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Asymptomatic Changes in Cardiac Function Can Occur in DCIS Patients Following Treatment with HER-2/neu Pulsed Dendritic Cell Vaccines

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Abstract

Background—Targeting HER-2/neu with Trastuzumab has been associated with development of cardiac toxicity.

Methods—Twenty-seven patients with ductal carcinoma in situ (DCIS) of the breast completed an IRB approved clinical trial of a HER-2/neu targeted dendritic cell based vaccine. Four weekly vaccinations were administered prior to surgical resection. All subjects underwent pre- and post-vaccine cardiac monitoring by MUGA/ECHO scanning allowing for a comparison of cardiac function.

Results—In 3 of 27 vaccinated patients (11%) transient asymptomatic decrements in ejection fraction of greater than 15% were noted after vaccination. Notably, evidence of circulating anti-HER-2/neu antibody was found prior to vaccination in all three patients, but cardiac toxicity was not noted until induction of cellular mediated immune responses.

Conclusions—This is the first description of HER-2/neu targeted vaccination associated with an incidence of cardiac changes, and the induction of cellular immune responses combined with antibody may contribute to changes in cardiac function.

Keywords

Trastuzumab; HER-2; vaccinations; DCIS; dendritic cells; cardiac toxicity

Introduction

HER-2/neu (HER-2) is a tyrosine kinase growth factor receptor encoded by the ErbB-2 gene and a member of the epidermal growth factor receptor (EGFR) family^{1–3}. HER-2/neu has attracted considerable attention as a therapeutic target in breast cancer therapy because its over-

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expression correlates with a poor prognosis in node positive patients. Additionally, it is differentially expressed in malignant cells and normal cells, providing for therapeutic specificity. Passive targeting of HER-2/neu with a recombinant humanized antibody (trastuzumab) has proven effective in patients with metastatic and high-risk invasive breast cancer⁴, particularly when combined with cytotoxic chemotherapy^{5–8}. Trastuzumab is currently being tested in early breast cancer settings for the treatment of Ductal Carcinoma In-Situ (DCIS) and may serve as an effective adjunct to other therapies⁹. The mechanisms by which trastuzumab influences tumorigenesis and disease progression are not completely understood. *In vitro* and *in vivo* work point to a number of potential mechanisms including: (1) diminished receptor signaling as a result of internalization and degradation of HER-2/neu¹⁰; (2) induction of cell cycle arrest in the G1 phase resulting in reduced tumor cell proliferation; (3) induction of apoptosis; (4) inhibition of angiogenesis and (5) inhibition of DNA repair¹¹. Additionally, there is evidence that trastuzumab may induce antibody dependent cytotoxic cell response directed against HER-2/neu over-expressing cells^{12–14}.

Despite promising clinical data supporting the efficacy of trastuzumab, the agent does have limitations. 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 year⁷. Additionally, trastuzumab has been associated with significant cardiac toxicity; cardiac events ranging from subclinical decrements in ejection fraction to symptomatic congestive heart failure occur in up to 30% of patients^{15, 16}. While HER-2/neu signaling is known to play a role in embryonic cardiogenesis¹⁷ as well as in the prevention of dilated cardiomyopathy¹⁸, the specific mechanisms of trastuzumab induced cardiotoxicity are poorly understood.

Efforts to develop vaccines directed at HER-2/neu are now underway. Potential benefits of immunization over passive immunotherapy include lower toxicity and induced immunologic memory obviating the need for long term therapy. This would be significant for preventing estrogen independent breast cancer. We have explored a neoadjuvant dendritic cell based vaccine strategy which targets HER-2/neu in an early disease setting. We report three cases of sub-clinical cardiac depression associated with HER-2/neu targeted vaccination and discuss the implications of this finding.

Methods

Clinical Trial

Patients with histologically confirmed DCIS with HER-2/*neu* overexpression (>2+ intensity) in at least 10% of cells [assayed by HercepTest and verified by single pathologist (P.J.Z.)] were recruited to this Institutional Review Board–approved clinical trial. Subjects were screened by magnetic resonance imaging (MRI) before enrollment to eliminate individuals with obvious areas of invasive disease in either breast. Only patients requiring further surgical therapy for DCIS were eligible for neoadjuvant administration of the study vaccine. All patients underwent cardiac evaluation with multigated acquisition (MUGA) scan or echocardiography to document adequate baseline cardiac function. These scans were done before the first dose of vaccine and within 2 weeks of the final dose. All patients underwent HLA class I tissue typing pre-enrollment and had routine history, physical exams, EKG, blood work, and urinalysis prior to vaccination. After obtaining informed consent, all patients had a prevaccine leukapheresis done to obtain sufficient numbers of monocytes for vaccine preparation. In a few cases, a second pheresis was required for additional monocytes. A postvaccination pheresis was also done, usually within 2 weeks of the final vaccination, to obtain postimmunization lymphocytes for evaluation. All patients underwent postvaccine mammography, MRI, and surgical resection of DCIS with either lumpectomy or mastectomy. Sentinel lymph node biopsy was also

