Immunotherapy and prevention of breast cancer

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Abstract

Breast cancer is the most commonly diagnosed cancer in women and is a leading cause of cancer death in women worldwide. Despite the significant benefit of the use of conventional chemotherapy and monoclonal antibodies in the prognosis of breast cancer patients and although the recent approval of the anti-PD-L1 antibody atezolizumab in combination with chemotherapy has been a milestone for the treatment of patients with metastatic triple-negative breast cancer, immunologic treatment of breast tumors remains a great challenge. In this review, we summarize current breast cancer classification and standard of care, the main obstacles that hinder the success of immunotherapies in breast cancer patients, as well as different approaches that could be useful to enhance the response of breast tumors to immunotherapies.

Key words: Immunotherapy; prevention vaccines; breast cancer

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Introduction

Breast cancer is the most common cancer and also the primary cause of mortality due to cancer in female around the World. About 1.38 million new breast cancer cases were diagnosed in 2008 with almost 50% of all breast cancer patients and approximately 60% of deaths occurring in developing countries. There is a huge difference in breast cancer survival rates worldwide, with an estimated 5-year survival of 80% in developed countries to below 40% for developing countries [1].

Developing countries face resource and infrastructure constraints that challenge the objective of improving breast cancer outcomes by timely recognition, diagnosis and management [2]. In developed countries like the United States, about 232,340 female will be diagnosed and death of 39,620 female will occur due to breast cancer in 2013 [3]. The lifetime risk of developing breast cancer in an American female is 12.38% [3]. The significant decline in morality due to breast cancer in the United States from 1975 to 2000 is attributed to constant enhancement in both screening mammography and management [4]. According

to the World Health Organization (WHO), enhancing breast cancer outcome and survival by early detection remains the foundation of breast cancer regulations. Different modern medicines are prescribed to treat breast cancer. Medical therapy of breast cancer with antiestrogens such as raloxifene or tamoxifen might avoid breast cancer in individuals who are at increased possibility of developing it [5]. Surgery of both breasts is an added preventative measure in some increased probability of developing cancer in female. In patients who have been identified with breast tumor, different strategies of management are used such as targeted therapy, hormonal therapy, radiation therapy, surgery and chemotherapy. In individuals with distant metastasis, managements are typically aimed at enhancing life quality and survival rate [6]. The unpleasant side effects of breast cancer treatment are one of the most motivating factors to find some alternative methods. The use of herbs for treating the patients having breast cancer is considered a natural alternative, because some plants may contain properties that naturally have the ability to treat breast cancer [7].

Breast cancer, perhaps more than any other solid tumor, was transformed by the progressive application of clinical hypothesis testing of basic biologic concepts. The revolutionary overthrow of the Halstedian hypothesis, with its emphasis on the primacy of locoregional control through extensive surgery, led to changes both in locoregional therapy as well as providing the intellectual basis for adjuvant systemic therapies. And, at a time when systemic therapies were dominated by rank empiricism, breast cancer led the way in the application of targeted biologic therapy, long before targeted therapy became an oncologic mantra.

This article will review a half-century of progress, focusing on the areas in which the greatest progress has been seen: the revolution in locoregional therapy; the application of cytotoxic chemotherapy in both local and advanced disease; the discovery and therapeutic exploitation of estrogen receptor biology; the use of estrogen receptor biology for breast cancer prevention; and the targeting of the human epidermal growth factor receptor complex [8]. Collectively, these constitute a revolution in breast cancer therapeutics that has occurred within the lifetime of an organization. Finally, we will touch on the remaining therapeutic challenges for this disease.

HER2-Positive Disease

In the late 1980s, HER2 gene amplification was recognized as a prognostic marker for poor clinical outcome in early-stage breast cancer. While retrospective studies suggested a preferential benefit with adjuvant anthracycline regimens, the true revolution in therapy for HER2-positive patients awaited the development of the targeted monoclonal anti-HER2

antibody trastuzumab. In 1998, a randomized clinical trial showed an unprecedented improvement in survival when trastuzumab was added to standard chemotherapy in metastatic disease, the use of adjuvant trastuzumab transformed the face of HER2-positive disease, substantially improving disease-free and overall survival [9].

