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Restoring anti-oncogene Th1 responses with dendritic cell vaccines in HER2/neu-positive breast cancer: progress and potential

HER2/neu is expressed in the majority of *in situ* breast cancers, but maintained in 20–30% of invasive breast cancer (IBC). During breast tumorigenesis, there is a progressive loss of anti-HER2 CD4^{pos} Th1 (anti-HER2Th1) from benign to ductal carcinoma *in situ*, with almost complete loss in IBC. This anti-HER2Th1 response can predict response to neoadjuvant therapy, risk of recurrence and disease-free survival. Vaccines consisting of HER2-pulsed type I polarized dendritic cells (DC1) administered during ductal carcinoma *in situ* and early IBC can efficiently correct anti-HER2Th1 response and have clinical impact on the disease. In this review, we will discuss the role of anti-HER2Th1 response in the three phases of immunoediting during HER2 breast cancer development and opportunities for reversing these processes using DC1 vaccines alone or in combination with standard therapies. Correcting the anti-HER2Th1 response may represent an opportunity for improving outcomes and providing a path to eliminate escape variants.

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The rationale for targeting HER2

Although HER2 (ERBB2) is transiently expressed during fetal and normal breast development, as well as during breast growth in pregnancy, overexpression of HER2/neu can contribute to breast tumorigenesis. HER2/neu has become the most studied tumor-associated antigen (TAA), as it is a molecular oncogene overexpressed in 20–30% of invasive breast cancers (IBC) and in about 13–56% of ductal carcinoma *in situ* (DCIS) lesions [1].

HER2/neu is an immunogenic protein that elicits both humoral and cellular immune responses in patients with HER2^{pos} tumors, but patients with HER2/neu overexpressing tumors often demonstrate diminished existing immunity directed against the protein [2]. In IBC, HER2/neu is associated with a heightened risk for invasion,

suggesting a crucial role for HER2/neu in stimulating a tumorigenic microenvironment. Whether by ‘immunoediting’ or other immune evasion mechanisms, a diminished cellular immune response to HER2/neu in the tumor microenvironment of HER2^{pos} breast cancer (HER2^{pos}BC) is associated with poorer prognosis [2]. On the contrary, an increased cellular and humoral response against HER2/neu has been associated with decreased tumor development and improved outcomes [3]. Clinical implications of HER2/neu expression in DCIS (HER2^{pos}DCIS) are not as clear, but present data suggest that it predicts the presence of invasive foci [4], and increases risk of disease recurrence, although only approximately 20% of HER2^{pos}DCIS recurs as HER2^{pos}BC [5]. This low rate of HER2^{pos}DCIS conversion to HER2^{pos}IBC may be due to

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pathway and phenotype instability in HER2^{pos} cancer cells, with natural or induced immunity possibly shaping the resulting tumor phenotype [6].

With the exception of antibody-based HER2 targeting, HER2-directed immunotherapy in BC has not been as successful as initially expected, there has only been limited success in the setting of advanced disease where tumor cells have already escaped immunosurveillance. The future of HER2-targeted vaccination trials should be geared toward early-stage HER2^{pos}BC, potentially halting progression of HER2^{pos}DCIS to IBC (Figure 1) or situations to prevent recurrence. HER2 vaccines must be created based on immunologic principles of circumventing tolerance, a primary mechanism of escape, by strengthening the weak pre-existent anti-HER2 CD4^{pos} Th1 (anti-HER2Th1) immune response [6].

Loss of anti-HER2 CD4^{pos} Th1 during breast tumorigenesis

Recently, we have identified a progressive loss of anti-HER2Th1 immune response in HER2^{pos}BC patients relative to healthy controls, with an early and progressive decrease in immune competence in patients with HER2^{pos}BC [7]. Specifically, there is a loss of

anti-HER2Th1 response during breast tumorigenesis, where healthy patients have a strong anti-HER2Th1 immune response that is diminished in patients with DCIS and nearly absent in patients with IBC [7]. This supports the hypothesis that the level of circulating anti-HER2Th1 response correlates to immune response in patients with HER2^{pos}BC after treatment [7]. It is likely that this immune deficit begins in DCIS and continues to decline with disease progression. This depressed anti-HER2Th1 response is driven predominantly by Th1 phenotypes. In patients who had undergone neo-adjuvant therapies, preserved anti-HER2Th1 response was associated with pCR and improved disease-free survival (DFS), while a depressed response was found in patients without pathologic complete response (pCR) and recurrent disease after treatment [8]. These findings may potentially lead to the use of anti-HER2Th1 monitoring for patients receiving HER2-targeted therapies to identify those at risk of clinicopathologic failure.

