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NON-INVASIVE BREAST CANCER DIAGNOSIS AND TREATMENT (ES HWANG, SECTION EDITOR)

Treatment from within: Ductal Carcinoma as an Opportunity to Harness the Immune System

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Abstract

Purpose of Review Breast Ductal Carcinoma in Situ (DCIS) is an increasingly common diagnosis and already accounts for ~20% of screen-detected breast cancers. A subset of patients with DCIS will experience disease recurrence and some will die from breast cancer. Tailored strategies for treatment are lacking at this time. Human Epidermal Growth Factor Receptor 2 (HER2) is a tumor associated antigen that is shown to correlate with poorer outcomes among patients with early breast cancer, including DCIS. Significant interactions between the humoral and cellular branches of the immune system were observed in tumorigenesis of HER2-expressing lesions. These can be leveraged through administration vaccines to improve outcomes among patients with $HER2^+$ DCIS.

Recent Findings Pre-clinical and clinical data support that immune response supported not only by $CD8^+$ cytotoxic T cells but also $CD4^+$ helper T cells can lead to antitumor activity in DCIS. These early studies have demonstrated prolonged, broad, activation of the immune system, and with a favorable toxicity profile.

Summary As nuances in our understanding of immune responses to early breast cancer begin to unveil, there is growing momentum in the development of preventative strategies. Clinical trials assessing the efficacy of vaccines for the treatment of DCIS are forthcoming.

Keywords HER2 · Immunity · Ductal carcinoma in situ · Breast

Introduction

Breast Ductal Carcinoma in Situ (DCIS), generally thought to be a pre-invasive cancer, has variable biology and prognosis. [1] DCIS is an increasingly common diagnosis and accounts for ~20% of screen-detected breast cancers (BCs). [2] Overall, most DCIS is relatively indolent, has a predictable course and favorable outcomes when compared with invasive cancer (i.e., 20-year BC-specific mortality rate of ~ 3%). [3] The treatment after lumpectomy is often whole-breast radiation and endocrine therapy for patients with hormone receptor positive tumors, as these adjuvant treatments have shown reduced recurrence rates but not mortality. Notwithstanding the efficacy of currently available treatments, certain patients have higher absolute risk (AR) of BC recurrence followed by death. In a large observational study of 108,196 participants diagnosed with DCIS, African American women and age < 35 years presented greater risk of death from BC (AR 7.8% and 7.0%; respectively). [3] The immediate corollary to these observations is that a more nuanced treatment approach to DCIS is needed, as some high risk patients could benefit from treatment escalation and low risk patients from de-escalation of adjuvant therapies. [4] The latter is occurring in several observational studies de-escalating surgery. [5] Moreover, appropriate management of high risk DCIS allows for an opportunity for prevention of ipsilateral invasive cancer which shows strong correlation with BC death (HR 18.1, $P < 0.001$). [3]

Development of tailored treatments for DCIS is challenging for numerous reasons which pertain to the obvious hurdles associated with clinical trial design (e.g., need for large sample size in order to design appropriately powered studies). In

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addition, the molecular characterization of subsets of DCIS defined by targetable aberrations remains an elusive goal due to intra-tumoral heterogeneity. [6] In an attempt to better select patients for adjuvant treatment genomic assays are being developed. [7] For instance, a biologic signature-based decision-making tool called DCISionRT, has been developed to help risk stratify DCIS patients to help patients make decisions regarding adjuvant radiation. Increased risk DCIS, as measured by DCISionRT is ~3 times more likely to recur as invasive breast cancer than low-risk, and risk can be substantially reduced with radiation. [8] Also, an observational study of 327 patients with DCIS treated in the ECOG5194 trial with breast conserving surgery showed that Oncotype Dx score shows correlation with the risk of ipsilateral cancer recurrence even when adjusted for tamoxifen therapy (Hazard ratio 2.31, $P = 0.02$). [9] Oncotype Dx remains underutilized in routine clinical practice likely given the absence of prospective trials with robust clinical validity in predicting benefit from adjuvant therapies for DCIS. [10] The clinical validity of biomarkers able to impart predictive information on adjuvant radiation therapy remains to be assessed in prospective studies prior to routine use. [11]