Trastuzumab resistance occurs in both the metastatic and adjuvant settings. Starting in 2007, several new drugs became available [10], including the small molecule tyrosine kinase inhibitor lapatinib, the anti HER2-HER3 dimerization antibody pertuzumab, and the antibody drug conjugate ado-trastuzumab emtansine or T-DM1 in 2013. These approvals were based on improvement in survival outcomes in metastatic patients with mostly trastuzumab-naive (pertuzumab74) or trastuzumab-exposed (lapatinib18 and T-DM175) breast cancer, and all these agents are now being tested in ongoing adjuvant trials [11].

In 2014, the Adjuvant Lapatinib and/or Trastuzumab Treatment Optimisation (ALTTO) trial will be the first trial to report on whether dual anti-HER2 therapy with trastuzumab and lapatinib (in sequence or in combination) improves outcomes compared to single agent trastuzumab [12]. The Addition to Chemotherapy and Herceptin (Trastuzumab) As Adjuvant Therapy in patients with HER2-Positive Primary Breast Cancer (APHINITY) trial is testing the addition of pertuzumab to standard nonanthracycline or anthracycline-based chemotherapy plus trastuzumab. In addition, A Study of Trastuzumab Emtansine Versus Trastuzumab as Adjuvant Therapy in Patients with HER2-Positive Breast Cancer Who Have Residual Tumor in the Breast or Axillary Lymph Nodes Following Preoperative Therapy (KATHERINE) is examining the role of postoperative T-DM1 versus trastuzumab in patients with HER2-positive disease and less than a pathologic complete response after preoperative therapy with a trastuzumab-based regimen [13].

The remarkable switch from a prognostic marker for worse survival in the absence of treatment to a predictive marker for improved outcome cemented the clinical utility of HER2 overexpression [14]. HER2 amplification occurs in approximately 15% of all newly diagnosed patients.76 Findings from the first generation of adjuvant HER2-targeted trials also led the American Society of Clinical Oncology and the College of American Pathologists to provide guidance on HER2 testing [15]. Earlier concerns about the high frequency of false-positive HER2 test results have diminished as a result of greater standardization of tissue handling, improved laboratory performance of HER2 testing, and more careful reporting of test results. Current guidelines examine less common clinical scenarios and expand the focus beyond specificity (false-positive results) to also address concerns about sensitivity (false-negative results) [8].

NSABP B-47 is now attempting to confirm retrospective, hypothesis-generating exploratory data from two of the adjuvant trastuzumab trials regarding a possible benefit in patients

confirmed on central testing to have HER2-negative disease but whose tumors had initially tested positive in a local laboratory. HER2-targeted therapy combined with radiation therapy is also the subject of another prospective trial in women with in situ disease (NSABP B43). In the meantime, prospective trials have shown no benefit from lapatinib78 or pertuzumab80 in HER2-negative metastatic disease [16].

Although few patients with node-negative disease and almost no patients with tumors measuring 1 cm or less were eligible for the first generation of adjuvant trials, retrospective institutional series suggest that patients with small node-negative, HER2-positive tumors have a high enough risk of recurrence in the absence of therapy to potentially support the use of adjuvant trastuzumab [17].

Smaller tumor size retains prognostic utility in small untreated HER2-positive tumors, and the first results from a single-arm study of 12 weeks of paclitaxel/trastuzumab followed by trastuzumab were recently reported with a short median follow-up), a with the suggestion that such therapy resulted in an exceptionally low relapse rate. A subsequent study (ATEMPT) will soon test this regimen against T-DM1 in a similar patient group [18].

The clinical landscape for HER2-positive breast cancer was forever altered with the approval of trastuzumab in 1998. Many, though not all, HER2-positive patients with metastatic disease face a manageable chronic disease. The development of metastases in sanctuary sites like the CNS has been seen more commonly as systemic therapy has improved [12].