Role of immune response & immunoediting during breast tumorigenesis

The breast parenchyma normally harbors immune cells such as cytolytic CD8^{pos} T cells, Th1 cells and NK cells

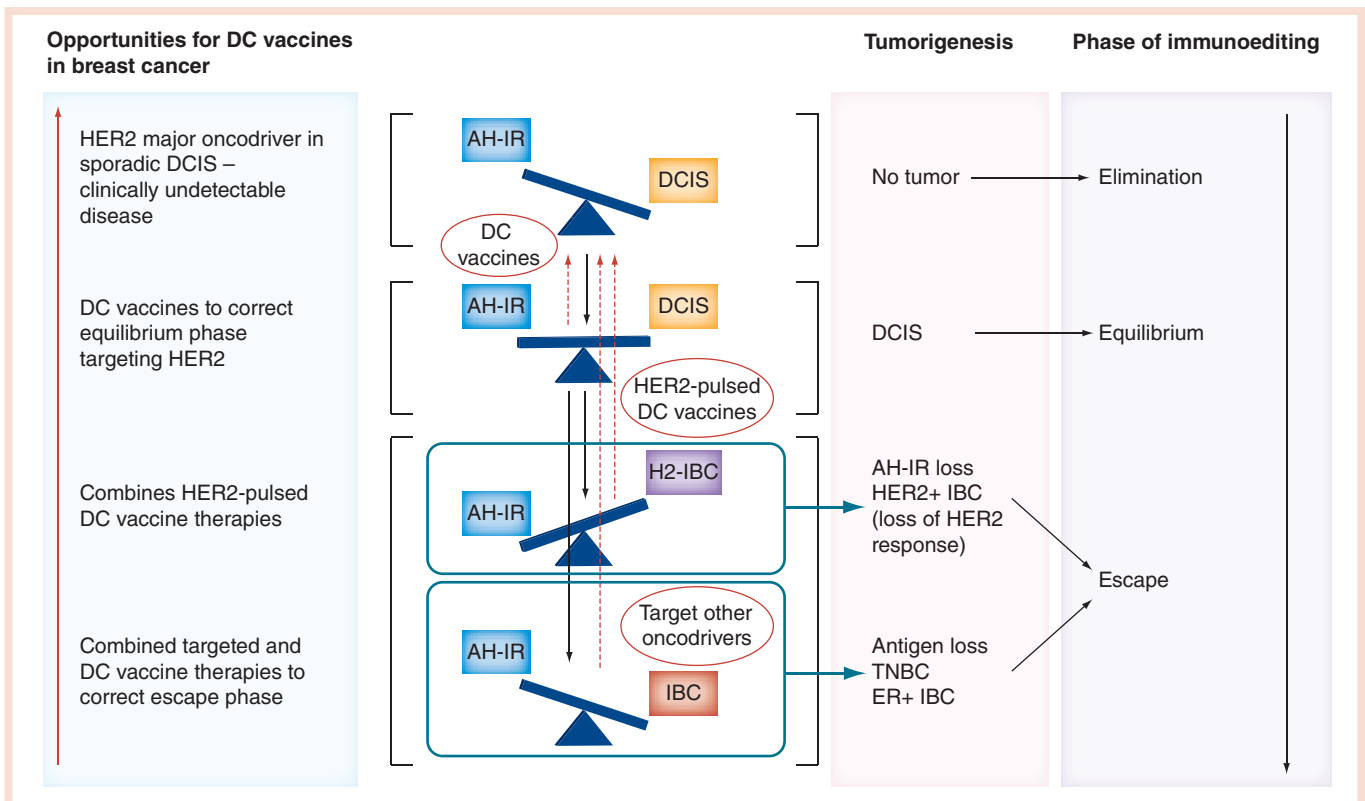


Figure 1. Immunoediting process in breast cancer with the potential sites of immunotherapy and targeted therapies in HER2-positive, triple-negative and ER-positive breast cancer. AH-IR: Anti-HER2 immune response; DC: Dendritic cell; DCIS: Ductal carcinoma *in situ*; ER + BC: Estrogen receptor positive breast cancer; H2IBC: HER2-positive invasive breast cancer; IBC: Invasive breast cancer; IR: Immune response; TNBC: Triple-negative breast cancer.

that aid in development, lactation, involution and may participate in immunosurveillance of the breast [9]. The presence of high-density CD8^{pos} T cells in tumors and nearby stroma is associated with improved prognosis in BC, suggesting that immune effector cells have identified the malignant cells and are actively mounting an appropriate immune response. Potent immune effectors act by eliminating tumors directly via exocytosis of cytotoxic granules, or indirectly, by producing INF- γ to activate Th1 immune response. It is thought that under immunosurveillance, the host eliminates evolving tumor cells continuously during elimination, until some malignant cells manage to outnumber and stress the immune response, evading immune recognition through a process described as ‘immunoediting’ [9]. Initially malignant clones are effectively eliminated by the immune response but as the tumor grows and expands a state of tumor equilibrium is reached (Figure 1). If this process becomes ineffective or fails, malignant cells may escape from immunological control to proliferate and manifest clinically as invasive and metastatic cancer [10].

The immunoediting phenomenon is evident during breast tumorigenesis mediated by HER2. HER2/neu is present in nonmalignant cells at basal levels, but overexpression leads to malignant transformation. We hypothesize that in the initial stage of malignant transformation, the mounted immune response is robust enough against this HER2/neu overexpression and may eliminate HER2^{pos}DCIS from becoming clinically apparent. If elimination is not achieved, HER2/neu overexpressing tumor cells and the immune response develop a state of equilibrium where the anti-HER2Th1 immune response is unable to completely eliminate malignant cells, but is able to prevent them from becoming invasive. This equilibrium state may be clinically manifested as HER2^{pos}DCIS presenting with microcalcifications on mammography [11]. These calcifications may be a reflection of necrosis caused by immune effectors cells (anti-HER2Th1 response) attempting to eliminate tumor cells during this period of homeostasis (Figure 1). The anti-HER2Th1 response although diminished remains somewhat active in DCIS [7]. This phase of tumor equilibrium in DCIS may be boosted using DC vaccines, which, by stimulating both CD4^{pos} and CD8^{pos} T cells and IL-12, can maximize INF- γ production and the functionality of Th1 cells thereby tip the equilibrium state toward elimination again (Figure 1).