conducted in the majority of patients secondary to suspicion of microinvasion or large areas of DCIS necessitating mastectomy.

Vaccine Production

Vaccine preparation proceeded according to the following methodology. *Ex vivo* activated DC1 (high IL-12-secreting dendritic cells) were prepared from autologous monocytes under Food and Drug Administration IND BB-11043. Dendritic cells were pulsed individually with six MHC class II peptides derived from HER-2/*neu* as described previously.²⁸ The dendritic cells of HLA-A2^{POS} subjects were additionally pulsed with two HLA-A2-binding peptides (369–377 and 689–697) shown previously to stimulate CD8^{POS} T cells. The intention of the combined MHC class II and class I peptide design was to promote CD4^{POS} antigen-specific help for CD8^{POS} T cells, partly by fostering an additional *in vivo* burst of IL-12 production through CD40 ligation of the administered DC1.

Vaccine Administration

Vaccines were administered in the NIH General Research Center at the University of Pennsylvania. The vaccines consisted of 10 to 20 million HER-2/*neu*-pulsed DC1 cells suspended in 1-mL sterile saline. The vaccines were administered by Ultrasound guidance into a single lymph node in each groin. Half of each vaccine was placed into each node with a 22-g needle. The first nine subjects were observed for 2 h after vaccination with routine vital signs obtained at 15-min intervals. Subsequent subjects were observed for 1 h. Vaccines were administered once weekly for 4 weeks. All subjects completed all four scheduled vaccines.

Assessment of Complement Fixing Antibody

This was conducted by testing pre and post vaccination serum with dilutions ranging from 1:6 to 1:256 on either HER-2/*neu*^{POS} or HER-2/*neu*^{NEG} breast cancer lines in tissue culture microplates. The serum was absorbed to the cells and 1:4 diluted guinea pig serum was added as source of complement. Heat inactivated complement served as control. After 4 h WST1 was added. The plates were analyzed by an ELISA reader at 1, 2 and 3 h afterwards at a wavelength of 450. Trastuzumab (Herceptin) was also used at 1:6 to 1:240 dilutions. The percentage cytotoxicity was calculated using the following formula: $[(a - b) / (a - c)] \times 100$ (where a = cells, antibody and heat inactivated complement, b = cells, antibody and complement; and c = Triton X lysed cells).

CD4+T cell ELISPOT

CD4^{POS} T cell anti-HER-2/*neu* interferon gamma (IFN- γ) responses were assessed by ELISPOT to quantify T cells in peripheral blood or sentinel lymph node without *in vitro* expansion. Peripheral blood CD4^{POS} T cells, obtained from subjects before and after vaccination, or T cells from sentinel lymph nodes post-vaccination were co cultured with dendritic cells either pulsed with HER-2/*neu* peptides or left unpulsed. Dendritic cells were also pulsed with tetanus toxoid and cocultured with T cells to monitor for nonspecific vaccine-induced changes in immune function. Individual peptide reactivities were measured in quadruplicate as previously described²⁶. Ratio of post to pre vaccines ELISPOTS were calculated.

Immunohistochemical staining of DCIS lesions

Formalin-fixed, paraffin-embedded tissue blocks were sectioned at 5 μ m on plus slides. Sections were heated for 1 h at 60°C to remove excess paraffin, cooled for 10 min, and subsequently deparaffinized and rehydrated in a series of xylenes and alcohols.

Immunohistochemistry was done using the DAKO Autostainer. All tissues were stained for HercepTest (DAKO), various CD markers, and IgG (DAKO).

Results

All 27 patients enrolled in the vaccine trial were required to have a minimum of 50 percent ejection fraction with no underlying history of cardiac disease. 26 patients underwent MUGA scan and one patient underwent ECHO to determine ejection fraction. Three of the 27 patients experienced at least an 18 percent decline in ejection fraction. (Table I.) All patients remained asymptomatic throughout the course of this study. One of these patients (08012-26) underwent a subsequent MUGA 30 days later demonstrating a return of her baseline cardiac function. Although there is not a documented repeat MUGA for the other two patients, there have been no instances of long-term symptomatic cardiac disease develop in any subject.