Questions remain about optimal sequence, duration, and combination of various anti-HER2 targeted agents, with and without chemotherapy. Our understanding regarding mechanisms of resistance to HER2-targeted therapy (including perturbations of the PI3 kinase pathway) is still limited, and clinical applications that exploit interactions with this and other growth factor pathways are still early in development.83 Despite the enormous accomplishments of the past 25 years, much remains to be learned about the optimal clinical management of HER2-positive breast cancer [19].

The past 50 years transformed the care of patients with breast cancer, reducing morbidity and mortality through the application of basic scientific principles to the clinic. Although enormous progress has been made, many important challenges remain. To name but a few of these: though effective prevention approaches exist, they have had little effect as a result of poor uptake in the general medical community [11]; improved breast imaging has revealed the existence of large populations that may never require treatment, yet we have no effective means of separating the dangerous from the innocuous; the majority of women relapsing and dying of ER-positive breast cancer do so as a result of dormant micro-metastases, which are largely untouched by initial adjuvant systemic therapies; resistance to all systemic therapies remains a major problem; triple-negative breast cancer, dominated

by genomic chaos, does not seem likely to be amenable to the targeted therapies that have transformed ER- and HER2-positive breast cancer; and the success of systemic therapies for HER2-positive disease has resulted in a progressive increase in symptomatic CNS relapses, uncontrolled by standard monoclonal antibody therapies [19].

Immunotherapy as an Option for Cancer Treatment

According to the cancer immunoediting model [15], the relation between tumor cells and the immune system is a dynamic process which consists of three main phases:

Elimination: During this phase, cancer cells are successfully recognized and destroyed by the body's immune system [20]. The success of the immune system to eliminate tumor cells depends on the ability of the antigen to trigger the immune response, or immunogenicity, which can be summarized as follows:

1. Genetic abnormalities lead to the production of new antigens by tumor cells, which are processed and presented as antigen-derived peptides on the cell surface in association with Human Leukocyte Antigen class I (HLA-I).
2. Neoantigens that are present in tumor microenvironment are recognized, processed, and presented on the surface of Antigen Presenting Cells (APCs) as antigen-derived peptides in association with Human Leukocyte Antigen class-II (HLA-II), which can be recognized by helper T-cell receptors and leads to B-cell and cytotoxic T-cell stimulation and maturation.
3. After T-cell activation by co-stimulatory signals provided by APCs, T-cells recognize neoantigens presented by HLA-I and attack the targeted tumor cell by the secretion of cytotoxic granules and/or via Fas cell surface death receptor (FAS) and caspase activation.
4. Equilibrium: During this phase, transformed cells with a resistant or non-immunogenic phenotype escape the elimination phase and proliferate, although the immune system is able to control the tumor growth [21].
5. Escape: The selective pressure caused by anti-cancer treatments or immune-surveillance promotes the uncontrolled proliferation of cells with a resistant or a non-immunogenic phenotype, leading to tumor progression and metastasis.

The first cancer immunotherapy treatments were based on the use of humanized monoclonal antibodies with the ability to bind and neutralize a targeted altered molecule expressed by cancer cells and on which their survival and proliferation depends. The approval in September 1998 of trastuzumab (Herceptin®, Genentech, Inc., South San Francisco, CA, United States) represented the release of the first antibody for the treatment of metastatic breast cancer patients with HER2 (Receptor tyrosine-protein kinase ERBB2,

CD340) overexpression and/or gene amplification, which represented a milestone in the treatment of breast cancer. After trastuzumab, other different anti-HER2 monoclonal antibodies including lapatinib (Tykerb®, GlaxoSmithKline, Brentford, United Kingdom), neratinib (Nerlynx®, Puma Biotechnology, Los Angeles, CA, United States), gefitinib (Iressa®, AstraZeneca, Cambridge, United Kingdom), or afatinib (Giotrif®, Boehringer Ingelheim Pharmaceuticals, Inc., Ingelheim am Rhein, Germany) [8] as monotherapy or in combination with conventional treatments have contributed to increasing the number of therapeutic options for breast cancer patients [22].

