

REVIEW ARTICLE

Cancer Vaccines Targeting HER2/neu for Early Breast Cancer

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Recent studies of immune responses to pathogens have identified pathogen-associated molecular patterns recognized by the innate immune system through specialized receptors called toll-like receptors (TLRs). Signaling through these receptors initiates robust immune responses. By exploiting TLR signaling pathways, immunity to tumor-associated antigens may be generated. Many tumor-associated antigens are involved in the regulation of tumor phenotype or carcinogenesis. Immune targeting of these antigens may either alter the tumor phenotype, yielding a more treatable tumor, or eradicate early tumor stem cells preventing tumor formation. The oncoprotein HER2/neu, which is often overexpressed in ductal carcinoma *in situ* (DCIS), may provide such a target. Immune responses directed against HER2/neu may eliminate the disease, make tumors more amenable to anti-estrogen therapy, or prevent escape of hormone-resistant tumor phenotypes. Effective breast cancer prevention in preclinical studies utilizing murine HER2/neu transgenic models has stimulated interest in, and optimism regarding, protective

breast cancer vaccines in humans. Induction of anti-HER2/neu T cell (CD4+ and CD8+) and B cell responses has been demonstrated in an ongoing clinical study targeting HER2/neu using a TLR agonist-primed dendritic cell vaccine. Moreover, these vaccinations lead to reductions in both HER2/neu expression and extent of DCIS. HER2/neu expression and aromatase activity have recently been linked through the intermediary cyclooxygenase 2 (COX-2). This convergence between growth factor and hormone mediated pathways provides additional support for the notion that a significant number of breast cancers may be prevented through effective immune targeting of HER2/neu. As progress is made towards the development of vaccines for breast cancer prevention, the contributions of immune-mediated effector and inhibitory mechanisms to the pathogenesis of HER2/neu overexpressing breast cancer will need to be better understood.

Key Words: Breast neoplasms, Dendritic cells, HER2/neu, Vaccines

INTRODUCTION

During the past decade, significant progress has been made in the development of new therapies for the treatment of breast cancer. Hormone receptor modulation agents, aromatase inhibitors, and the monoclonal antibody trastuzumab, have been incorporated into treatment paradigms. Nonetheless, approximately 40% of women with breast cancer fail primary treatment.⁽¹⁾ Active immunization against breast cancer employing a number of different vaccine strategies has been investigated both in murine models and in humans. While the potential

benefits of active immunization are numerous and include low toxicity, high specificity, and induced immunologic memory, efficacy of tumor vaccines has thus far been quite limited. A number of factors likely contribute to this poor efficacy. Tumor associated antigens are most often overexpressed non-mutated self proteins and the ability to sensitize the immune system to recognize and respond to these antigens is, therefore, inherently limited. In addition to this tolerance, T cell dysfunction and suppressive T cell function appear to contribute to cancer pathogenesis and progression. Finally, some evidence supports a deleterious effect of the immune response in sculpting tumor phenotypes and selecting for more aggressive disease. In this article, we will review the foundation for recent efforts to design effective vaccines for breast cancer, including the rationale for selecting HER2/neu as a target, advantages of active immune therapies targeting

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HER2/neu over passive therapies such as trastuzumab, preclinical vaccination data from murine models, and strategies for overcoming the immunologic obstacles.

HER2/NEU AS A TARGET FOR IMMUNOTHERAPY

HER2/neu as a critical molecule in breast cancer pathogenesis

HER2/neu, a tyrosine kinase growth factor receptor encoded by the ErbB-2 gene, is a member of the epidermal growth factor receptor (EGFR) family. (2) This family also includes those receptors encoded by the genes ErbB-1, ErbB-3, and ErbB-4. Although no specific ligand for HER2/neu has been identified, HER-2/neu functionality is derived from the receptor's ability to form heterodimers with other members of the EGFR family, and homodimers. (3) Dimerization of HER2/neu initiates intracellular signals via numerous molecular pathways, including those involving PI3 kinase and MAP kinase, which influence cell-cycle and apoptotic regulation. (4)

