Greenwich LifeSciences

Final five year median follow-up data from a prospective, randomized, placebo-controlled, single-blinded, multicenter, phase IIb study evaluating a time series of immune responses using HER2/neu peptide GP2 + GM-CSF vs. GM-CSF alone after adjuvant trastuzumab in HER2 positive women with operable breast cancer

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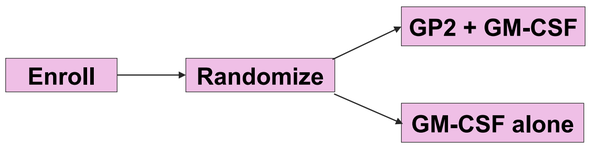
BACKGROUND

The final analysis of the GP2 prospective, randomized, placebo-controlled, single-blinded, multicenter Phase IIb trial (*NCT00524277*) investigating GP2+GM-CSF versus GM-CSF alone in HLA-A02 patients administered in the adjuvant setting to node-positive and high-risk node-negative breast cancer patients with HER2 status (IHC 1-3+) is now complete with 5 year follow-up. It has been previously reported that completion of the GP2+GM-CSF Primary Immunization Series (PIS) reduced recurrence rates to 0% over a 5 year follow-up period in HER2 3+ patients, who received a standard course of trastuzumab after surgery. Here we present the final 5 year immune response results, assessing peak immunity compared to baseline and between patients treated with GP2+GM-CSF versus GM-CSF alone, including by HER2 status. Interim analyses for this trial have been previously reported by Mittendorf et al.

METHODS

Each GP2-treated patient was scheduled to receive 6 GP2+GM-CSF intradermal injections over the first 6 months as part of the PIS and 4 GP2+GM-CSF booster intradermal injections every 6 months thereafter. Placebo patients received intradermal injections with GM-CSF alone. Immune responses to GP2 were measured over time using delayed-type-hypersensitivity (DTH) skin tests and CD8 T cell dimer binding assays.



Primary Objective

- Determine if GP2+GM-CSF treatment reduces recurrence rates vs. GM-CSF alone

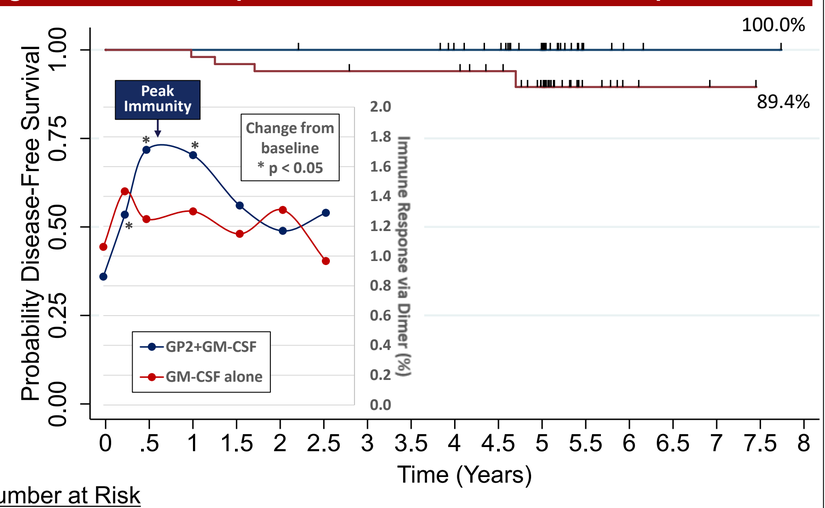
Secondary Objective

- Monitor immune response and correlate with clinical outcomes
- Monitor for any unexpected toxicities

RESULTS

This basket trial explored HER2 3+ patients, who received a standard course of trastuzumab after surgery, and HER2 1-2+ patients, who did not receive trastuzumab after surgery. A DTH reaction was used to assess in vivo immune responses in patients (n=150). The DTH orthogonal mean was measured 48-72 hours after injection using the sensitive ballpoint-pen method and are compared using a Wilcoxon Rank-Sum. For GP2 treated patients, there was a significant increase in DTH reactions after the PIS compared to baseline DTH reactions. The DTH orthogonal mean in GP2 treated patients at baseline had a median 0.0 mm versus 10.8 mm after the PIS. For patients receiving GM-CSF alone, the DTH orthogonal mean prior to and after the PIS had a median of 0.0 mm. In addition, the DTH reactions after the PIS were significantly greater in GP2 treated patients than in placebo patients (10.8 mm vs. 0.0 mm, p = 0.009) and the DTH immune response in GP2 treated patients was similar between HER2 3+ and HER2 1-2+ patients. Ex vivo immune responses were assessed by phenotypic clonal expansion assays in the majority of patients (n=113). GP2-specific CTLs were quantified using the Ig:A2 dimer assay and demonstrated an expansion over time, showing an increase over baseline after the 3rd vaccination and remaining elevated for the entire course of follow-up ($p < 0.05$) in patients treated with GP2.