If the process of equilibrium continues, the tumor may remain stagnant and suspended as DCIS with no disease progression to invasion. The alternative to equilibrium in DCIS would be tumor progression and developing invasion (IBC). If progression occurs, there

are likely two scenarios where the immune response would be ineffective at halting disease progression. First, the anti-HER2Th1 immune response further erodes over time [7] and becomes virtually absent allowing tumor escape. This hyporesponsive state may be due to a chronic exhaustion of the immune system leading to increased inhibitory receptors [11], decreased effector cytokines or impaired cytotoxicity, allowing for tumor escape. The result is continued expression of HER2 on the IBC. The second scenario is one where anti-HER2Th1 response is sustained, but tumor cells manage to evade immunosurveillance via phenotypic shifting. In this scenario, the immune system remains responsive, but tumor cells lose or down-regulate HER2/neu expression resulting in a new phenotype, such as triple-negative breast cancer (TNBC) or estrogen receptor positive BC (ER^{pos}BC) (Figure 1). There are numerous examples of ER^{pos} non-HER2 primary tumors developing systemic metastasis that are HER2^{pos} and also examples of HER2^{pos} primary tumors that lose expression of HER2 [12]. Using our understanding of immunoediting and immune escape, there are numerous opportunities to use DC vaccines to boost anti-HER2 immunity when needed and boost immunity against oncodrivers involved with escape to prevent recurrence.

Activation of type I polarized DC drive strong anticancer Th1 responses

Dendritic cells (DCs) are a heterogeneous group of specialized APCs [13], found in lymphoid and nonlymphoid tissue. They are classified according to their ability to elicit a specific immune response based on polarization of a T-cell response. DCs are typically found in immature form, and maturation is triggered by proinflammatory cytokines such as IL-1, IL-6, IFN- γ and TNF- α [14]. Additional signals such as bacterial lipopolysaccharide and IFN- γ drive IL-12 production leading to pulsed type I polarized DC1 [15]. Once maturation and activation occurs, migration to nearby lymph nodes is facilitated [16], where activated DCs deliver costimulatory signals essential for T-cell activation such as CD40, CD80, CD86, CD46 ligands and Toll-like receptors [17]. DCs signal activation and polarization of T cells is crucial for the differentiation of CD8^{pos} T cells into cytotoxic T lymphocytes (CTLs) [18] and for the polarization of CD4^{pos} T cells into their different effectors (Th1, Th2 and Th17) [19].

During BC development and progression, CD4^{pos} T cells may be a crucial element in the tumor ‘immunoediting’ process. Therefore, there is increasing interest in activating CD4^{pos} T cells because of their role as helpers in maintaining CD8^{pos} cells as functionally active [20] and indirect effects on other innate cells such

as NK, macrophages and DCs [21]. CD4^{pos} are significantly increased during breast cancer and the subsets dynamically change with disease progression. In early-tumor stages, Th1 cells are the dominant population of CD4^{pos} T cells, while in the advanced tumor stages, FoxP3^{pos} Treg and Th17 cells become the dominant populations [22]. Th1 cells secrete high-level cytokines such as IFN- γ and TNF- α , both key components of initiating Th1-polarized response and to the antitumor function of DC1 vaccines [19]. DC1s are very efficient in the presentation of antigens and production of IL-12 [23] that polarizes T cells toward the IFN- γ Th1 phenotype [24]. IL12 has multiple roles with inherent antitumor effect, antiangiogenic capabilities, activation of NK cells as well as enhancing adaptive immunity and improving sensitization to tumor antigens [25].

Despite their ability to prime an immune response, results have been disappointing when testing DC vaccines in late-stage BC. One reason may be because, in advanced disease, DCs are unable to mount a strong enough immune response to overcome the overwhelming immunosuppressive tumor microenvironment present in tumors that have escaped immunosurveillance. This may be one reason why, in early stages of BC, DC vaccines have enjoyed some clinical successes [3]. Another potential reason for the ineffectiveness of DC vaccines in late-stage disease is that inflammatory type DC vaccines have been used in most studies that do not necessarily drive strong Th1 response [15]. Increasing understanding of the dynamics of DCs activation, treatment of early-stage BC and different adjuvant settings will allow for improvements that warrant further investigation.

Effectiveness of DC1 vaccines administered in the equilibrium phase of DCIS

We suggest that HER2^{pos}DCIS represents the equilibrium phase of immunoediting, where Th1 immune response is in homeostasis with cancer cells in the ducts. Supporting this argument, data suggest that a strong Th1 proinflammatory signature in tumor microenvironment is associated with improved outcomes [13]. Therefore, if we can stimulate a strong Th1 immune response during the equilibrium phase of disease, it may be possible to prevent recurrence of the preinvasive lesions or halt disease progression to IBC.