Several observations support an important role for HER2/neu in breast cancer pathogenesis and progression. HER2/neu overexpression in transgenic mice results in development of breast cancers in all mammary glands. (5) In humans, HER2/neu overexpressing node-positive cancers are characterized by more rapid progression and relative refractoriness to chemotherapy, and are associated with poorer overall survival when compared to node-positive cancers that do not overexpress HER2/neu. (6) Significantly, 50–60% of high grade ductal carcinoma *in situ* (DCIS) lesions, compared to 20–30% of invasive breast cancers, overexpress HER2/neu, suggesting a more prominent role in early tumor progression. (7)

HER2/neu has attracted considerable attention as a therapeutic target in breast cancer therapy for a number of reasons: 1) As stated above, overexpression of HER2/neu correlates with poor prognosis in node positive patients, 2) HER2/neu overexpression appears to be involved in early breast carcinogenesis and 3) HER2/neu is differentially expressed in malignant cells compared to normal cells, providing for specificity of therapies targeting

HER2/neu. Targeting of HER2/neu using the recombinant humanized antibody trastuzumab in patients with high-risk node-positive disease has proven effective, particularly when combined with chemotherapy. (8) Thus selective targeting of HER2/neu for either treatment of early breast cancer or primary prevention, to interrupt breast cancer development may be promising.

Evidence for HER2/neu as a tumor antigen

Fundamental to efforts to immunologically target HER2/neu is the notion that HER2/neu is intrinsically immunogenic. This immunogenicity has been established in a number of studies. (9–11) Disis et al. (11) documented HER2/neu specific antibody formation in 11% (12/107) of patients with breast cancer. The antibody responses measured in some cases were quite substantial. Of note, the HER2/neu antibody-positive patients all had stage I or II disease. In a second study by Disis et al. (12) 45 women with advanced HER2/neu over-expressing breast or ovarian cancer were evaluated for specific T-cell and antibody immunity. Five of 45 patients (11%) had a specific T-cell response, while three of 45 (7%) had detectable HER2/neu specific antibodies. Kuerer et al. (10) documented proliferative cellular immune responses to HER2/neu-derived peptide in lymphocytes isolated from the axillary nodes of patients with breast cancer. HER2/neu peptides induced a predominantly T helper type 1 pattern of cytokine expression. Thus HER2/neu shows a degree of immunogenicity in at least some patients with breast cancer.

Passive immunologic targeting of HER2/neu using trastuzumab

The differential expression of HER2/neu by malignant and benign cells has allowed for passive targeting of HER2/neu with recombinant humanized antibodies (trastuzumab). (13) This agent has proven effective in patients with metastatic and high-risk invasive breast cancer, (14) particularly when combined with cytotoxic chemotherapy. (8, 15, 16) Trastuzumab is currently being tested in early breast cancer settings for the treatment of DCIS and may serve as an effective adjunct to other therapies in the treatment of early disease. (17) However, it remains to be deter-

mined whether passive transfer of trastuzumab will induce an active immune response that protect against long term development of breast cancer.

Despite some promising clinical data supporting the efficacy of trastuzumab, the agent has several significant limitations. Cardiotoxicity associated with trastuzumab is well described. Cardiac event ranging from subclinical decreases in ejection fraction to symptomatic congestive heart failure occur in up to 30% of patients.(18) Additionally, response rates to trastuzumab as monotherapy are low, and despite significantly improved rates of response when administered in combination with chemotherapy, the majority of patients treated with these combinations acquire resistance within a year.(5)

For these reasons, ongoing efforts to develop vaccines directed to HER2/neu are under way. Potential benefits of active immunization over passive immunotherapy include lower toxicity and induced immunologic memory that obviate the need for long term therapy.