In this scenario, the development of strategies aiming to activate cellular immunity against BC could lead to improved outcomes and a more targeted treatment approach which could avoid treatment-associated burden. BC vaccines are compelling as they can lead to more prolonged, broad, activation of the immune system, and they have been associated with favorable toxicity profile. [12•]

DCIS, along with other early or pre-invasive cancers, is particularly apt for this approach due to its indolent growth, and immune responsiveness, which may be at its highest in this pre-cancerous stage. While early studies of vaccine therapy focused on invasive or metastatic tumors, it has been shown that very early BC has the propensity to disseminate, possibly even more so than once it has become an overtly invasive cancer. [13] Both circulating tumor cells in the blood and disseminated cancer cells in the bone marrow can be detected in very early invasive ductal carcinoma. [13, 14] This, along with the lack of antitumor efficacy of vaccines in response to later tumor antigens, makes DCIS an appropriate target in which to develop vaccine therapy.

Immune Response to Breast Cancer

Immune system recognition of BC as non-self, is generally associated with better prognosis. Interactions between the two arms of the immune system (humoral and cellular) have been shown in $HER2^+$ breast cancer tumorigenesis. The humoral immune response is sensitized to a specific antigen and drives adaptive immunity, where memory B cells secrete targeted antibodies and cytotoxic $CD8^+$ T lymphocytes and helper $CD4^+$ T lymphocytes are recruited leading to cytotoxicity (Fig. 1). A large retrospective case-control study demonstrated that patients with high levels of auto-antibodies against HER2 have a decreased risk of developing both DCIS and invasive BC. [15] In parallel, evidence supporting the importance of cellular immune responses has been identified as potential prognostic and predictive indicators in $HER2^+$ BCs. This immune response is characterized pathologically by infiltration of tumors by tumor infiltrating lymphocytes (TILs). BC with high levels of TILs have better prognosis than those with low numbers of TILs, with 28% hazard reduction in triple-negative BC and HR 0.54 when tumors treated with anthracyclines demonstrate CD8 positivity. [16] As much as 48% of BCs demonstrate TIL positivity. [17] There is some predictability in the likelihood of TIL response in invasive BC as $HER2^+$ and TNBC have higher proclivity for TILs than hormone receptor (HR) positive BCs.

Cellular immunity, via TILs, is primarily mediated by cytotoxic $CD8^+$ T cells and helper $CD4^+$ T cells. [18, 19] The predominance of $CD4^+$ Th1 response over CD8 cytotoxic response goes against some bias in the belief that CD8 cytotoxic cells are the predominant driver in T cell cytotoxicity. Evidence suggests that $CD4^+$ Th1 cells are critical in the tumor for successful immunotherapy. [20] In transgenic mice expressing HER-2 oncogene in the mammary gland, stimulation with IL-12 results in a profound mammary infiltration of reactive cells, leading to delay in tumor development. [21] This response is abolished with $CD4^+$ depletion, but only halved with $CD8^+$ depletion. [22] $CD4^+$ Th1 cell cytokines (i.e., IFN-γ) directly induce senescence, a state of permanent tumor growth arrest, and regression in vivo, [23] while also being able to induce tumor cell apoptosis in vitro. [24] Through complex cellular signaling, [25] Th1 cytokines will shut down angiogenesis and chemokine expression, resulting in sustained tumor regression upon oncogene inactivation. [26–28] Thus, oncogene inactivation appears to induce senescence, apoptosis, and activates the immune system, while at the same time; the immune system may also be inducing senescence and apoptosis. Based on this idea, combined treatments of BC cell lines in vitro with Th1 cytokines TNF-α and IFN-γ may cause oncogene inactivation of HER2 and subsequent senescence and apoptosis. [23, 29]