Although the use of monoclonal antibodies targeting altered proteins has definitely improved the outcome of cancer patients, modest response rates and resistance development [23] remain as the major impediments for treatment success and require the search for new approaches apart from combined therapies, among which antibody-drug conjugates (ADC) such as the recently FDA approved ado-trastuzumab emtansine (Kadcyla®, Genentech, Inc., South San Francisco, CA, United States) [24] and T cell bispecific antibodies stand out among the most promising strategies for breast cancer patients [25].

Checkpoint Inhibition in Triple-Negative Breast Cancer

Triple-negative breast cancer is a highly heterogeneous breast cancer subtype that has been defined by the lack of a target. It has been subdivided into 6 different subgroups based on its molecular heterogeneity that include basal-like, mesenchymal-like, mesenchymal stem-like, luminal androgen receptor expression, immunomodulatory and an unstable type [26]. For years it was thought that this disease is resistant to immunotherapy, however recent studies have shown evidence of significant immune infiltration of TILs (tumor-infiltrating lymphocytes) in a subset of patients with triple-negative breast cancer. Triple-negative breast cancer seems to have a high expression of PDL1 and harbors a strong infiltration by immune cells in the actual tumor bed. TILs seem to have both a prognostic as well as predictive power, with high numbers correlating with better outcome and better response to therapy. Elevated TIL scores were proven to correlate with increased pathological complete response to neoadjuvant chemotherapy. This proves that the immune system plays a pivotal role in this subgroup of patients [27].

Based on that, efforts were generated to prime the immune system to elicit an immune response capable of fighting off those cancer cells. Immune checkpoint blockade exploited this mechanism at its best through targeting the PD1/PDL1 pathway. PD1 (programmed cell death-1) receptor is a cell surface membrane protein, member of the B7 family of checkpoints that is expressed on the surface of activated T cells [28]. PD1 is activated by its ligands PD-L1 and PD-L2 that are commonly expressed on the surface of dendritic cells

or macrophages as well as on tumor cells. When activated, the PD1/PDL1 pathway leads to the suppression of the T-cell-mediated immune response, which normally can minimize states of chronic inflammation and help control autoimmune diseases [29].

Unfortunately, Tumor cells can exploit this pathway to evade the immune detection system or what is called the cancer immunity cycle. Tumor cells overexpress PDL1 and trigger the PD1/PDL1 pathway, which leads to the inhibition of the cytotoxic T cells. These deactivated T cells remain inhibited in the tumor microenvironment that leads to the unopposed proliferation of cancer cells [30].

Anti-PD-1 antibodies (like Pembrolizumab and Nivolumab) and anti-PDL-L1 antibodies (like Atezolizumab and Durvalumab) have been developed and are currently being investigated. Those monoclonal antibodies aim to restore the immune system by disrupting the PD1/PDL1 interaction.48 However, it remains to be seen whether PD1 or PDL1 blockade is better [22].

A similar pathway being investigated is targeting the Cytotoxic T lymphocyte-associated protein 4 (CTLA-4). CTLA4 is a T cell inhibitory receptor that is expressed on activated CD4+ and CD8+ T cells that specifically overexpress CD25 and foxp3. CTLA4 is upregulated by activation of T cell receptor and cytokines such as Il-12 and IFN gamma, which usually forms a negative feedback to, activated T cells leading to a physiologic break of the immune response [31]. CTLA-4 was initially implicated in cancer when in vivo it was demonstrated that blockade of the inhibitory effects of CTLA-4 can release the brake and potentiate the immune response against tumor cells leading to tumor regression in mouse models of sarcoma and colon adenocarcinoma. Anti CTLA-4 drugs are currently being investigated. Ipilimumab has already been approved in melanoma and is currently being investigated in breast cancer.17 Tremelimumab another anti-CTLA4 is also being investigated [32].