ACTIVE VACCINATION TARGETING HER2/NEU AS A TREATMENT FOR BREAST CANCER

Several groups have developed vaccine strategies that target HER2/neu for patients with advanced cancer. There is substantial evidence that administration of peptides derived from HER2/neu can induce HER2/neu specific CD4+ and CD8+ T cells.(19–22) Although most of these vaccines have been utilized in the adjuvant setting, there is some evidence that induction of HER2/neu-specific CD8+ T cells may also help to prevent or delay recurrence of tumors.(21) Vaccine approaches using HER2/neu peptides not only induce anti-HER2/neu specific T cells, but also can generate anti-HER2/neu antibodies.(23) These endogenously produced antibodies may have therapeutic effects similar to those of trastuzumab. Peptide vaccines have frequently been administered with GM-CSF as an adjuvant. Whether the use of other adjuvant systems such as dendritic cells (DCs) and toll-like receptors (TLRs) agonists can improve efficacy has not been definitively addressed.(24)

CLUES TO DEVELOPING PROTECTIVE BREAST CANCER VACCINES

Murine data thus far suggest that anti-HER2/neu immunoglobulins are principle effectors of protective immunity, as is the cytokine IFN- γ . CD8+ CTLs appear to play a relatively dispensable role and CD4+ T cells play an early ‘helper’ role, rather than a later ‘effector’ role.(25–27) In human trials of patients with either HER2/neu overexpressing in invasive breast cancer or DCIS, there are a significant number of patients who develop HER2/neu antibodies following vaccination with HER2/neu peptides.(21,28) In addition, following vaccination with HER2/neu peptide pulsed DCs, there is evidence of complement fixing anti-HER2/neu antibody development in the majority of the patients.(28) Whether this will protect patients from subsequent recurrence remains to be determined. These findings suggest that the tools have been developed to begin contemplating protective breast cancer vaccination.

OBSTACLES TO EFFECTIVE ACTIVE IMMUNOTHERAPY

HER2/neu tolerance

Given that HER2/neu is a non-mutated self protein, it is not surprising that its mere overexpression does not lead to vigorous immune responses in most patients. A number of observations, however, point to alterations in the immune response to HER2/neu which may contribute to breast cancer pathogenesis. Studies have demonstrated blunted cellular immune responses to HER2/neu in the lymph nodes of patients with metastatic disease compared to those observed in the lymph nodes of patients without metastatic disease.(17) Czerniecki et al.(28) have identified the presence of HER2/neu-tetramer reactive CD8+ T cells in patients with HER2/neu overexpressing DCIS. These cells are impaired in their capacity to secrete IFN- γ in response to HER2/neu, and they highly express the B7 ligand, CTLA-4. The presence of these cells suggests that tumor antigen sensitization yields antigen specific T cells with anergic phenotypes. The

resultant tolerance allows the persistence of the HER2/neu overexpressing tumor and is a major obstacle for attempts to elicit an effective active immunologic response to HER2/neu.

Regulatory T cells

A subpopulation of CD4⁺ T cells with a known anergic suppressor phenotype has been identified; these cells have been termed regulatory T cells (T_{reg}) and are thought to play a role in the maintenance of peripheral tolerance.⁽¹⁷⁾ The suppressive function of T_{reg} requires cell-to-cell contact but a role for immuno-inhibitory cytokines including TGF- β and IL-10 has emerged.⁽²⁹⁾ Depletion of these cells in normal hosts results in autoimmunity. Treg also appear to play a role in cancer pathogenesis and progression, and limit responses to cancer vaccines.^(30,31) Evidence for this includes: 1) accumulation of Treg at tumor sites and in the peripheral blood of patients with cancer,⁽³²⁾ 2) enhanced anti-tumor immune responses through vaccination following depletion of T_{reg},⁽³³⁾ and 3) recent reports identifying tumor-specific Treg that suppress the proliferation of CD4⁺ effector cell.⁽³⁴⁾ CD4⁺ T cells that secrete TGF- β in response to HER2/neu were sometimes found in patients with HER2/neu overexpressing DCIS suggesting that tumor antigen sensitization may be accompanied by specific T_{reg} development.