We assessed both the humoral and cellular immune response in patients prior to and following vaccination. The results demonstrated the presence of complement fixing antibody in all three of the subjects demonstrating cardiac dysfunction (Figure 1). However, these antibodies were also seen pre-vaccine in the majority of the subjects and there was an interesting decline in the serum activity post-vaccination. These results suggest that many DCIS patients have existing complement fixing antibodies that may mediate lysis of HER-2/neu expressing breast cancer cells. However, these appear to be clinically insufficient to induce cardiac toxicity.

We next determined the development of CD4 response in patients post vaccination. All patients demonstrated evidence of CD4+ T cell response to at least one peptide (data not shown). Of the 3 patients exhibiting declines in cardiac ejection fraction, 08012-09 had only blood derived CD4 T cell response, 08012-08 had both blood and sentinel lymph node CD4 T cell reactivity, and 08012-26 had only lymph node reactivity (Table II.). This data supports evidence of a cellular immune response with CD4 T cells in all patients either in the blood or lymphatic compartments. In addition, there were no differences in specific peptide reactivity between those that experienced a decline in cardiac ejection fraction and those that did not.

All three subjects with cardiac dysfunction demonstrated a decline in HER-2/neu expression in residual DCIS or in the radiographic area of microcalcification associated DCIS²⁶. We conducted immunohistochemistry staining to determine whether endogenous antibody actually bound to areas of tumor *in vivo*. Pre-vaccine and post-vaccine DCIS samples from one subject (08012-09) with residual high-grade HER-2/neu-expressing DCIS were stained with anti-human IgG. We found minimal evidence of IgG bound to tumor before vaccination. However, post vaccination DCIS cells showed strong anti-IgG staining. (Figure 2) This demonstrates that in a certain subset of vaccinated patients, endogenous and vaccine-induced antibodies bind directly to HER-2/neu expressing tumor targets. These antibodies may theoretically also be able to bind to cardiac tissue expressing HER-2/neu.

Discussion

Breast cancer is the second most common form of cancer in women and the leading cause of death caused by malignancy. The HER-2 gene is amplified in approximately 25% of breast cancers. HER-2 over-expression is commonly associated with poorly differentiated, high-grade tumors commonly associated with lymph node involvement and resistance to chemotherapy agents²⁰. Targeting HER-2/neu with trastuzumab has revolutionized therapy for these patients with this aggressive phenotype of breast cancer. In addition, HER-2/neu appears now to be expressed in breast cancer stem cells.²⁷ Therefore targeting HER-2 may play a large role in preventing non-hormone dependent breast cancer.

Cardiac dysfunction in patients receiving trastuzumab was first reported in the clinical trials of metastatic breast cancer. Because this dysfunction had not been identified as a potential side effect in either preclinical or clinical studies, cardiac monitoring was not included in the protocols of the larger, later-stage clinical trials. After trastuzumab-associated cardiac dysfunction had been recognized, a retrospective analysis of 7 phase II or III metastatic trials was carried out by an independent Cardiac Review and Evaluation Committee (CREC).²⁵ CREC identified cardiac dysfunction in 3–7% of patients and an incidence of symptomatic cardiac dysfunction (NYHA Class III, IV) of 2–4% in patients who had received trastuzumab alone. The incidence increased to 27% and 16% respectively in patients who received trastuzumab concurrently with an anthracycline.²⁵

More recently, 4 large-scale adjuvant trastuzumab trials have been conducted: the National Surgical Adjuvant Breast and Bowel Cancer Project (NSABP trial B-31)³¹; the North Central Cancer Treatment Group (NCCTG) trial N9831³¹; the Herceptin Adjuvant (HERA) trial⁹; and the Breast Cancer International Research Group (BCIRG) trial 006.²⁴ The 4 trials all excluded patients with a baseline history of increased risk or actual heart disease and included monitoring of LVEF before, during, and after the trial, using echocardiogram or multiple gated acquisition (MUGA) scanning (Table III.). Direct comparisons between the trials should not be made, due to the different definitions used for cardiac events across the trials. In all 4 trials, however, the incidence of cardiac events in the trastuzumab-containing treatment arms remained below 4% (the safety cut-off set by the independent data monitoring committees). The incidence of cardiac events was lowest in the BCIRG 006 trial; the incidence in the docetaxel, carboplatin, and trastuzumab (TCH) arm (0.4%) and the control arm (AC→docetaxel; 0.3%) were almost identical.