Other targets that are being investigated for potential checkpoint inhibition include the BTLA, VISTA, TIM3, LAG3, and CD47 proteins but are very early in development. We will review next the active and completed clinical trials exploring those drugs specifically in triple-negative breast cancer [33].

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 [34]. 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 [35]. 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) [4] and mucin 1 (MUC1) [36]. While peptide vaccines are customizable, easy and relatively inexpensive to produce, injected peptides are readily degraded and have variable immunogenicity [22]. Additionally, limitations on interaction with the chosen peptide and the MHC haplotypes variants must be considered when designing a peptide vaccine [12]. 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 [2]. 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 [11]. 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 downregulating expression of one specific antigen [23]. 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 [19]. Additional work with whole tumor cell lines has shown that modification of cell surface antigens can improve immunogenicity [2].

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 [37], and gamma-delta T cells [12], 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 [38]. 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.

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 [39]. Studies in animals have demonstrated the nability Treg depletion to synergize with immunotherapy in a murine model of mesothelioma [16]. 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 [18].

Cellular Therapy

Adoptive cell therapy is based on isolation of immune cells (T cells in most cases) from a patient. These cells are then enriched ex vivo for tumor-specific cloning, expanded, activated, and autologously readministered to the patient [40]. Another adoptive cell therapy involves the use of chimeric antigen receptor (CAR) T cells, for which T cells are genetically designed to express receptors against specific targets [1]. CAR T cells are being used currently for the treatment of hemato-oncological malignancies, such as lymphoma [92]. Their application in solid tumors is being examined in experimental and clinical research [22]. Assessed the effect of HER2-specific CAR T-cell therapy in vitro and in xenografts with trastuzumab-resistant breast cancer. They observed that HER2-specific CAR T cells induced tumor regression and proved that antibody resistance can be overcome by targeting the same epitope with CAR T cells [41]. Promising therapeutic approaches of this type are also available for triple-negative tumors. Other showed that T cells expressing folate receptor-α CAR inhibited the outgrowth of triple-negative breast cancer in vitro and in xenografts. In an early-phase clinical study, CAR T-cells specific for mesenchymal-epithelial transition factor (cMets) were injected into accessible cutaneous or lymph-node metastases in patients with metastatic breast cancer [42]. The researchers observed extensive necrosis

and immune cell invasion in the excised lesions and concluded that the intratumoral injection of cMet-specific CAR T cells generated an inflammatory response within the tumors.

Cytokine-Activated Mediation Therapy

Another treatment strategy is based on the assumption that monoclonal antibody therapy activates a signal that stimulates the release of type 1 IFN and INF-γ-producing CD8+ T cells [43]. Continuing this thought, concepts are being developed and early studies are being conducted to examine the combination of IFN-γ and anti-HER2 antibodies [9]. Produced an anti-HER2 single-chain variable fragment-IFN-γ fusion protein that showed activity superior to that of anti-HER2 antibodies in xenografts and was even effective on tumors with anti-HER2 resistance [7]. In a phase 1 study, assessed the effect of IFN-γ administered weekly in combination with paclitaxel, trastuzumab, and pertuzumab in patients with HER2-positive breast cancer. The 9 enrolled patients tolerated the therapy well, but oncological outcomes from that study remain to be published.

Conclusion

Immunotherapy is a rapidly emerging field in breast cancer, as evidenced by the plethora of preclinical and clinical concepts and ongoing trials. Initial studies established the role of immunotherapeutic agents as checkpoint inhibitors in the metastatic setting. As immunogenic factors, such as PD-L1 and TIL expression, decrease over the course of disease, an important aspect of immunotherapy will be its effectiveness for early-stage breast cancer. Another important aspect will be the identification of suitable biomarkers to identify patients who will benefit from certain treatment approaches. Understanding of the tumor microenvironment, the roles of the innate and adaptive immune systems in the development and progression of breast cancer, and factors that account for responses to immunotherapeutic agents is necessary to enable immunotherapy to come of age fully in breast cancer treatment.

Ethical Approval

The study was approved by the Ethical Committee.

Conflicts of Interest

The authors declare that they have no competing interests.

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