Figure 1: Immune Response in HER2 3+ Patients Who Completed PIS



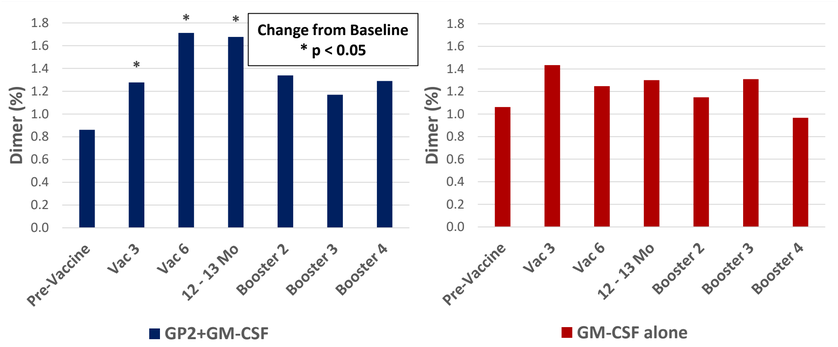
Number at Risk

GP2	46	46	46	46	46	45	45	45	42	40	30	4	2	1	1	1	0
Placebo	50	50	49	48	47	47	46	46	46	43	33	7	3	2	1	0	0

GP2 Placebo

GP2-specific CTLs were quantified using the Ig:A2 Dimer Assay. The assay was assessed over time and the results are presented in Figure 1 for those HER2 3+ patients along with their associated disease-free survival. Immune response in GP2-treated patients increased quickly during the 6 primary vaccinations (PIS) and remained statistically significantly above baseline for 6 months after the PIS ended. Some patients received booster vaccinations beginning at 12 months and the immune response was assessed one month after vaccination.

Figure 2: GP2-specific CTLs by Dimer Assay for HER2 3+

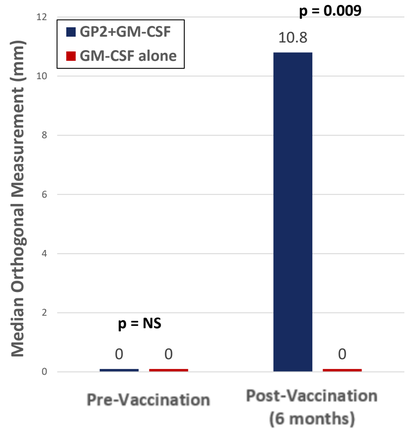


GP2-specific CTLs were quantified using the Ig:A2 Dimer Assay. The Dimer (%) increased in a statistically significant manner over baseline (pre-vaccination) over the course of exposure for HER2 3+ patients treated with GP2.

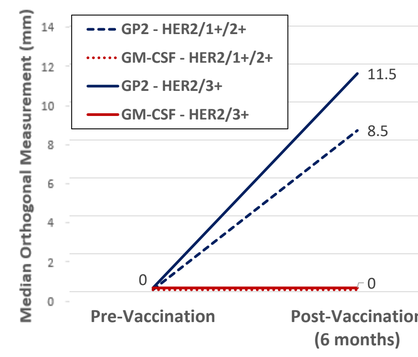
Figure 3: Delayed Type Hypersensitivity Skin Test (DTH)

Immune response was also assessed by DTH following the 6th vaccination after 6 months of exposure to GP2.

The median DTH increased to 10.8 mm at 6 months for GP2+ GM-CSF treated patients, versus 0 mm at baseline. The median DTH response was 0 mm at both baseline and 6 months for GM-CSF alone patients. The treatment effect reached statistical significance (p = 0.009).



The change from baseline in DTH at 6 months was more robust in the GP2+GM-CSF treated patients. Those patients had an 11.6 mm mean increase in DTH after 6 months of exposure while GM-CSF alone patients mean increase was 5.2 mm (p = 0.023).



Immune response by DTH was similarly robust in the HER2 3+ and HER2 1-2+ populations, independent of prior trastuzumab treatment and HER2 expression levels. Thus, GP2's robust immune response in the HER2 1-2+ population suggests the potential to apply GP2 immunotherapy to HER2 low to intermediate breast cancer expressors as well as to other HER2 1-3+ expressing cancers.

CONCLUSIONS

Immunological data comparing peak immunity to baseline and GP2 treated patients to GM-CSF showed that GP2 treated patients, independent of HER2 status, experienced a significant increase in their immune response while those receiving GM-CSF alone did not. Future studies may explore the use of immune responses to assess: immunogenicity of GP2 by HLA type, timing of boosters to sustain immunity, clinical site performance, and the discontinuation of treatment for non-responders.

ACKNOWLEDGEMENTS

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