A Phase I Study of a DNA Plasmid Based Vaccine Encoding the HER-2/neu (HER2) Intracellular Domain (ICD) in Subjects with HER2+ Breast Cancer

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INTRODUCTION

• Breast cancer patients can develop antigen specific immune responses to HER2 immunization.[1]
• DNA vaccines are not MHC-restricted and can elicit both CTL and T helper immune responses.[2]
• Plasmid DNA has been shown to stay present at the vaccine site after immunization has ended.
• Intradermal delivery with GM-CSF may improve APC transfection in vivo.

We asked if intradermal vaccination with different doses of plasmid DNA and GM-CSF was safe and able to generate HER2 specific immunity; and if plasmid DNA persists at the vaccine site after immunization has ended.

METHODS AND PATIENTS

Patient Population. A Phase I dose escalation study of a DNA plasmid based HER2 ICD vaccine was approved by The University of Washington IRB and the FDA. The purpose of this study was to immunize patients against the HER2 ICD domain using 3 different doses of pNGVL3-ICD (10 mcg/ARM, 100 mcg/ARM, 200 mcg/ARM) and to evaluate both the safety and immunogenicity of the approach. Patients with stage III and IV HER2 positive breast cancer were eligible for study if they: (1) had completed standard treatment and were NED with the exception of stable bone disease, (2) were off chemotherapy and steroids for 1 month prior to enrollment, and (3) had normal AST, ALT, and alkaline phosphatase. Treatments were administered 1.5 months for 3 months to the same regional draining lymph node site. Toxicity was evaluated by physical exam and clinical grade and ranked according to the CTCAE v3.0. Toxicity immunologic monitoring occurred at baseline, prior to each vaccine, and 1, 2, 4, and 6 months after the last vaccine. The study was divided into 2 Arms: 1) ARM1 and 2) ARM 2. 19 subjects were enrolled in ARM1 and 20 subjects (ARM 1 and 2) received all 3 vaccinations.

Plasmid DNA Vaccine. The plasmid, pNGVL3, was obtained from the National Gene Vector Laboratory Program. The HER2 ICD sequence was inserted and sequence confirmed by the plasmid's manufacturer (QIAGEN Inc). Plasmid DNA (pNGVL3-ICD) for clinical use was amplified, quantified and validated by the QIAGEN Plasmid Production Facility under GMP. 1.2 ml single-dose vials containing 100µg or 100µg DNA/0.6 ml were subjected to microbial, sterility and endotoxin testing. DNA was isolated using the QIAamp DNA Mini Kit (QIAGEN Inc) as per manufacturer's protocol. Primers used to amplify the vectors are coding primer PRDM36 GACTAACAGACTGTTCCTTTCCATGG and PRDM52 GCCAGAAGTCAGATGCTCAA for the complementary primer. A 1% agarose gel is used to verify the 1790 bp pNGVL3-PCR product.

Evaluation of DNA plasmid persistence at vaccine site. 3-mm skin punch biopsies are obtained from the vaccine site 1 and 6 months after the last vaccine and samples are stored at -70°C until analysis. When analyzed, tissue biopsy specimens are allowed to reduce to room temperature and DNA is isolated using the QIAamp DNA Mini Kit (QIAGEN Inc) per manufacturer's protocol. Primers used to amplify the vectors are coding primer PRDM36 GACTAACAGACTGTTCCTTTCCATGG and PRDM52 GCCAGAAGTCAGATGCTCAA for the complementary primer. A 1% agarose gel is used to verify the 1790 bp pNGVL3-PCR product.

Evaluation of HER2 T cell immunity. HER2 T cell immunity was assessed by 10 day IFN-γ assay as previously described.[3] Antigens evaluated included: 10 µg of p776 HER2 peptide and 10µg of overlapping peptides to the HER2 ICD or ECD domains respectively. No antigen wells, blank (0) (0.5µm), and PMB (0.5 µg/ml) stimulated wells were used as controls. ELISPOT data are presented on individual patients as antigen specific T cells/10^6 PBMC. The best immunologic analysis was done 6 months after the last vaccine for all subjects who completed all 3 vaccinations.

RESULTS

• Majority of toxicity related to vaccination was Grade I and II in both ARM 1 (low dose) and ARM 2 (intermediate dose).
  - Arthralgias
  - Fatigue
  - Vaccine site reaction (induration/pruritis)
• 7 subjects (6 in ARM 1 and 2 in ARM 2) developed asymptomatic transient low-level ANA titers (1:160).
• 6/19 (32%) subjects in ARM 1 had persistent plasmid DNA at site of vaccination after immunizations ended.
• The majority of patients developed HER2 specific immunity in ARM 1 and ARM 2
  - ARM 1 pre- vs max–vaccine immune responses:
    - p776 (p=0.110), ICDpm (p=0.091), and ECDpm (p=0.044)
  - ARM 2 pre- vs max–vaccine immune responses:
    - p776 (p=0.001), ICDpm (p=0.003), and ECDpm (p=0.0005)
• However, the magnitude of post- vs pre vaccination immunity was greater in ARM 2 and immunity was detected months after completing the last vaccine.

CONCLUSIONS

• Immunization with plasmid DNA is safe and well tolerated at low- and intermediate-dose.
• Plasmid DNA persists at the vaccine site after immunization with low-dose pNGVL3-ICD which may provide a constant source of antigenic stimulation.
• PCR analysis of DNA plasmid persistence at vaccine site for ARM 2 is on-going.
• While both low-dose (10µg) and intermediate dose (100µg) pNGVL3-ICD delivered i.d with GM-CSF is able to elicit HER2 specific immunity; the higher dose elicited a greater magnitude of immunity in most patients.
• Enrollment to study ARM 3 (high dose, 500µg pNGVL3-ICD) has completed and immunologic analysis is ongoing.

REFERENCES


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