Development of Immune Resistance in Tumorigenesis

During breast tumorigenesis, the immune system has decreasing responsiveness to certain breast-cancer associated antigens, including HER2. Despite continually looking less like "self" through mutagenesis, DCIS is shielded from the immune system through specific adaptations. Augmentation of HER2 immune response has shown to thwart development of

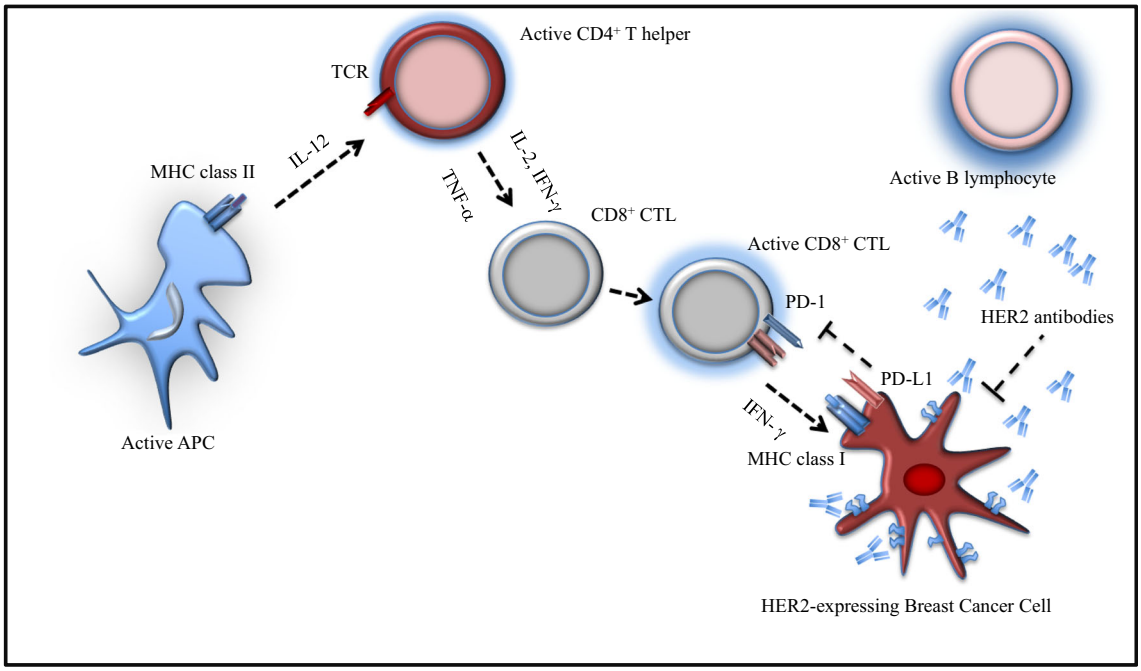

Fig. 1 Depiction of immune response to breast cancer expressing HER2. Legend: HER2 antigen is processed by APC leading to activation of cellular cytotoxic adaptive response (i.e., Th1 response mediated by $CD4^+$ helper lymphocytes). $CD8^+$ CTL can recognize HER2 antigen through MHC I leading to cytotoxic activity; breast cancer PD-L1 inhibitory activity and B cell activation and function are also shown. Line with arrow head depicts stimulatory effect and line with sharp ending depicts inhibitory effect. Abbreviations: Antigen Presenting Cells (APC), Cytotoxic T Cell (CTL), Human Epidermal Growth Factor 2 (HER2), Interleukin (IL), Interferon (IFN), Major Histocompatibility Complex (MHC), Program Cell Death Protein Ligand 1(PD-L1), Tumor Growth Factor (TGF), T Cell Receptor (TCR), Tumor Necrosis Factor (TNF)

