Targeted Immunoediting HER-2/neu Phenotypes in Early Breast Cancer: A New Approach to Cancer Vaccines

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ABSTRACT

Hypothesis: HER-2/neu expression in ductal carcinoma in situ (DCIS) lesions is associated with an increased incidence of synchronous invasive disease and a higher rate of recurrence. We postulated that vaccination against HER-2/neu in DCIS could potentially eradicate these lesions but more importantly may also protect against the development of the more aggressive HER-2/neu phenotype in invasive breast cancers (IBC).

Methods: A Phase I clinical trial was undertaken in patients with HER-2/neu^{pos} DCIS. Four weekly vaccines consisting of autologous type I polarized dendritic cells (DC1) pulsed with MHC class I and class II peptides were administered intranodally prior to definitive surgery. DC1 were activated by combined treatment with interferon gamma (IFN-γ) and the toll-like receptor 4 agonist, lipopolysaccharide. Peripheral blood and sentinel node lymphocytes were evaluated for evidence of anti-HER-2/neu responses. Expression of HER-2/neu in DCIS was assessed prior to and following surgery.

Results: Twenty five patients have completed treatment on this protocol. Toxicities were limited to mild (grade I) fever, chills, fatigue, and injection site reactions. Ninety percent of patients developed evidence of anti-HER-2/neu CD4 and CD8 T cells as measured by IFN-γ ELISPOT or in vitro sensitization assays. These responses persisted for a prolonged time period following vaccination. Additionally, anti-HER-2/neu reactive CD4 T cells were identified in sentinel nodes in 80% of the patients post-vaccination. Complement fixing antibodies were detected in 66% of evaluated patients post-vaccination. CD4, CD8 and B cells accumulated in the breast of many subjects post-vaccine. Sixty percent of the patients with residual DCIS demonstrated significant reductions in HER-2/neu expression post-vaccination often associated with declines in the area of DCIS as measured by microcalcifications on mammography. One patient with DCIS and microinvasion developed contralateral DCIS and within two years of vaccination that was HER-2/neu^{neg} and estrogen receptor positive. Serum HER-2/neu measurements in this patient were elevated post-vaccine and remained so until the time of diagnosis of the second primary cancer. Anti-HER-2/neu T cell responses also persisted at the time the second tumor was diagnosed.

Conclusions: HER-2/neu pulsed DC1 vaccines are safe, well tolerated and highly effective in inducing long lasting anti-HER-2/neu immune responses. These vaccines are also effective in diminishing expression of HER-2/neu in DCIS lesions. Evidence from one patient suggests successful immunoediting of breast cancer from a HER-2/neu^{pos} to a HER-2/neu^{neg} phenotype. Such immunoediting may prevent disease progression and recurrence and reduce breast cancer mortality. Immunologic targeting of pathways critical to cancer development may represent an important new direction for cancer vaccination.

METHODS

1. Study design

Patients with biopsy-proven DCIS (ductal carcinoma in situ) were received weekly 4 intranodal HER-2/neu pulsed DC1 vaccine injections. All patients had a minimum of 2-3% HER-2/neu staining on 10% of DCIS cells for entrance. Post treatment they underwent surgical resection of the area of DCIS. Pre and post vaccination PBMC of patients were collected for comparative studies. .

2. Preparation of dendritic cell vaccine

Patients provided informed consent and underwent leukapheresis. Blood product was then elutriated to obtain monocyte – rich and lymphocyte – enriched fractions. Elutriated monocytes were culture in Serum-free Medium in presence of GM-CSF and IL-4 and pulsed with HER-2/neu ECD p42-56, p98-114, p328-334 and ICD p776-784, p927-941, p1166-1180 separately. IFN-γ and LPS were used to generate IL-12 secreting - DC1. For HLA-A*0201 patients, HER-2/neu MHC class I peptides P369-377 and P689-698 were added to DC1 vaccines.

3. Tetramer staining and Flow Cytometry

Pre or post vaccination CD8+ T cells were stained with APC-labeled HER-2/neu p369-377 tetramer and anti-CD8-FITC, anti-CD28-PE or anti-CTLA-4-PE for 30min. Cells were then washed and subjected to Flow Cytometry analysis. APC labeled MART-1(27-35) peptide tetramer was used for background control.