A group of 27 patients with HER2^{pos}DCIS received HER2-pulsed DC1 vaccines in the neoadjuvant setting [2]. These patients all required surgical resection of their HER2^{pos}DCIS. In vaccinated subjects pCR was achieved in 18.5% of all patients (ER^{neg} = 40 vs ER^{pos} 5.9%) suggesting shift from equilibrium to elimination. Among those patients without a pCR in about 50% of HER2 expression was eradicated in residual

DCIS (sustained HER-2/neu expression ER^{neg} = 10% vs ER^{pos} = 47.1% [$p = 0.04$]). Postvaccination phenotypes were significantly different between ER^{pos} and ER^{neg} subjects ($p = 0.01$). The conversion of HER2^{pos}-DCIS to HER2^{neg} phenotypes after vaccination demonstrated the potential process of tumor escape in that a single target may not be sufficient to completely treat or eradicate disease. Interestingly, most of the DCIS lesions that changed were ER^{pos} and subsequent trials have demonstrated this group can have similar complete response rate by combining antiestrogen therapy with DC1 vaccination (Lowenfeld L, *Press Oncoimmunology* 2016, Manuscript submitted). This suggests that future experimental BC vaccines may be more effective by either targeting multiple oncodrivers or combining with other therapies that block additional pathways to prevent the escape phase of immunoediting [26].

Postimmunization, sensitization of T cells to at least one class II peptide was observed in 22 of 25 evaluable subjects, while 11 of 13 subjects were successfully sensitized to class I peptides. Perhaps most importantly, anti-HER2 peptide responses were observed up to 52 months postimmunization. These data show even in the presence of early-stage BC, such DC1 are potent inducers of durable Th1-polarized immunity, suggesting potential clinical value for development of cancer immunotherapy [27]. There is no significant difference in immune response detected systemically after vaccination in patients with HER2^{pos} or ER^{neg} and ER^{pos} tumors, but complete tumor regression was significantly more common in patients with ER^{neg} compared with ER^{pos}DCIS [28]. This proposed the concept of ER signaling as an escape pathway in HER^{pos}BC resistance to anti-HER2-targeted therapies. When looking at the effect of antiestrogen therapy in combination with DC1 vaccination, there is an increase rate of pCR and decreases recurrence in patients with ER^{pos}/HER^{pos}BC. Interestingly, the addition of antiestrogen therapy increased the anti-HER2Th1 response in regional sentinel nodes (Lowenfeld L, *Press Oncoimmunology* 2016, Manuscript submitted).

The increased interest in targeted vaccination against HER^{pos}BC has shown promising results. This data provide rationale for developing vaccinations to reduce recurrence in patients with ER^{neg} and HER2^{pos}-DCIS in whom there personalized therapies other than standard surgery and radiation do not exist. There is a large push in the community to develop novel, rationale-targeted therapies for DCIS that also provide protection against new breast events.

The other area where DC1 vaccines may have clinical benefit is in patients with HER2^{pos} IBC where the anti-HER2Th1 response is severely compromised (Figure 1) [7]. These patients have substantial risk of

recurrence and include those patients with residual disease following neoadjuvant therapy, and advanced stage III and IV patients that are no evidence of disease (NED). Ongoing research with DC and non-DC vaccines in early-stage trials are currently opened and results are pending. (Table 1). We have demonstrated that the anti-HER2 Th1 response can be restored using DC1 vaccines in these patients [29]. Whether or not restoration of Th1 response translates to diminished recurrence must await larger trials.

Use of DC1 vaccines during tumor escape in combination with anti-HER2 therapies

Loss of anti-HER2Th1 immune response is associated with lack of pCR to adjuvant chemotherapy and HER2-targeted therapies [7] as well as predicts increased of recurrence and diminished DFS [8]. Though DC vaccination has not yet proven to be an effective treatment of BC, other adjuvant treatment modalities have been shown to aid in promoting the immune response to tumor cells when given in combination with DC vaccines. These treatments often work synergistically with vaccination by aiding T-cell recognition of tumor cells. Some therapies, such as chemotherapy or

radiation, may induce changes in the tumor microenvironment, allowing a more complete response to DC vaccination. In combination with other therapies, such as HER2-targeted therapies, DC vaccination can aid in overcoming resistance [29,30]. Hence, pCR may be best achieved using combination therapy with DC vaccination (Figure 2).

Adjuvant chemotherapy

The tumor microenvironment may play a role in the recognition and response of the immune system to tumor cells. It has been observed that a high level of lymphocytic infiltration in breast tumors predicts a better prognosis and a better response to chemotherapy [31]. Chemotherapy has the potential to augment the tumor microenvironment by increasing CD4^{pos} T-cell infiltration (Figure 2), demonstrating a positive correlation to pCR [32]. Because vaccination, especially with DCs, can stimulate activation of T cells and potentially improve lymphocytic infiltration of breast tumors, concomitant or sequential administration of vaccines with low-dose chemotherapy may improve treatment outcomes. Sequential administration of a low dose of the chemotherapeutic drug, cyclo-

Table 1. Clinical trials of immunotherapy in early-stage breast cancer.