HER2 tumor in vivo. HER2-directed MHC class II peptide-based vaccine has shown to suppress tumorigenesis from early stage of mammary carcinoma in MMTV-PyMT transgenic mice model. [30] At the clinical level, patients with advanced BCs have been found to have a significantly reduced development of T cells into Th1 cells, resulting in decreased production of Th1 cytokines. [31] These patients tend to have diminished response to neoadjuvant chemotherapy and poorer prognoses. [31] For instance, compared to healthy subjects, peripheral blood anti-HER2, and anti-HER3 $CD4^+$ T-helper 1 (Th1) response is significantly decreased in patients with DCIS, and it is decreased more so in patients with invasive cancer. [32] Low anti-HER2 $CD4^+$ Th1 in peripheral blood is associated with an increased rate of metastasis in $HER2^+$ patients. This may be because either there is a loss of the cells or they are leaving the blood entering the tumor thus serving more as a marker of response.

Other antigenic alterations in breast cancer cells, such as increased Programmed Death Ligand 1 (PD-L1) and Cytotoxic T Lymphocyte-Associated protein 4 (CTLA-4) decrease immune response and TIL. PD-L1 is a transmembrane protein expressed on tumor cells that decreases Type 1 immune response and is known to be upregulated in TNBC. Patients with the combination of decreased TILs and increased PD-L1 have worse prognosis than those with high TILs and low PD-L1. [33] In $HER2^+$ breast cancer, PD-L1 expression has been associated with a higher tumor grade and TILs. [34] An In vivo model, showed that PD-1 and CTLA-4 inhibition improves the immune-mediated effects of HER2 therapies through activation of $CD8^+$ T cells. [35, 36]

Indeed, the initial immune-response-promoting environment of pre-cancerous lesions evolving into an immune-suppressive environment, has been demonstrated in other cancers, such as the evolution of low-grade Intraductal Papillary Mucinous Neoplasm (IPMN) to invasive adenocarcinoma, [37] the progression of bronchial pre-malignant lesions, [38–40] Monoclonal Gammopathy of Undetermined Significance (MGUS) to Multiple Myeloma [41], and colon polyps developing into invasive adenocarcinoma. [42] During this period of immune activation in pre-cancerous lesions, dysplastic cells demonstrate tremendous heterogeneity in antigen presentation, driving immune activation and, importantly, selecting for cells that escape recognition. [43] This immunoediting selection process, which results in decreased antigen-derived activation, likely occurs via multiple mechanisms, including hypermethylation of promoter regions of antigens. [40] Mouse models have demonstrated that these early stage antigens in BC elicit more potent immune response than in more advanced tumors, and are likely more effective targets for immune therapy. [44] It is impossible to know how many

pre-cancerous lesions occur in a lifetime that demonstrated neoantigens that the immune system recognized and destroyed before becoming clinically detectable.

Simultaneously, the bone marrow T cell compartment leaves clues to the systemic immune reaction to pre-cancerous and cancerous lesions. Pre-malignant lesions demonstrate an enrichment of T cell factor 1 (TCF-1) expressing memory T cells which bear resemblance to stem-like T cells, having the ability to self-renew and produce more differentiated effector cells. This is not dissimilar to the enrichment seen in response to chronic viral infections. The resident T cell compartment demonstrates loss of these stem-like cells and increase in terminal effector T cells (demonstrated in progression of MGUS to Multiple Myeloma), suggesting an attrition of sorts. [41] What drives this attrition is unclear. One clue may be the increasing presence of myeloid derived suppressor cells (MDSCs) present in circulation, and at tumor sites, during evolution from pre-cancerous lesions to invasive cancers. Decreased spontaneous or vaccine-associated response to antigen is associated with increasing presence of MDSCs. [42] Understanding this escape or evasion of the immune system through adaptive mutation is key to re-activating the immune system to cancers to which it has been blinded.

