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Immunotherapy in breast cancer: An introduction

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ABSTRACT

The field of breast cancer immunology has progressed tremendously over the last decade. Twenty years ago immunotherapy was not considered for the treatment of breast cancers because breast cancer was not considered immunogenic. Today we know that most patients with breast cancer have some evidence of an adaptive immune response against their tumors, detectable either in the peripheral blood or in the tumor. Moreover, immunity to breast cancer begins at the earliest stages of the disease, in some patients prior to diagnosis. Recent evidence suggests that lymphocytes infiltrating breast cancers and found in the tumor stroma are strong prognostic indicators of a beneficial disease outcome. These observations now pave the way for the integration of immunomodulation into standard of care therapy for the treatment of breast cancer.

The success of immune checkpoint inhibitor therapy in immunogenic cancers such as melanoma has underscored the importance of the adaptive immune system in cancer eradication. The adaptive immune system is composed of lymphocytes, both T-cells and B-cells, and is defined by the ability of those cells to specifically respond to immunogenic, i.e. antigenic, proteins expressed on and in cancer. Immune checkpoint inhibitor agents allow tumor educated T-cells to recognize cancer, proliferate, and limit tumor growth. The adaptive immune response is also associated with the development of immunologic memory, the ability of lymphocytes to respond again at a distant time point if ever exposed to tumor cells. Moreover, T-cells capable of killing tumors cells are of a Type I phenotype; CD4 T-cells in the tumor microenvironment secrete Type I cytokines such as interferon-gamma (IFN-g) and tumor necrosis factor-alpha which activate antigen presenting cells and support the development of cytotoxic CD8 T-cells needed to induce cancer death [1].

1. Antigen specific adaptive immunity in breast cancer

Twenty years ago, little was known about the immunogenicity of breast cancer. Today, breast cancer is one of the most commonly studied tumors for the presence of immune system cells in the lesion and scores of ongoing clinical trials are evaluating the role of immunotherapy in breast cancer treatment and prevention. The study of the immune microenvironment in breast cancer has progressed at a rapid pace and started with the identification of breast cancer associated antigens. One of the first breast cancer associated antigens identified was the glycoprotein MUC-1. Investigators determined that under glycosylation of MUC-1 resulted in the exposure of new epitopes to the immune system which were normally shielded in the fully glycosylate state [2]. Although MUC-1 was a "self" antigen and theoretically tolerance to self should prevent immune recognition, these studies demonstrated that proteins aberrantly expressed in cancer could trigger an immune response and that some self-epitopes could be "neo-antigens". Existing low level MUC1 specific T-cells could be identified in the peripheral blood of both breast cancer patients and volunteer donors suggesting the potential to boost these meager immune responses to therapeutic levels [3]. In a recent study of sera derived from 288 non-metastatic breast cancer patients prior to therapy, the presence of increased titers of MUC1 specific IgG antibodies was shown to be an independent predictor of improved overall survival ($p = 0.002$, median follow-up 148 months) [4]. Overexpression of a protein also enhances immunogenicity. The HER2 protein is overexpressed in approximately 25% of all breast cancers. Evaluating HER2 specific T-cells and serum antibodies derived from over 100 women with varying levels of HER2 overexpression in their breast cancers, investigators determined that both HER2 specific T-cells and antibody levels increased with increasing levels of expression of the HER2 protein [5]. Multivariate analyses demonstrated that

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Abbreviations

HER2	human epidermal receptor 2
IFN-g	interferon gamma
IL	interleukin
MUC	mucin
NK	natural killer
PD	programmed death
PDL	programmed death ligand
Th	T helper
TIL	tumor infiltrating lymphocytes

only the level of HER2 protein overexpression was a significant predictor for the development of an immune response ($p = 0.016$). Similar to MUC1, HER2 specific antibodies can also show clinical importance. Investigators assessed HER2 antibody levels in 500 untreated patients with invasive breast carcinoma [6]. The presence of HER2 specific antibodies was associated with a significantly improved recurrence free survival as compared to patients who did not have detectable HER2 specific serum antibodies ($p = 0.015$). This association was significant in both univariate and multi-variate analysis. Studies have shown that endogenous HER2 specific antibodies can bind to intact protein expressed on breast cancer cells and impact signaling through the tyrosine kinase [7]. Indeed, incubation of HER2+ breast cancer cells with patient antibodies specific for an epitope in the HER2 extracellular domain could significantly inhibit growth of those cells in 3-dimensional culture.