Phase	Study	Vaccine type	NCI identifier	Status
I	Folate receptor binding peptide vaccine	Peptide/protein	NCT02019524	Open
I	GP2-GM-CSF versus AE37 + GM-CSF versus GM-CSF alone	Peptide/protein	NCT00524277	Open
I	NY-EOS-1 vaccine + sirolimus	Peptide/protein	NCT01522820	Open
I	MUC1-peptide for triple-negative breast cancer	Peptide/protein	NCT00986609	Open
II	Trastuzumab + GM-CSF/HER-2 E75 peptide	Peptide/Protein	NCT01570036	Open
III	NeuVax TM	Peptide/protein	NCT01479244	Open
I	Pilot-breast cancer vaccine + Poly-ICLC	Celullar vaccine	NCT01532960	Open
II	GSK2302024A (Wilms tumor 1-specific therapy) + adjuvant therapy	Celullar vaccine	NCT01220128	Open
II	Preoperative cryotherapy +/- ipilimumab (anti-CTLA-4)	Checkpoint inhibitors	NCT01502592	Open
I/II	HER-2/Neu-pulsed DC1 vaccine combined with trastuzumab for patients with DCIS	Pulsed dendritic cells	NCT02336984	Open
I/II	Randomized trial of HER-2/Neu-pulsed DC1 vaccine for patients with DCIS	Pulsed dendritic cells	NCT02061332	Open
I/II	Nelipepimut-S E75 (HER2) NeuVax TM	Peptide/protein		Closed
I/II	GP2 + GM-CSF	Peptide/protein		Closed
	AE37 + GM-CSF	Peptide/protein		Closed
	AE37/GP2 + GM-CSF	Peptide/protein		Closed
I	A HER-2/Neu-pulsed DC1 vaccine for patients with DCIS	Pulsed dendritic cells	NCT00107211	Closed

DCIS: Ductal carcinoma *in situ*; MUC-1: Mucin-1.

phosphamide, along with vaccination against HER2 has been shown to induce *de novo* HER2-specific T helper-dependent immunity [33]. Low-dose doxorubicin, paclitaxel and methotrexate have also been shown to enhance HER2-specific immunity with vaccination as well as stimulate DC maturation and differentiation [33–35]. Therefore, chemotherapy may be better utilized in order to maximize the effectiveness of the antitumor immune response.

Combinations of vaccines with HER2-directed therapy

Trastuzumab is a monoclonal antibody that targets the HER2/neu protein by binding to the extracellular domain, triggering HER2 internalization and degradation. The use of trastuzumab induces passive immunity to HER2, although a major limitation of this therapy after continued use is the development of drug resistance. About 70% of patients who initially respond to therapy experience progression of the disease within approximately a year, suggesting the development of an acquired resistance to the antibody [36]. Patients undergoing neoadjuvant trastuzumab therapy plus chemotherapy may not show pCR due to depressed anti-HER2Th1 cell response [29]. Conversely, combination use of trastuzumab with vaccination against HER2 can significantly sensitize CTLs to tumor cells expressing the peptide and result in increased tumor cell lysis specific to HER2 [30,37]. In addition, it has recently been demonstrated that the anti-HER2Th1 response is positively correlated with pCR in patients given neoadjuvant chemotherapy plus trastuzumab. Those patients with a depressed anti-HER2Th1 response did not show pCR, but it was shown that this could be augmented with use of HER2-targeted DC vaccination [29]. Additionally, there is a strong association between immune gene expression and DFS following treatment with adjuvant trastuzumab, suggesting that there is a subset of HER2^{pos} tumors with a high level of immunologic activity [38]. Thus, DC vaccination may prove to be a useful adjunct to trastuzumab therapy by restoring immune response to HER2.

Pertuzumab another anti-HER2 mAb, similar to trastuzumab, targets the extracellular dimerization domain of the HER2 receptor. Developed in attempts to combat resistance to trastuzumab, pertuzumab prevents HER2/HER3 heterodimerization [39,40]. Co-administration of the two therapies has been shown to enhance clinical effectiveness compared with either one administered singly [40,41]. Pertuzumab has not yet been studied in combination with vaccination against cancer, but, considering its similar mechanism of action and synergistic effects with trastuzumab,

DC vaccination could prove to be a clinically effective adjunct.

Similarly, lapatinib, a tyrosine kinase inhibitor (TKI) that disrupts the HER2/neu and EGFR, is used to treat HER2^{pos}BC. Like trastuzumab, patients may develop resistance fairly early. In attempts to combat this, vaccination against HER2 was given concomitantly with lapatinib in a clinical trial, which demonstrated safety of the combined treatment, but due to small sample size, no additional immunologic benefits were observed [42]. However, another TKI, axitinib, showed improved therapeutic efficacy when combined with DC vaccination in a preclinical model of murine melanoma [43]. Although DC vaccination combined with TKIs has not yet shown improved outcomes in breast cancer, improved anti-HER2 immunity acquired by vaccination may improve pCR and overcome resistance seen with continued use of TKIs [29,37].