Immune Barriers and Vaccine Therapy

Vaccination is a novel approach to increasing immune response to DCIS early in the course of disease. HER2 is a cancer-associated oncodriver antigen which is overexpressed/amplified in 30%–50% of cases of DCIS and has been associated with high-grade, shorter disease free survival (DFS), higher likelihood for microinvasion, and early metastasis. [1, 13, 45, 46] These cells have a propensity to act like stem cells, so if they do become invasive and disseminate, their concentration in the bone marrow is ~5-fold higher than that in the blood. Albeit rare, half of all mortalities from DCIS occur secondary to disseminated disease without local recurrence, suggesting that these malignant cells in the marrow may be ceding later metastases. Cancer cell positivity in these compartments is associated with decreased disease-specific survival, distant disease-free survival, and overall survival among patients with early breast cancer, re-enforcing the possible impact of improved therapy for DCIS. [47]

HER2 overexpression/amplification is predictive of benefit from HER2-targeted agents in a wide range of breast cancers including small HER2$^+$ BCs (i.e., < 1 cm). [48, 49] Many of these cancers develop resistance to HER2-targeted therapies, spawning trials of combination therapy to regain/prolong antitumor activity. At the cellular level, evidence supports the re-sensitization of resistant HER2$^+$ cancers in the presence of CD4$^+$ Th1 cells or Th1 cytokines. [50, 51] Furthermore, preliminary evidence already supports that active immune therapy with HER2 vaccines can induce regression of HER2$^+$ DCIS. [52, 53] Taken together these results supported that HER2 is a biomarker predictive of antitumor activity in small BCs including HER2$^+$ DCIS.

Activation of this CD4$^+$ TIL response can be achieved via treatment with type 1-polarized dendritic cells (DC1) pulsed with MHC class II HER2 peptides, which has been the most common vaccine studied for the treatment of patients with DCIS. Through either intralesional or intranodal injection of these HER2-pulsed DC1 cells patients have demonstrated a significant CD4$^+$ T cell response. [32]

Lowenfeld et al. conducted a phase I/II trial of 54 patients with HER2$^+$ BCs (including patients with tumors with immune histochemistry 2–3+ scores) were treated with six weekly injections of autologous DC1 HER2 vaccines. The pathologic complete response (pCR) rate was 28% among the 42 patients with DCIS. [54••] Peripheral blood HER2 CD4$^+$ and CD8$^+$ immune responses were observed in 81% of the patients. Patterns of response to HER2 DC1 vaccines have been explored. Patients with estrogen receptor (ER) negative DCIS had more potent response to vaccine than ER$^+$ patients as patients with ER$^+$ DCIS have decreased CD4$^+$ Th1 cytokine-induced metabolic suppression and decreased pathologic complete response (~4%). However, dual therapy with estrogen receptor blockade of ER$^+$ patients shows essentially equivalent pCR to ER$^-$ patients (29–31%) [55]. Thus far, this vaccination strategy has proven safe. Results of systematic review and meta-analysis of toxicity endpoints of HER2-targeted vaccines pooled data from eight prospective studies and 248 patients with HER2$^+$ BC (see Table 1). [12•] A total of six trials assessed the efficacy of DC1 vaccines and two of a recombinant HER2 peptide (dHER2). No grade 3–5 events were observed. At the most common adverse events were manageable, including infusion site reaction (23%), fever or chills (31%), and fatigue (33%). This may encourage combining with other anti-cancer strategies such as statin drugs and anti-estrogens. [55, 56]