4. ELISPOT assay

ELISPOT was performed according to manufacturer's instructions. Briefly, 96-well plates were coated with anti-IFN-γ antibody overnight at 4°C and then blocked with medium in addition of 10% human serum for 2h at RT. 100ul purified pre or post vaccination CD4+ T cells (10^5 cells) were co-cultured with 100ul DCs (10^4 cells) pulsed with HER-2/neu ECD or ICD peptides respectively in a well of 96-well plate in triplicate overnight. PMA and CI stimulated CD4+ T cells were used as a positive control. CD4+ T cells were also co-cultured with unpulsed DC for background control. Plates were then washed and incubated with biotinylated anti-IFN-γ for 2h. Next plates were washed again and TMB substrate solution was added. The colorimetric reaction was stopped by washing with running water. The plates were allowed to dry and the number of spots was read on an ELISPOT reader.

5. In vitro CD4+ T cell sensitization assay

Purified pre or post vaccination CD4+ T cells were sensitized by DCs pulsed with HER-2/neu ECD or ICD peptides respectively at a ratio of 10:1in 24-well plate. IL-2 (60 IU/ml) was added to the cultures next day. T cells were harvested on day 10 and tested for their antigen specificity. 100ul CD4+ T cells (10^5 cells) were re-stimulated with 100ul DCs (10^4 cells) pulsed with HER-2/neu peptides accordingly or co-cultured with DCs pulsed with control peptides in a well of 96-well plate in triplicate. After 24 h stimulation, supernatants were harvested for IFN-γ release measurements by ELISA.

6. In vitro CD8+ T cell sensitization assay

Autologous DCs were pulsed with HER-2/neu p369-377 or P689-698 at 10ug/ml 2h prior to harvest. They were co-cultured with purified pre or post vaccination CD8+ T cells at a ratio of 10:1 in a well of 48-well plate. 30IU IL-2/ml was added to the cultures on day 2. After 10 day sensitizations, the CD8+ T cells were harvested and re-stimulated with T2 cells pulsed either relevant or irrelevant peptides, or they were tested against breast cancer cell lines MAD-MB-231 (HER-2/neu +, HLA-A2 +) or MAD-MB435S (HER-2/neu +, HLA-A 2 -). Supernatants were harvested after 24h and analyzed by ELISA.

RESULTS

HER-2/neu Pulsed DC1 for Treatment of DCIS

- Neoadjuvant study
- 4 Intranodal DC1 Vaccinations weekly
- MHC class II and class I HER-2/neu peptides
- Follow-up immune response
- Surgical Therapy

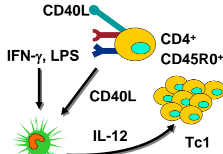
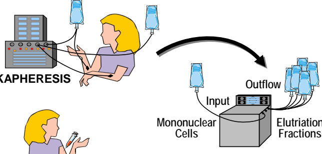


FIGURE 1. Study design and dendritic cell vaccine preparations. Patients with DCIS (ductal carcinoma in situ) were received weekly 4 intranodal HER-2/neu pulsed DC1 vaccine injections. DC vaccine was generated by rapid culture of monocytes in 40 hours in serum free medium with IFN-γ and LPS.

Protocol for DC1 Preparation



DC1 Activated with GM-CSF, IL-4, IFN-γ, LPS, pulsed with MHC class II and MHC class I HER-2/neu peptides, 40 hours total culture

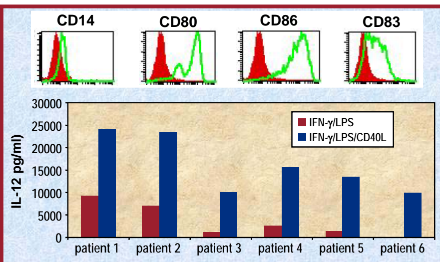


FIGURE 2. Quality analysis of DC vaccine. A) An aliquot of DC was stained with anti-CD14, anti-CD80, anti-CD86 and anti-CD83 for DC surface phenotype analysis. B) Culture supernatants were analysis by ELISA for IL-12 measurement. Grey bars represent DC treated with IFN-γ and LPS and black bars represent IFN-γ/LPS treated DC were further activated by CD40L.