Radiation

Radiation is an important part of anticancer therapy, with nearly two-thirds of patients with cancer receiving it at some point during the course of their treatment [44]. Radiation is known for its ability to damage DNA of tumor cells, eventually causing enough injury to prohibit cellular proliferation and/or cause tumor cell death. Along with the antitumor properties that radiation has on its own, it may also have the ability to amplify the effects induction of apoptosis and necrosis; so APCs are ultimately attracted to this environment. This radiation-induced tumor microenvironment increases expression of Fas, MHC class I molecules and several other cell surface proteins [45], which provoke a DC-mediated antigen-specific immune response [46], therefore stimulating a CTL response. Correspondingly, the goal of vaccination is to induce an antigen-specific immune response to tumor cells, so addition of radiotherapy to vaccination should have a synergistic effect. This effect has been shown in a preclinical model of subcutaneous murine colorectal carcinoma where, after combination therapy of local radiation and vaccination, there was a massive infiltration of T cells that was not seen in either modality alone [47]. Prostate cancer patients treated with radiotherapy in combination of anti-PSA DC vaccines were able to increase immune response against PSA, but there was no clear synergistic effect of the combination of radiation with vaccination [45]. At this point, there have been no studies examining the effect of radiation therapy in combination with DC vaccines in the treatment of BC, however the ability of radiation to enhance MHC class I expression and consequently CTL infiltration of tumor has been shown in other forms of cancer immunotherapy using monoclonal antibodies against molecules such as cytotoxic T-lymphocyte associated

tion of DC–T-cell interactions, restore APC function, repair T-cell function by increasing IFN- γ production, promoting T-cell proliferation [52] and T-cell targeting of tumors (Figure 2).

The restoration of T-cell function with the use of mAb against these checkpoint inhibitors presents a synergistic opportunity for administration of DC vaccines. Using PD-1 mAb in conjunction with DC vaccination has shown an increase in CD4^{pos} and CD8^{pos} T-cell responses [56]. Along with restoring function of disabled immune cells, blocking the PD-1/PD-L1 checkpoint may further enhance the effectiveness of DC vaccination by preventing suppression of DC maturation [52]. In a murine breast cancer model, administration of a monoclonal antibody against PD-L1 in combination with DC vaccination induced more potent protective immunity than just DC vaccination alone [52]. Anti-HER2 DC vaccination combined with anti-CTLA4 therapy in a murine mammary carcinoma model also exhibited a significant increase in the frequency of tumor infiltrating CD4^{pos} and CD8^{pos} T cells [62]. A probable mechanism for the success in this therapeutic combination is the restoration and promotion of a robust Th1 response, encouraging cytotoxic CD8^{pos} T-cell response and infiltration into the tumor [62]. Checkpoint inhibitors and DC vaccination seem to be an advantageous synergistic combination for activation of the immune system in cancer therapy for which there are ongoing trials [58]. Ultimately, the immune response needs help targeting oncodrivers, and the combination of checkpoint inhibitors with DC vaccines may prove to be the most successful combination.

DC vaccines for breast cancer escape with loss of HER2 onco-driver expression

Genetic profiling has allowed us to identify different oncodrivers associated with IBC as well as the four principal subtypes. HER2^{pos} expressing tumor cells, as previously mentioned, may undergo phenotypic shifting or loss of immune response under anti-HER2 pressure (Figure 1) which may allow for disease progression to HER2^{neg}IBC with subtypes such as basal like (TNBC) or Luminal A (ER^{pos}BC). With this in mind, modulating the immune responses using DC pulsed with antigens against other oncodrivers may aid in eliminating residual BC cells and prevent recurrence of escape variants that lose HER2/neu expression. We will discuss some of the potential onco-driver targets in the different subtypes of BC.

ER-positive breast cancer oncodrivers MUC1

Epithelial Mucin-1 (MUC1) is a large transmembrane protein and is the most widely expressed of the mucins,

located on the apical surface of human epithelial cells lining glands or ducts. It is an overexpressed antigen found in about 90% of BC and is a cancer-specific class of vaccine target [63]. Elevated levels of MUC1 on the tumor have been associated with invasiveness, tumor growth, metastatic properties leading to poor prognosis in colon, pancreas, breast and bladder cancer [64,65]. MUC1 has been found to stimulate ER-mediated transcription, antagonize tamoxifen and contribute to ER-induced growth and survival of BC cells [66]. These findings support for the idea that MUC1 is crucial to ER function in BC. It contributes to tumorigenesis by reducing degradation of EGFR, increasing cell proliferation and inhibiting cell death by protecting against oxidative and genotoxic stress [67–69]. MUC1 has been studied as a target in cancer immunotherapy due to the ability to induce humoral immune responses in healthy subjects and in cancer patients [70]. Patients vaccinated with MUC1 core peptide-pulsed DC vaccines with MUC1-positive cancer were also found to have a reduction in tumor size or tumor marker levels as well as prolonged survival, suggesting that MUC1 is sufficiently immunogenic to be used as a target for DC vaccination [71]. More recently, MUC1 peptide vaccine study was able to induce INF gamma CD4^{pos} and CD8^{pos} T cells that recognized tumor-associated MUC1. Although only T-cell preactivation outside the tumor bed, either in culture or by repetitive vaccination, showed to overcome escape phase of tumor ‘immunoediting’ [72].