Evidence for immunoediting in breast cancer

The term immunoediting describes the role that the immune response plays in sculpting a tumor's phenotype. Dunn et al.⁽³⁵⁾ and Schreiber⁽³⁶⁾ have argued that, in some instances, the immune system eliminates clones with greater immunogenicity and leaves less immunogenic ones to continue to grow. Such immunoediting may select for tumors with more aggressive phenotypes. In breast cancer there is some evidence (both preclinical and clinical) that immunoediting influences tumor phenotype. There are a small number of patients with breast cancers that do not express HER2/neu but nonetheless are found to have anti-HER2/neu antibodies in their blood.⁽¹¹⁾ Though the existence of these antibodies is suggestive of a role for immunoediting, this observation

has led some investigators to speculate that immune responses generated against HER2/neu select a non-HER2/neu-expressing phenotype. Only less than 5% of DCIS are triple negative tumors, whereas 20–30% of invasive cancers are triple negative. It is possible that these changes reflect immunologic selection for a more aggressive tumor phenotype and correlate with a progression to invasive cancer. Clearly, in situations where strong immune responses are generated, initial regression of tumors with subsequent regrowth is sometimes observed. The regrowth of tumors often heralds a transition to a more aggressive phenotype.⁽³⁷⁾ Understanding the events leading to this transition will be important if our goal is to eliminate all clones of residual breast cancer or, as we will discuss below, to alter the phenotype of breast tumors to render them curable.

STRATEGIES FOR OVERCOMING OBSTACLES TO EFFECTIVE ACTIVE IMMUNOTHERAPY

Taking advantage of innate immunity in cancer vaccines

If mechanisms of immunologic tolerance and suppression allow, and possibly even promote, the pathogenesis of breast cancer, novel strategies must be employed to elicit effective immune response against these cancers. The immune response evolved primarily to eliminate pathogens including viruses and bacteria. Medzhitov and Janeway⁽³⁸⁾ first suggested that the innate immune system has the capacity to respond to biomolecular patterns common to many pathogens. It is now known that this is achieved in part through specialized receptors known as TLRs that recognize a set of pathogen-associated molecular patterns (PAMPs).⁽³⁹⁾ These TLRs recognize relatively common pathogen-associated molecules such as lipopolysaccharide (TLR4), lipotechoic acid (TLR2), ds RNA (TLR3), ss RNA (TLR7 and 8), unmethylated deoxynucleotides (TLR9), and flagellin (TLR5). TLRs allow the innate immune system to rapidly respond to microbial invaders prior to the development of an adaptive immune response.^(38–40) Activation of these receptors initiates at least one of two intracellular pathways, the

MyD88 adaptor protein pathway(41) and the TRIF pathway(42), and ultimately leads to DC maturation through NF κ B activation and cytokine secretion.(43) Significantly, TLR agonist maturation of DCs results in proliferation of naive and effector T cells and an escape from T_{reg} control.(44)

Previous studies have established the importance of IL-12 secretion of DCs in sensitizing T cells to tumor.(45) Significantly, the pharmacologic agent R848, a TLR8 agonist, has the capacity to prime DCs for high level IL-12p70 production when paired with additional maturation agents.(45) In fact, several combinations of paired TLR ligands(46) or single TLR ligands plus IFN- γ can also induce IL-12p70.(47) Interestingly, it has been shown *in vitro* that IFN- γ paired with the TLR4 agonist LPS can not only induce an intense burst of IL-12 approximately 6 hr after stimulation, but can prepare DCs for a second, more intense burst of IL-12 up to 36 hr later if the DCs subsequently encounter recombinant CD40 ligand. These two bursts of IL-12 secretion allow for: 1) rapid IL-12 secretion when DC precursors encounter pathogens in peripheral tissues which can activate NK cells and, 2) delayed secretion of IL-12 when DCs migrate, as part of their maturation process, to the regional lymph nodes and encounter CD40 ligand on the surface of antigen-specific T cells. This second burst can polarize CD4+ T cells toward the Th1 phenotype and enhance the functional avidity of CD8+ T cells,(45) allowing them to recognize and kill target cells. This TLR agonist activation of DCs represents a link between innate and adaptive immunity that can be exploited to increase the efficacy of vaccine strategies targeting tumors.

NEW STRATEGIES IN BREAST CANCER VACCINE DEVELOPMENT

Vaccines targeting HER2/neu in early stage breast cancer

As noted above, clinical breast cancer vaccine trials have largely focused on patients with advanced disease or patients who have already undergone surgical resection of all apparent disease. Vaccines are less likely to

be effective in the former patients as a result of compromised immune function, declining overall health, and the frequent presence of extensive, difficult to control disease already refractory to multiple therapies.(48) In the latter patients, the absence of residual detectable disease makes it exceedingly difficult to gauge vaccine efficacy.

