel to doxorubicin with doxorubicin-based chemotherapy: BIG 02-98. J Clin Oncol. 2013;31(7):860-7.

90. Ingold Heppner B, Untch M, Denkert C, Pfitzner BM, Lederer B, Schmitt W, et al. Tumor-infiltrating lymphocytes: A predictive and prognostic biomarker in neoadjuvant-treated HER2-positive breast cancer. Clin Cancer Res. 2016;22(23):5747-54.

91. Carbognin L, Pilotto S, Nortilli R, Brunelli M, Nottegar A, Sperduti I, et al. Predictive and prognostic role of tumor-infiltrating lymphocytes for early breast cancer according to disease subtypes: Sensitivity analysis of randomized trials in adjuvant and neoadjuvant setting. Oncologist. 2016;21(3):283-91.

92. Bense RD, Sotiriou C, Piccart-Gebhart MJ, Haanen JBAG, van Vugt MATM, de Vries EGE, et al. Relevance of tumor-infiltrating immune cell composition and functionality for disease outcome in breast cancer. J Natl Cancer Inst. 2016;109(1):1-9.

93. Piccart-Gebhart M, Holmes E, Baselga J, de Azambuja E, Dueck AC, Viale G, et al. Adjuvant lapatinib and trastuzumab for early human epidermal growth factor receptor 2-positive breast cancer: Results from the randomized phase III adjuvant lapatinib and/or trastuzumab treatment optimization trial. J Clin Oncol. 2016;34(10):1034-42.

94. Bracci L, Schiavoni G, Sistigu A, Belardelli F. Immune-based mechanisms of cytotoxic chemotherapy: Implications for the design of novel and rationale-based combined treatments against cancer. Cell Death & Differentiation. 2014;21(1):15-25.

95. Zitvogel L, Galluzzi L, Smyth MJ, Kroemer G. Mechanism of action of conventional and targeted anticancer therapies: Reinstating immunosurveillance. Immunity. 2013;39(1):74-88.

96. Sevko A, Michels T, Vrohlings M, Umansky L, Beckhove P, Kato M, et al. Antitumor effect of paclitaxel is mediated by inhibition of myeloid-derived suppressor cells and chronic inflammation in the spontaneous melanoma model. J Immunol. 2013;190(5):2464-71.

97. Ge Y, Domschke C, Stoiber N, Schott S, Heil J, Rom J, et al. Metronomic cyclophosphamide treatment in metastasized breast cancer patients: Immunological effects and clinical outcome. Cancer immunology, immunotherapy. 2012;61(3):353-62.

98. Schiavoni G, Sistigu A, Valentini M, Mattei F, Sestili P, Spadaro F, et al. Cyclophosphamide synergizes with type I interferons through systemic dendritic cell reactivation and induction of immunogenic tumor apoptosis. Cancer Res. 2011;71(3):768-78.

99. Galluzzi L, Buque A, Kepp O, Zitvogel L, Kroemer G. Immunological effects of conventional chemotherapy and targeted anticancer agents. Cancer cell. 2015;28(6):690-714.

100. Alizadeh D, Trad M, Hanke NT, Larmonier CB, Janikashvili N, Bonnotte B, et al. Doxorubicin eliminates myeloid-derived suppressor cells and enhances the efficacy of adoptive T-cell transfer in breast cancer. Cancer Res. 2014;74(1):104-18.

101. Tsavaris N, Kosmas C, Vadiaka M, Kanelopoulos P, Boulamatsis D. Immune changes in patients with advanced breast cancer undergoing chemotherapy with taxanes. Br J Cancer. 2002;87(1):21-7.

102. Demaria S, Volm MD, Shapiro RL, Yee HT, Oratz R, Formenti SC, et al. Development of tumor-infiltrating lymphocytes in breast cancer after neoadjuvant paclitaxel chemotherapy. Clin Cancer Res. 2001;7(10):3025-30.

103. Sistigu A, Viaud S, Chaput N, Bracci L, Proietti E, Zitvogel L. Immunomodulatory effects of cyclophosphamide and implementations for vaccine design. Semin Immunopathol. 2011;33(4):369-83.

104. Liu WM, Fowler DW, Smith P, Dalgleish AG. Pre-treatment with chemotherapy can enhance the antigenicity and immunogenicity of tumours by promoting adaptive immune responses. Br J Cancer. 2010;102(1):115-23.