Introduction

• The HER-2/neu (HER2) ICD vaccine immunizes patients with CD4+ T helper epitope peptides from HER2.
• Immunity against the HER2 ICD correlates with anti-tumor responses in animal models.[1]
• HER2 breast cancer patients demonstrated the ability to generate immunity to the HER2 ICD in Phase I studies.[2]
• HER2 ICD vaccine-induced T helper responses resulted in:
  - Development of immunologic memory & persistent immunity
  - Development of epitope spreading
• Vaccinating advanced-stage HER2 breast cancer patients while on trastuzumab may:
  - Allow tumor specific immunity to develop and evolve while the cancer is controlled.

We questioned whether addition of active immunization against HER2 to maintenance trastuzumab would result in a HER2-specific immune response that would prolong remission and potentially prolong survival.

Methods

Patient population. A Phase II trial of a HER2 peptide-based vaccine was approved by the University of Washington and DOD IRB and the US FDA. Patients with stage IIIB and IV HER2+ breast cancer were eligible for study entry if (1) they received standard treatment which included trastuzumab chemotherapy; (2) were in remission defined as HER2 with the exception of stable bony disease, and (3) were within 6 months of starting maintenance trastuzumab.

Vaccines were composed of 100 mcg GM-CSF and administered intradermally monthly for 6 months. Toxicity was evaluated by physical exam and clinical labs. Toxicity grading was defined by the CTCAE V3.0. Toxicity and immunologic monitoring occurred at baseline, prior to each vaccine, and 1, 2, and 6 months after the last vaccine. 10 subjects were enrolled. A total of all patients; 1 has received 4 vaccines and 1 has received 2 vaccines, that is, 2.5 vaccines.

HER2 peptide-based vaccine. The HER2 ICD vaccine included peptides p776-p797 (p776, p797), p927 (p927), and p1166-1180 (p1166) derived from the intracellular domain of HER2. The peptides are manufactured by Multiple Peptide Systems, San Diego, CA as previously described.[2]

Evaluation of HER2 T cell immunity. HER2 T cell immunity was measured using a 10 day ELISPOT assay. PBMC pre- and post-vaccinations were stimulated with the 3 ICD peptides included in the vaccine (p776, p927, and p1166) and overlapping peptide pools for the HER2 intracellular domain (ICDpm) on Day 1. The overlapping peptide pools for the HER2 extracellular domain (ECDpm), which were not included in the vaccine, was also used to stimulate the cells.

On Day 5, 10μl of 10000 IU/ml of rhIL-2 was added into each well. On Day 8, the cells were re-stimulated with a biotinylated mAb 7-B6-1 (Mabtech) was added into each well at 5 μg/ml. The IFN-γ secreting cells were then counted using an ELISPOT reader (Cell Technology). The results are presented as mean spot forming cells.

Evaluation of serum TGF-β levels. TGF-β serum levels were measured pre- and post-vaccinations using a TGF-β ELISA kit (eBioscience, San Diego, CA). The lowest TGF-β in serum was acid-treated and then neutralized to activate the antibodies and was serially diluted (1000 ng/ml). Standards were plated into triplicate 96-well microplate pre-coated with anti-human TGF-β1 antibody was added into each well and incubated at room temperature in dark. Stop solution was then added into each well. The optical density (OD) of each well was read using a microplate reader set to 450 and 570nm, and determined as OD450nm - OD570nm.

Results

• Immunization with HER2 ICD vaccine and concurrent trastuzumab was well tolerated.
• Toxicity was primarily Grade 1 and 2 pain (myalgias), fatigue, leukopenia, and lymphopenia.
• No cardiovascular AEs have been observed.

A planned interim analysis will be done after 25 patients are enrolled. We continue to actively accrue patients to study (target enrollment = 52).

Conclusions

• Vaccination with HER2 ICD peptide-based vaccine and concurrent trastuzumab monotherapy elicited HER2-specific peptide and protein immunity.

The majority of patients developed HER2-specific immunity to the ICD. Moreover, these patients also developed epitope spreading which was associated with improved overall survival in our previous study.

We continue to actively accrue patients to study (target enrollment = 52).

A planned interim analysis will be done after the first 25 patients are enrolled.

References/Acknowledgements


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