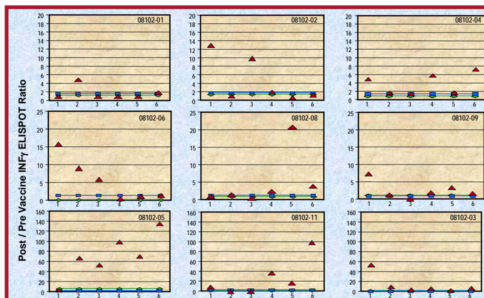


FIGURE 3: Post/pre vaccine IFN-γ ELISPOT ratios are shown from nine patients. Responses were tested against six peptides (1-6 on the x axis).

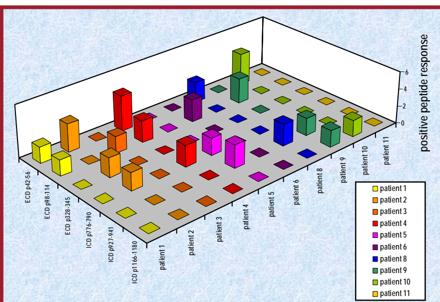


FIGURE 4. In vitro stimulation analysis of HER-2/neu specific CD4 T cell responses induced by DC1 vaccination. Each bar represents a peptide specific response index of post-vaccination.

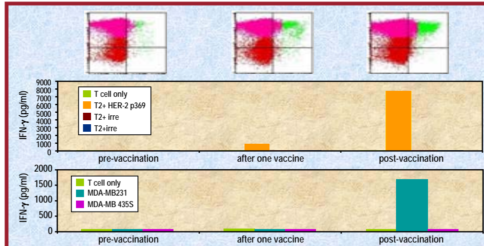
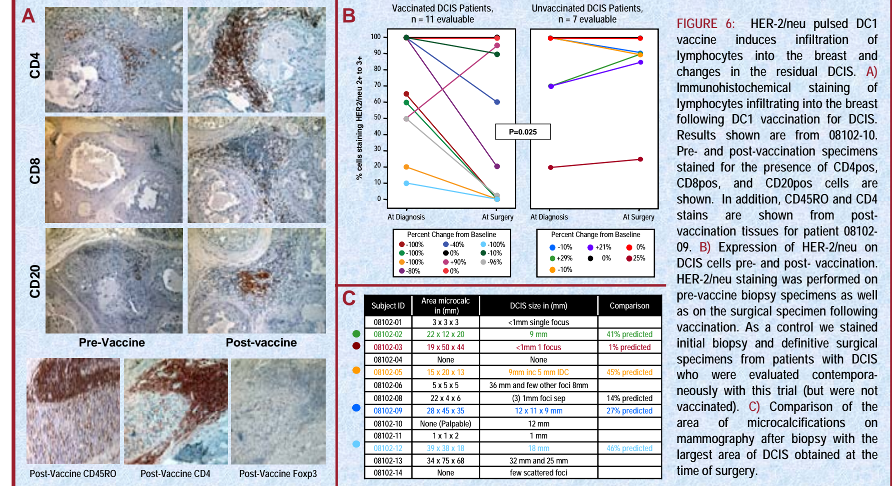


FIGURE 9. Induction of tumor recognizing CD8+ T cells require multiple vaccinations. CD8+ T cells were purified from pre-vaccine, after one vaccine, or after 4 vaccines and cultured with immature DC pulsed with HER-2/neu p369-377 for 10 days. A. The cells were stained with anti-CD8 (FITC: y-axis) and HER-2/neu p369-377 tetramer (APC: X-axis), and subjected to FACS analysis. B. Sensitized CD8+ T cells were cocultured with target T2 cells pulsed HER-2/neu p369-377 or irrelevant peptides or C. with HLA-A2+/HER-2/neu^{pos} tumor cell MDA-MB231 and HLA-A2-/HER-2/neu^{pos} cells MDA-MB435S for 24h, culture supernatants were harvested for ELISA IFN-γ release measurements.