HER2-positive breast cancer – other oncodrivers hTERT

Human telomerase reverse transcriptase (hTERT) expression has been found to be increased in radio-resistant HER2^{pos}BC, while HER2 reduction has been shown to cause hTERT/telomerase activity (TA) downregulation. A widely expressed tumor antigen, hTERT, is expressed in more than 85% of all human cancers [73] and absent in most normal human cells [73], making it a favorable target for immunotherapy. hTERT is not a cellular growth receptor or signal transducer, but rather overexpression, prolongs tumor cellular survival by maintaining chromosomal integrity and protecting telomeric DNA [74]. TA has been found in up to 75% of breast carcinoma *in situ* lesions. Proto-oncogenes and growth factors, such as p53 and HER2, have been associated with the regulation of TA. Studies have shown that HER2 mediates hTERT expression through activation of NF- κ B and c-myc [75]. In the setting of immunotherapy, it has been observed that the hTERT peptide, I540, binds with high affinity to HLA-A2, and can be used to generate specific CTLs *in vitro* that lyse a wide range of hTERT-positive tumor cell lines [73]. Overall, hTERT-specific

immunity has been achieved with induction of CD8^{pos} tumor infiltrating lymphocytes [76,77]. Although there was some immunity to the TAA peptide hTERT, trials have not yet proven DC vaccination against hTERT to be an effective therapeutic strategy.

HER3

HER3 is another member of the EGFR family. Some studies have suggested that combination of HER2 and HER3 are crucial in cell growth, tumorigenesis and directly contribute in acquired resistance to therapies in HER2^{pos}BC and ER^{pos}BC. Although HER3 overexpression and activation is seen in invasive BC, a few studies have looked at it in the setting of DCIS, in which HER2^{pos} status is frequently seen [78]. HER3 can channel ErbB signaling to PI3K pathway leading to its adeptness to favor tumor growth. Murine models have shown that overexpression of HER3 is seen in conjunction with high levels of HER2 [79]. This is thought to occur via gene amplification, transcription or translation. In cells with high HER2 and HER3, downregulation of HER3 affects both receptors, implying association of the two for signal maintenance [79]. This may have impact in HER2-targeted therapies and provides resistance against pertuzumab, mTOR inhibitors and tamoxifen. In the case of tamoxifen, downregulation of ERBB3 in MCF-7 cells rendered resistant by HER2 overexpression promotes Tamoxifen-induced apoptosis. This relationship allows for a chance at improving efficacy of HER2-targeted therapies, by hindering linking of HER2 to HER3 potentially using DC vaccines. HER-3 expression is also a poor prognostic factor in TNBC [80] as it can heterodimerize with EGFR as well as transduce signals with c-MET.

Triple-negative breast cancer oncodrivers

c-MET & EGFR

c-MET and EGFR amplification and overexpression have been associated with TNBC and basal-like subgroups. EGFR is found in 50% of BC and c-MET is overexpressed in 20–30% of cases. c-MET is a tyrosine kinase receptor that binds to ligand EGFR or HGF for organ development, but when an anomalous activation occurs it can lead to tumor development [81]. Overexpression has been associated with increasing tumor size, increased nodal involvement [82], decreased DFS, decreased overall survival and poor response to chemotherapy [83].

c-MET amplification is associated with treatment failure to trastuzumab and shorter time to disease progression [84] in HER2^{pos}BC, and resistance to anti-EGFR therapies in TNBC [85]. Currently, Phase II trials of anti-c-MET therapies are underway in the set-

ting of advanced BC using targeted therapies such as tyrosine kinase inhibitors and monoclonal antibodies.

Combined c-MET/EGFR therapies have been studied in head and neck, lung and colon cancer and more recently for TNBC. EGFR/MET inhibition is synergic and therefore targeting both may provide for insight to establishing an effective therapeutic strategy in the setting on TNBC where limited therapies are available [85]. This role of c-MET and EGFR in BC merits further investigation and potentially using them as a DC vaccine target to block both pathways.

P53

p53 is a tumor suppressor gene, regulator of the cell cycle, cellular proliferation and apoptosis, which is found to be mutated in 25% of BC. It is most commonly seen in TNBC in about 50–80% [86]. Overexpression is associated with poor response to chemotherapy [87], DFS and overall survival [88]. Tumor cells are able to bypass the G1 checkpoint and complete the cell cycle, even in the presence of DNA damage in the setting of a p53 mutation [86]. Th1 response is significantly more prevalent in patients with tumors exhibiting high expression of p53. With this in mind, future immunotherapeutic approaches may potentially be geared toward Th1 polarization to allow a more effective immunotherapy in patients with advanced disease with p53 mutation [89].