Since HER2/neu appears to play a role in breast carcinogenesis, and overexpression of HER2/neu can lead to breast cancer development, strategies to develop vaccines targeting HER2/neu may be useful to treat early disease and potentially prevent breast cancer. Preclinical models suggest that targeted HER2/neu vaccination in HER2/neu transgenic mice can prevent the subsequent development of invasive breast cancer. This prevention of breast cancer is dependent upon the activation of HER-2/neu-specific CD4+ T cells and complement-fixing anti-HER2/neu antibodies.(26,27) CD8+ T cells may play a role in the elimination of HER-2/neu-expressing tumors, especially in later, metastatic disease, but appear to be less essential than CD4+ T cells and antibody in preventative settings.(26) If effectively translated to humans, vaccines targeting HER2/neu are likely to possess more distinct clinical advantages in patients with DCIS or early breast cancer than in ones with advanced cancer. Vaccinating patients with early disease represents an incremental step towards preventative vaccination and an appropriate area for investigation given our current knowledge.

HER2/neu peptide-pulsed DC vaccines for neoadjuvant therapy in DCIS

DC vaccines represent one of several approaches for vaccinating patients against tumor-associated antigens such as HER2/neu. DCs polarized toward the type 1 dendritic cell (DC1) phenotype may produce cytokines and chemokines critical for maximizing antitumor immunity and may therefore enhance the efficacy of antitumor vaccines. Ligation of these TLRs also primes DCs for multiple bursts of IL-12 secretion, which can be triggered when DC1s interact with CD40 ligand expressed by CD4+ T cells. In addition to IL-12, DC1s also produce other

cytokines and chemokines involved in antitumor CD4+ T cell and CTL activity.(49)

Using technologies for producing DC1s, Czerniecki et al.(50) initiated a clinical trial for patients with HER2/neu overexpressing DCIS. DC1s are pulsed with MHC class I and MHC class II peptides previously described by Murray et al.(19) and Disis et al.(20) The vaccines with autologous HER2/neu peptide-pulsed DC1s are administered in a neoadjuvant setting before surgical resection of the patients' DCIS. Early results of this trial are highly encouraging and suggest robust sensitization of CD4+ and CD8+ T cells against HER2/neu. Additionally, complement-dependent, tumorlytic antibody was induced. This suggests that high avidity CTLs are being generated *in vivo* as was predicted in studies where IL-12-secreting DCs similarly sensitized T cells *in vitro*.

Thus, HER2/neu pulsed DC1 vaccines generate apparently vigorous immune responses in patients with DCIS despite the presence of existing tolerance to HER2/neu. The non-specific actions of TLR agonist are here utilized to overcome tolerance to a specific self antigen.

Targeted Immunoediting of HER2/neu in breast cancer

Although the eradication of existing disease remains the ultimate goal of vaccine therapy for breast cancer, vaccine-induced alterations in tumor phenotype yielding a more treatable tumor, or one with a better prognosis, would be a significant achievement. This approach has been termed as targeted immunoediting. Cells within the tumor mass are heterogeneous for the expression of a number of surface proteins, including HER2/neu and estrogen receptors. Therefore, it appears possible to utilize a DC vaccination to target selectively a discrete population of tumor cells. Because patients with DCIS tumors that overexpress HER2/neu are at higher risk for local recurrence, and because HER2/neu is a poor prognostic indicator in more advanced breast cancer, HER2/neu is a logical target for immunoediting. Indeed, Czerniecki et al.(50) found that subjects vaccinated with HER2/neu pulsed DC1 demonstrated a dramatic decrease in HER2/neu expression.(28) It remains the formidable challenge

predicting which individuals are at risk for developing breast cancer and how different tumor phenotypes evolve.