Subject ID	Area microcalc in (mm)	DCIS size in (mm)	Comparison
08102-01	3 x 3 x 3	<1mm single focus	
08102-02	22 x 12 x 20	9 mm	41% predicted
08102-03	19 x 50 x 44	<1mm 1 focus	1% predicted
08102-04	None	None	
08102-05	15 x 20 x 13	9mm inc 5 mm IDC	45% predicted
08102-06	5 x 5 x 5	36 mm and few other foci 8mm	
08102-08	22 x 4 x 6	(3) 1mm foci sep	14% predicted
08102-09	28 x 45 x 35	12 x 11 x 9 mm	27% predicted
08102-10	None (Palpable)	12 mm	
08102-11	1 x 1 x 2	1 mm	
08102-12	39 x 38 x 18	18 mm	46% predicted
08102-13	34 x 75 x 68	32 mm and 25 mm	
08102-14	None	few scattered foci	

FIGURE 6: HER-2/neu pulsed DC1 vaccine induces infiltration of lymphocytes into the breast and changes in the residual DCIS. A) Immunohistochemical staining of lymphocytes infiltrating into the breast following DC1 vaccination for DCIS. Results shown are from 08102-10. Pre- and post-vaccination specimens stained for the presence of CD4pos, CD8pos, and CD20pos cells are shown. In addition, CD45RO and CD4 stains are shown from post-vaccination tissues for patient 08102-09. B) Expression of HER-2/neu on DCIS cells pre- and post- vaccination. HER-2/neu staining was performed on pre-vaccine biopsy specimens as well as on the surgical specimen following vaccination. As a control we stained initial biopsy and definitive surgical specimens from patients with DCIS who were evaluated contemporaneously with this trial (but were not vaccinated). C) Comparison of the area of microcalcifications on mammography after biopsy with the largest area of DCIS obtained at the time of surgery.

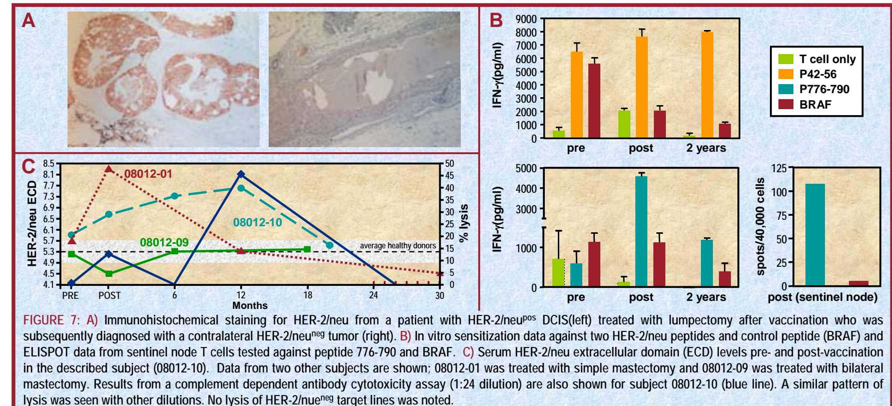


FIGURE 7: A) Immunohistochemical staining for HER-2/neu from a patient with HER-2/neu^{pos} DCIS(left) treated with lumpectomy after vaccination who was subsequently diagnosed with a contralateral HER-2/neu^{neg} tumor (right). B) In vitro sensitization data against two HER-2/neu peptides and control peptide (BRAF) and ELISPOT data from sentinel node T cells tested against peptide 776-790 and BRAF. C) Serum HER-2/neu extracellular domain (ECD) levels pre- and post-vaccination in the described subject (08012-10). Data from two other subjects are shown; 08012-01 was treated with simple mastectomy and 08012-09 was treated with bilateral mastectomy. Results from a complement dependent antibody cytotoxicity assay (1:24 dilution) are also shown for subject 08012-10 (blue line). A similar pattern of lysis was seen with other dilutions. No lysis of HER-2/nue^{neg} target lines was noted.

CONCLUSIONS

- **HER-2/neu pulsed DC1 vaccines are safe, well tolerated and highly effective in inducing long lasting anti-HER-2/neu immune responses.**
- **HER-2/neu pulsed DC1 vaccines are effective in diminishing the expression of HER-2/neu in DCIS lesions**
- **Immunoediting of breast cancers from a HER-2/neu^{pos} to a HER-2/neu^{neg} phenotype may prevent disease progression and recurrence and reduce breast cancer mortality.**