It may be that many resistance mechanisms to targeted therapies relate to decreased tumor infiltration by lymphocytes. Recent data from our group also shows improved CD4+ and CD8+ cell tumor infiltration to checkpoint blockade with Class II HER2-DC1 vaccination. [57••] The results could support further development of immune strategies for the treatment of HER2$^+$ breast as monotherapy with PD-1 inhibitor (pembrolizumab) showed modest clinical benefit in an early phase clinical trial. [58•] It is also possible that development of HER2-directed immune therapies including vaccines may improve outcomes among patients with HER2$^-$ tumors exhibiting low levels of HER2 expression. For example, the HER2 antibody drug conjugate DS8201a has shown to improve outcomes of heavily pretreated patients with HER2$^+$ MBC and is now under development for treatment of patients with HER2- tumors (NCTNCT03734029). [59•] Based on this notion, other vaccine strategies (H2NVAC) are

Table 1 Selected clinical trials assessing the efficacy and toxicity profile of HER2-directed vaccines for the treatment of $HER2^+$ breast cancer

PMID	Author	Study phase	Stage	Evaluable patients, *n*	HER2 Vaccine
17293384	Czerniecki	2	DCIS and microinvasive	13	Dendritic cells
27965306	Lowenfeld	2	DCIS and I-III	54	Dendritic cells
22252842	Sharma	1	DCIS	29	Dendritic cells
26975189	Curigliano	2	Stage IV	40	dHER2
17822557	Morse	Pilot	Stages I-IV	6	Dendritic cells
22130160	Koski**	Pilot	DCIS	27	Dendritic cells
17704416	Park	1	Stage IV	18	Dendritic cells
26993131	Limentani	1	Stages I-III	61	dHER2

DCIS, Ductal Carcinoma In situ; dHER2, a truncated recombinant HER2/neu peptide; NA, Not Applicable; NR, Not Reported; PMID, PubMed Identification

under development for the treatment of DCIS with low expression of HER2 (NCT04144023).

Future Perspectives for Breast Cancer Preventive Vaccines

The development of preventive strategies in DCIS can help those patients with high risk from developing secondary local recurrence or metastatic disease. The clinical development of vaccines has the potential to fill the gap in targeted preventive strategies for these patients. There are obvious hurdles ahead such as: (i) the need to identify neoantigens or overexpressed proteins that can be effectively and safely targeted by vaccines and immune therapies, (ii) better understanding as how the immune response becomes compromised during breast tumorigenesis, and (iii) assessment of clinical validity of markers of immune response. In addition, in high risk DCIS lesions, we need better understanding of the relationship between the immune response and disseminated cancer cells and whether these can be eliminated or kept in a state of permanent tumor dormancy (senescence). Furthermore, the design of clinical trials will need to take into account the timing and safety of administration of vaccines with regard to pregnancies will need to be considered. Notwithstanding these challenges, there is now great interest in developing vaccines for DCIS (NCT02636582, NCT03793829) as well as invasive cancer (NCT02780401) as evidenced by the multiple trials ongoing. It should be noted however that breast cancer vaccines have been associated with much lower absolute risk of adverse events when compared to other immune therapies being developed for the treatment of cancer. Hence these are more appealing preventative strategies for DCIS. As the ultimate goal of research aims to achieve cure, the development of preventive vaccines for breast cancer is an important endeavor given that resistance to approved treatments is a virtually universal challenge faced by patients with metastatic recurrent disease.

Conclusion

The field of immune modulation for DCIS is relatively young but promising progress has been made. Tailored HER2 vaccines can result in improved anti-tumor immune responses that can impact DCIS lesions and improve outcomes. This may prove to be a cost-effective way to prevent breast cancer recurrence in a subset of patients with DCIS. Thus far, the latter strategy has shown more favorable benefit-toxicity ratio compared to other modalities of immunotherapies currently approved for the treatment of various types of cancer. [60]

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Compliance with Ethics Guidelines

Conflict of Interest Brian Czerniecki reports intellectual property on DC1 vaccine with ImmunoRestoration. Justin Wilkes declares no conflict of interest relevant to this manuscript. Ricardo Costa received consulting honoraria from Bristol Myers Squibb, Pfizer, Daiichi Sankyo; and recieved research grant from Bristol Meyers Squibb.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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- • Of importance
- •• Of major importance

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