The level of HER-2/neu (HER2) gene amplification in breast cancer impacts the persistence of antigen specific T cell immunity achieved after HER2 vaccination

M. Disis¹, D. Wallace¹, A. Coveler¹, D. Higgins¹, J. Childs¹, N. Bates¹, L. Salazar¹, M. Sgota¹, Y. Dang¹, J. Wisman²
¹University of Washington, Seattle, WA and ²Breastlink Medical Group Inc., Long Beach, CA

Introduction

- Studies by our group have shown that HER2 specific immunity, generated endogenously, is more common in patients whose tumors highly overexpress HER2 (Goodell et al, 2008).
- Multiple investigations have suggested that trastuzumab therapy is more effective in HER2 breast cancer patients with higher levels of gene amplification.
- Breast cancer patients can be immunized against HER2 and the immune responses generated can vary greatly in incidence, magnitude, and persistence (Figure 1).
- We questioned whether HER2 specific vaccine efficacy could be predicted by the level of HER2 gene amplification in the primary tumor.
- Patients enrolled on an ongoing dose escalation study of a HER2 ICD plasmid based vaccine were evaluated to assess whether pre-existent HER2 specific T cell immunity, the magnitude of response achieved with immunization, or the persistence of response after immunization was impacted by the level of HER2 gene amplification in their breast cancers.

Methods

Study Population
Twenty-two subjects with stage III or IV breast cancer meeting these criteria: (1) primary tumor or metastases with HER2 overexpression by FISH or 3+ by IHC (2) NED or stable disease of cytotoxic chemotherapy for 30 days (3) ECOG performance status 0 (4) normal baseline labs and (5) normal baseline LVEF by MUGA were enrolled on a HER2 vaccine study. Subjects received 10 mcg of a HER2 ICD DNA plasmid based vaccine admixed with GM-CSF as an adjuvant (lowest dose of a dose escalation study). The vaccine was administered intradermally once a month for three months and the patients were followed via intermittent immune response evaluations.

The patients enrolled all had evaluation of HER2/CEP17 ratios available from either primary, recurrent or metastatic lesions. Thes data were correlated with immune response parameters measured by ELISPOT.

Assessment of T cell immunity via ELISPOT. IFN-g enzyme-linked immunosorbent (ELISPOT) assay was performed as previously described (Park et al, 2008). Antigens included one ring of overlapping peptide-pools (10 mer) peptides overlapping by 7 residues for the HER2 ECD and 9.36 nmol tetanus toxoid. Each antigen was assessed in 6 replicates of 250 IFU PMBC. With PMBC alone as negative controls and used to establish the background. All samples for each patient were cryopreserved, then thawed and analyzed simultaneously to ensure comparability. Data are presented as IFN-g SPW corrected for background (Corrected SPW). The number of patients presented in each analysis was based on individuals having a pre-vaccine ELISPOT; at least one post-vaccine analysis greater than 6 months after the end of immunization. Summary data demonstrating vaccinated responses in this group are presented in individuals as a calculated frequency of IFN-g secreting cells in 10^6 PMBC and discussed as the ratio of responding cells to PMBC.

Statistical Analysis
 Differences in median immune responses were compared using a non-parametric Mann-Whitney test, with a significance level set at 0.05. Linear regression was used to assess the correlation between HER2/CEP17 ratio and immune responses. Progression-free survival (PFS) was defined as the time elapsed between first vaccine and disease progression as reported by the patients' primary physicians. Analyses were performed with GraphPad Prism v.5.01 (GraphPad Software, San Diego, CA).

Pre Existent Immunity

Persistence of Response After Vaccination

Conclusions

- The level of HER2 gene amplification did not impact the level of pre-existent T cell immunity to HER2 in this patient population.
- The level of HER2 gene amplification did not impact the magnitude of HER2 specific T cell immunity generated with vaccination.
- Inability to maintain immunity was not associated with greater risk of progressive disease or death in patients with higher levels of gene amplification.
- Higher levels of HER2 gene amplification may adversely impact the development of significant antigen specific memory T cell responses after vaccination.

References

- Goodell, V., Winsto, J., Gdaski, J., Dutta, J., Ries, S., Linke, J., Conover, R., Fedele, W., Chida, S., Frisell, P.A., Fager, D., and discrete. Based on the level of expression of HER2, the vaccine could be selected for the immunization of breast cancer patients.

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