Breast cancer patients can have low levels of antigen specific T-cells capable of recognizing their tumor in the peripheral blood [8]. Recent studies, however, have demonstrated that the development of a polyclonal autoantibody response is a very early event in breast cancer pathogenesis. Utilizing case and control sera from the Women's Health Initiative Observational Study, investigators probed for antibody immunity in sera derived from women 6 months prior to a breast cancer diagnosis compared to controls [10]. Women who would go on to develop breast cancer had dozens of autoantibodies specific for tumor associated proteins in their sera. Moreover, those antibodies were directed against proteins in growth regulatory networks that could be pathognomonic for their disease. As an example, in patients who eventually developed triple negative breast cancer, autoantibodies were found 6 months before diagnosis directed against proteins in the p53, BRCA1, and cytokeratin networks [10]. In post-menopausal women the autoantibody repertoire of those who would go on to develop breast cancer marked glycolysis and spliceosome protein networks [11]. These studies highlight the inherent immunogenicity of breast cancer, but also underscore a defect in the immune response. Breast cancer is defined by a Type II immune microenvironment that supports the development of a primary antibody response, but such an environment will not support the proliferation and maintenance of CD8 cytotoxic T-cells which are needed for tumor eradication.

2. The tumor immune environment in breast cancer

The identification of specific tumor antigens allowed researchers to begin to probe the tumor specific immune response in breast cancer and determine whether there are any functional defects in that response. An evaluation of cytokine release by antigen specific T-cells directed against HER2, CEA, and MAGE3 demonstrated that breast cancer patients were lacking CD4 T-cells that were capable of secreting IFN-g [9]. Additional investigations have shown that as breast cancer progresses, Type I immune responses that had developed to breast cancer antigens such as HER2 or HER3 began to diminish and eventually decrease to the point of no detection [12]. Loss of immunity was associated with persistent disease after standard therapy. High levels of IL-10 in the tumor microenvironment are associated with incomplete response after neoadjuvant chemotherapy [13]. Studies in murine models of mammary cancer have shown that the presence of Th2 CD4 T-cells and macrophage mediate resistance to radiotherapy [14]. These data suggest that immunotherapeutic strategies in breast cancer should have some focus on reversing the phenotype of the immune response to one that is rich in Type I cells; with T-helper 1 CD4 T-cells and cytotoxic T-cells which work together to promote tumor destruction.

An additional issue with the adaptive immune response in breast cancer is that breast cancer is poorly immunogenic. In the last decade, studies of tumor infiltrating lymphocytes (TIL) in breast cancer have shown that the higher the level of lymphocytic infiltration a patients has in their tumor, the better their outcome particularly for the triple negative breast cancer subtype. An evaluation of over 1000 patients in the neoadjuvant setting found that if patients demonstrated “lymphocyte predominant disease” these patients were more likely to achieve a pathologic complete response after neoadjuvant therapy than patients who did not demonstrate high levels of TIL [15]. An evaluation of two randomized Phase III adjuvant breast cancer trials for triple negative disease demonstrated that for every 10% increase in stromal TIL there was a significant reduction in the risk of disease recurrence, distant recurrence, and death [16]. Most recently, for HER2+ disease, analysis of TIL from the CLEOPATRA study (comparing the addition of either pertuzumab or placebo to first-line therapy with trastuzumab and docetaxel for patients with metastatic HER2+ breast cancer), each 10% increase in stromal TIL was also associated with a longer overall survival [17]. The level of TIL has a prognostic impact in some breast cancer patients and studies are ongoing to determine whether measurement of lymphocytic infiltrate should be incorporated into the standard staging of breast cancer.

Although TIL can be prognostic in breast cancer, there are clearly differences between subtypes. Both the incidence and magnitude of TIL can vary depending on the type of breast cancer [18]. The triple negative breast cancer is the subtype most likely to have the greatest number of patients with lymphocyte predominant disease. However, that number is still less than a quarter of all patients. Hormone receptor positive breast cancer is the subtype least likely not to be associated with a robust adaptive immune infiltrate. HER2+ disease is intermediate between the two. What is evident from these studies is that most patients have some level of an adaptive immune infiltrate in their tumors, but for the majority of patients that level of TIL is low. There is good evidence that TIL can be modulated. A retrospective analysis of tumor tissue taken from patients with HER2+ breast cancer before and after specific treatments demonstrated that patients had little to no evidence of Tbet + T-cells in their tumors prior to therapy [19]. Tbet is a marker for Type I T-cells. In this series, about half of the patients were treated with anthracycline based neoadjuvant therapy and half were treated with trastuzumab-taxane. The presence of T-bet + lymphocytes in the tumor after chemotherapy was significantly more frequent in patients treated with trastuzumab-taxane ($p = 0.0008$). As trastuzumab induces antibody dependent cell mediated cytotoxicity and can significantly increase IFN-g production by natural killer (NK) cells, the antibody is modulating the environment to begin to support Type I T-cells [20,21]. Trastuzumab can elicit these effects in both the adjuvant and metastatic setting [22]. There may be peripheral blood biomarker that can be used to reflect the predominance of Tbet + TIL. Investigators have

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