Conclusion

Cancer immunotherapy is an important feature in the rapidly changing landscape of cancer treatment. Because breast cancer may represent a classic example of immunoediting, DC-based cancer vaccines can harness the potential to reinvigorate the immune system, moving toward elimination of tumor cells and shifting away from the equilibrium and escape stages. However, a one-size-fits-all theory will not likely be possible with DC vaccines. The use of vaccines in early stages of breast cancer, such as in DCIS, has claimed more success than in late-stage use, likely because, in DCIS, the tumor cells and the immune response have achieved a state of equilibrium. Vaccines may be able to more readily tip the scales in favor of the immune system, thereby eliminating equilibrium and moving toward elimination of the tumor. Although DC vaccines have enjoyed some success in early stages of breast cancer, they still have been limited success when used in later stages. This limited success has shifted focus toward using DC vaccines in the adjuvant setting to prevent recurrence. Vaccination in combination with targeted molecular and immune therapies that provide an additive or synergistic response, as well as using DC with cytotoxic therapies such as chemotherapy and radiation

remain underexplored. Combinations with checkpoint inhibitors are rapidly moving forward. DC vaccination therapy may also be used to target numerous TAAs, giving it the potential to be a personalized tumor treatment of tumor immune escape for essentially any phenotypic subtype of breast cancers. The versatility of DC vaccines lends itself to being a powerful tool in the future of cancer therapy.

Future perspective

In the past, monotherapy with DC vaccines did not meet the expectations as a standalone therapy in

advanced breast cancer. As we move forward, the focus of DC vaccines is being shifted to early disease and use in adjuvant settings. In addition, DC vaccines are being incorporated in combination with conventional therapies such as radiation, chemotherapy and antiestrogen therapy as well as in combination with other immune adjuvants in the treatment of early-stage breast cancer. It is presumed that such uses of DC vaccines will reduce recurrence and could potentially be developed for primary prevention. The synergistic effects will yield improved outcomes as the era of personalized medicine continues.

Executive summary

Targeting HER2 & loss of anti-HER2

- HER2/neu is associated with a heightened risk for invasion and metastasis, suggesting a crucial role for HER2/neu during breast tumorigenesis. We have observed a progressive loss of anti-HER2 CD4^{pos} Th1 (anti-HER2Th1) immune response during tumorigenesis in patients with HER-2 expressing breast cancer. HER2 vaccines can restore anti-HER2Th1 immune responses.

Role of immune response & immunoediting during breast tumorigenesis

- Since healthy women have robust anti-HER-2 CD4 Th1 responses, we hypothesize that in the initial stage of malignant transformation, the mounted immune response is robust enough against this HER2/neu overexpression and may eliminate HER2^{pos}DCIS or invasive breast cancer (IBC) from becoming clinically apparent. If elimination is not achieved, HER2/neu overexpressing tumor cells and the immune response develop into a stalemate of equilibrium where the anti-HER2Th1 immune response is unable to completely eliminate malignant cells, but is able to prevent them from becoming invasive. This equilibrium state may be clinically manifested as HER2^{pos}DCIS and may remain in this state of equilibrium and the anti-HER-2 Th1 response further erodes allowing tumor escape as HER-2^{pos}IBC or anti-HER2Th1 response is sustained but ineffectual and tumor cells manage to evade immunosurveillance via phenotypic shifting.

Activation of type I polarized DC drive strong anticancer Th1 responses

- CD4^{pos} T cells may be a crucial element in the antitumor immune response because of their role as helpers in keeping CD8^{pos} cells functionally active. Th1 cells secrete high-level cytokines such as IFN- γ and TNF- α , both key components in antitumor function. These cytokines can induce apoptosis of HER-2 expressing breast cancer cells and upregulate MHC class I expression making the cells more susceptible to CD8-mediated cytotoxicity. Dendritic cell (DC) polarized toward high levels of IL-12 production specifically drive anti-HER-2 CD4 Th1.

Effectiveness of DC1 vaccines administered in the equilibrium phase of DCIS

- We suggest that HER2^{pos}DCIS represents the equilibrium phase of immunoediting where Th1 immune response is in homeostasis with cancer cells in the ducts. Stimulating a strong Th1 immune response may lead to eradication or return to the elimination phase. Results from ductal carcinoma *in situ* patients treated with HER-2-pulsed DC1 demonstrated that some patients who received HER2-pulsed DC1 vaccines alone achieved pCR. In addition, others have remaining tumor with loss of HER-2 suggesting tumors can escape. Combining vaccines targeting multiple oncodrivers or combination therapies may prevent immunoediting.

Use of DC1 vaccines during tumor escape in combination with anti-HER2 therapies

- Persistent diminished anti-HER2Th1 immune response is associated with lack of pCR to neoadjuvant chemotherapy and HER2-targeted therapies. Ongoing clinical trials are being performed to assess whether restoring anti-HER-2 CD4 Th1 can prevent recurrence or combined with initial therapies to cause more complete responses to standard therapy. Combining the anti-HER-2 therapies and other immune modulating agents such as checkpoint inhibitors are subjects of future trials to augment effective anti-HER-2 CD4 Th1 responses.

DC vaccines for breast cancer escape with loss of HER2 oncodriver expression

- Ineffectual anti-HER-2 Th1 responses may lead HER2^{pos} expressing tumor cells to undergo phenotypic shifting or loss of immune response under anti-HER2 pressure which may allow for disease progression to HER2^{neg}IBC with subtypes such as basal like (triple-negative breast cancer) or Luminal A (ER^{pos}BC). Modulating immune responses using DC pulsed with antigens against other oncodrivers such as Mucin-1, c-MET, human telomerase reverse transcriptase, HER3 may aid in eliminating residual BC cells and prevent recurrence of escape variants that lose HER2/neu expression.

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