Inhibition of HER-2 signaling is implicated as a central mechanism of trastuzumab-related cardiotoxicity.¹⁹ The HER-2 gene belongs to a family of epidermal growth factor receptors (EGFRs) that regulate cell growth, proliferation, and survival. Restricted HER-2 expression in mouse models have demonstrated dilated cardiomyopathy and poor contractility, and showed enhanced susceptibility to anthracycline-induced cardiotoxicity. In addition, reduction of HER-2 signaling induces cardiomyocyte apoptosis.²⁰ In contrast, HER-2 signaling in myocytes confers protection and improves myocardial function. Trastuzumab blocks the extracellular domain of HER-2, thereby reducing the myocardial homeostasis and exposing the myocardium to unopposed damaging effects of stress-signaling pathways. Prior treatment with anthracyclines increases cell surface receptors that are subsequently blocked by trastuzumab. However, toxic effects of trastuzumab, unlike those of anthracyclines, are not cumulative or dose related.

Several clinical trials have completed or are in progress using vaccines targeting HER-2.^{28, 29} The results of this trial report for the first time documentation of subclinical cardiac dysfunction associated with HER-2 based vaccines. This suggests a clear role for pre and post treatment monitoring of cardiac function in all patients undergoing these vaccinations. In this study, 3 of the 27 (11%) patients exhibited a subclinical decline in cardiac ejection fraction. All three patients showed evidence of pre-circulating anti-HER-2 antibodies. Complement-fixing antibody has been shown to be important for successful vaccination against HER-2/neu breast cancers in mouse models.²² However, the decline in ejection fraction was not evident until vaccination initiated the cell-mediated immune response. Our demonstration of anti IgG bound to DCIS cells suggest that perhaps a similar phenomenon may be occurring in cardiac myocytes. This cannot be demonstrated from our current data and is a potential area for future investigation. The induction of cell-mediated response in the presence of anti-HER-2 antibody may offer an underlying mechanism for the resulting subclinical cardiac dysfunction seen with both trastuzumab and this vaccine.

Though most of the HER-2 vaccines studies did not assess cardiac dysfunction, other studies with anti-HER-2 vaccinations have not demonstrated EF suppression to be the case.³⁰ Morse et al recently discussed HER-2 based vaccination in the adjuvant setting with stage 2, 3, or 4 HER-2 positive breast cancers. These vaccines were dendritic cell based and given to patients following surgical resection, adjuvant systemic therapy, and in some cases concomitant with hormonal treatment. There were no changes in MUGA or ECHO in the 6 of the 7 patients enrolled in the study. Perhaps the small subset of patient used in this study may have not been sufficient to demonstrate significant changes in cardiac function.

HER-2 directed vaccinations pose a unique systemic treatment approach to the HER-2 positive breast cancer patient. From prior studies, we are aware of the reversible cardiotoxicity that can occur in the setting of trastuzumab infusion. In our study involving HER-2 vaccination of HER-2 positive DCIS patients, we were able to demonstrate a statistically significant decline in ejection fraction in a subset of vaccinated patients. We believe that this is an immune mediated phenomenon, supported by the evidence of complement fixing antibody, CD 4 response and anti IgG binding. While all patients demonstrated pre-vaccination antibodies, only a small subset showed declines in ejection fraction post vaccination. While this could be related to anti IgG binding to myocytes, further studies would need to validate this. In conclusion, this is the first study to show changes in cardiac function within HER-2 vaccination trials, suggesting that MUGA/ECHO should be performed on all patients pre and post vaccination, regardless of the absence or presence of symptoms.

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FIGURE 1.
Presence of Complement Fixing antibody in both pre and post vaccination subjects. The 3 subjects with post-vaccination cardiotoxicity are 08102-08, 09, and 26.

Pre-Vaccination **Post Vaccination****FIGURE 2.**

DCIS specimens obtained before and after vaccination from patient 08102-09 were treated with anti-human IgG. In pre-vaccine samples, there was minimal evidence of anti-human IgG staining (brown staining), except in necrotic cells in the central duct; however, post-vaccine, there was evidence of over 50% of ductal cells staining with anti human Ig. Cells were counterstained with H&E staining (blue staining). Magnifications, $\times 10$ (pre vaccine) and $\times 20$ (post vaccine).