POTENTIAL MULTIMODALITY THERAPIES INCORPORATING VACCINES

HER2/neu and estrogen receptor cross-talk

A growing body of clinical and laboratory research points to a complex interplay between hormone and growth factor receptor mediated pathways in the pathogenesis and progression of breast cancer. An inverse correlation between estrogen receptor status and HER2/neu expression is well established. Furthermore, anti-estrogen therapy has been demonstrated to induce increased expression of HER2/neu and EGFR in tumor cells. Indeed, conversion to HER2/neu overexpression has been observed in breast cancers following sustained anti-estrogen therapy and has been correlated with rapid disease progression.(51) Laboratory evidence supporting a dynamic interaction between growth factor receptor and hormone mediated signaling includes: decreased expression of HER2/neu in breast cancer cells after estrogen stimulation,(52) and refractoriness to anti-estrogen therapy in cells transfected with HER2 cDNA that overexpress HER2/neu.(53)

Progress has been made in identifying connections between HER2/neu and hormone mediated pathways.(54, 55) Subbaramaiah et al.(56) have demonstrated that modulation of COX-2 expression is associated with coordinate alterations in aromatase activity in a murine model and have proposed that COX-2 in an intermediary between HER2/neu pathways and estrogen production. HER2/neu overexpression has been correlated with increased levels of COX-2 and prostaglandin E2 (PGE2). PGE2 in turn, stimulates aromatase activity especially in stromal cells which enhances estrogen biosynthesis and resultant estrogen driven tumorigenesis. Given the interaction between these pathways and suggestive evidence that clinical resistance to hormone therapies is mediated through HER2/neu overexpression and growth factor mediated pathways, preclinical and clinical studies of combination therapies targeting multiple components of these path-

ways have been undertaken with promising results. Combined trastuzumab and tamoxifen have been shown to inhibit tumor growth to a greater degree than either agent alone in a mouse xenograft model.(57) The combination of letrozole and trastuzumab was also studied in a recent phase II clinical trial and yielded good clinical responses, albeit in a minority of patients.(58)

Further study will clarify which clinical settings are most appropriate for application of these combined therapies. Effective immunotherapies and, in particular, vaccines that generate active immunity directed against HER2/neu, may be of particular utility in interrupting the circuit between HER2/neu, COX2 and aromatase and potentially preventing a substantial number of breast cancer. As discussed above, it is conceivable that appropriate HER2/neu vaccination will eliminate HER2/neu expressing tumor cells. Given the known inverse correlation between HER2/neu and estrogen receptor, it is likewise reasonable to assume that such an approach would leave behind tumors that are estrogen receptor positive and, therefore sensitive to hormone receptor modulating therapies. The notions that targeted immunoeediting of a tumor utilizing a vaccine directed at HER2/neu might render DCIS more vulnerable to hormonal therapies. It is enticing that subsequent administration of estrogen receptor modulating agents will have greater clinical efficacy.

Preclinical work suggests that the administration of chemotherapy in combination with vaccination may result in greater vaccine efficacy.(33) Chemotherapy may deplete T cells with suppressor phenotypes (T_{reg}), thereby enhancing the functionality of newly sensitized effector T cells. Vaccination combined with trastuzumab has also been investigated in a preclinical model with promising results.(59) The combinations of vaccination against HER2/neu with chemotherapy, passive immunotherapy or hormone therapy are areas for continued exploration.(60)

CONCLUSION

The recent introduction of a preventative vaccine for

cervical cancer represents a significant advance in the field of cancer immunotherapy. But the development of an effective vaccine for breast cancer remains a very distinct challenge. Cervical cancer is linked to a single causal virus, human papilloma virus, which the cervical cancer vaccine targets. The diversity of factors which contribute to the pathogenesis of breast cancer makes the identification of an optimal target for breast cancer immunotherapy more challenging. HER2/neu is emerging as an important target in human. The application of lessons learned in innate immunity and utilization TLR agonists to direct the immune response towards HER2/neu may allow us to overcome immune tolerance. A TLR agonist primed DC vaccine targeting HER2/neu, which elicits both a cellular and humoral immune response, is beginning to yield promising results in patients with early breast cancer. The translation of a similar strategy to a preventative setting is a logical next step. By finding not only appropriate tumor associated antigens but also mechanisms by which can induce robust immune response overcoming tumor tolerance, cancer vaccines may advance to the next higher level of effectiveness.

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