TABLE I
Pre and Post Vaccination Ejection Fractions measured by MUGA/ECHO

SUBJECT ID	AGE	Race	ER	PR	HER-2	CONSENT DATE	ELIGIBLE (Y/N)	Pre EF%	Post EF%	% DECLINE
08102-01	44	White	-	-	2+	9/13/04	Y	71	71	
08102-02	64	White	+	+	2+	2/14/05	Y	55	56	
08102-03	48	Black	-	-	3+	4/6/05	Y	60	63	
08102-04	67	White	-	-	3+	4/27/05	Y	58	66	
08102-05	45	Black	+	+	2+	4/27/05	Y	51	54	
08102-06	45	White	-	-	3+	6/20/05	Y	59	60	
08102-07	54	Black	+	-	3+	9/1/05	N			
08102-08	55	White	+	-	3+	10/19/05	Y	62	50	19%
08102-09	53	White	+	-	3+	10/20/05	Y	69	55	20%
08102-10	48	White	+	+	3+	11/1/05	Y	56	65	
08102-11	51	White	+	+	3+	11/2/05	Y	65	72	
08102-12	58	Black	-	-	2+	2/22/06	Y	50*echo	55*echo	
08102-13	43	White	+	+	3+	4/12/06	Y	51	54	
08102-14	38	White	+	-	3+	8/14/06	Y	67	67	
08102-15	59	White	-	-	3+	9/20/06	Y	57	62	
08102-16	69	White	-	-	3+	9/13/06	Y	73	75	
08102-17	45	White	+	-	3+	10/10/06	N			
08102-18	49	White	+	-	3+	11/21/06	Y	58	69	
08102-19	87	White	+	+	2+	11/28/06	Y	72	73	
08102-20	44	White	+	+	2+	1/25/07	Y	69	73	
08102-21	57	Black	+	-	3+	2/6/07	Y	61	65	
08102-22	43	White	-	-	3+	4/23/07	Y	64	60	
08102-23	57	White	+	+	3+	5/29/07	Y	63	66	
08102-24	64	White	+	-	2+	6/12/07	Y	67	68	
08102-25	64	White	-	-	2+	11/5/07	Y	61	71	
08102-26	40	White	+	+	2+	12/12/07	Y	66	54	18%
08102-27	57	Black	+	+	3+	1/9/08	Y	68	63	
08102-28	47	Black	+	+	3+	3/19/08	Y	69	72	

SUBJECT ID	AGE	Race	ER	PR	HER-2	CONSENT DATE	ELIGIBLE (Y/N)	Pre EF%	Post EF%	% DECLINE
08102-29	45	White	+	+	2+	4/2/08	Y	56	62	
08102-30	77	Black				7/8/08	Y	60	pt withdrew	

Table II
ELLISPOT Ratio demonstrating CD 4 reactivity within serum or lymph node

	Serum			Lymph Node		
	08102-08	08102-09	08102-26	08102-08	08102-09	08102-26
HER2neu-p42-56	0.87	7.28	0.00	-	-	+
HER2neu-p98-114	1.47	1.24	0.00	-	-	-
HER2neu-p328-345	0.33	0.00	0.00	-	-	-
HER2neu-p776-790	2.27	1.89	0.00	-	-	+
HER2neu-p927-941	20.66	3.32	0.00	-	-	+
HER2neu-p1166-1180	3.75	1.62	0.00	+	-	+

TABLE III

Incidence of CHF in the Adjuvant Trastuzumab Trials*

Trial	Treatment Regimen	Incidence of CHF	
		Trastuzumab	Control
NCCTG N9831 and NSABP B-31 (combined results) [†]	AC → paclitaxel and trastuzumab	2% (32/1677)	0.4% (7/1600)
HERA	Chemotherapy → trastuzumab	2% (30/1678)	0.3% (5/1708)
BCIRG 006	AC → docetaxel and trastuzumab	2% (20/1068)	0.3% (3/1050)
BCIRG 006	Docetaxel, carboplatin, and trastuzumab (TCH)	0.4% (4/1056)	0.3% (3/1050)

Combined results of NCCTG N9831 and NSABP B-31 trials: Cumulative incidence of time to first LVEF decline ≥ 10 percentage points from baseline and to below 50% with death as a competing risk factor. (Herceptin Prescribing Information, 2008)

[†] Includes 1 patient with fatal cardiomyopathy