

# Results of the First Phase 1 Clinical Trial of the HER-2/*neu* Peptide (GP2) Vaccine in Disease-Free Breast Cancer Patients

United States Military Cancer Institute Clinical Trials Group Study I-04

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**BACKGROUND:** HER-2/*neu*, overexpressed in breast cancer, is a source of immunogenic peptides that include GP2 and E75. Phase 2 testing of E75 as an adjuvant vaccine has suggested a clinical benefit. GP2, derived from the transmembrane portion of HER-2/*neu*, has differing binding characteristics and may be more immunogenic than E75. Results of the first phase 1 trial of GP2 peptide vaccine are presented. **METHODS:** Disease-free, lymph node-negative, human leukocyte antigen (HLA)-A2<sup>+</sup> breast cancer patients were enrolled. This dose escalation trial included 4 groups to determine safety and optimal GP2 peptide/granulocyte-macrophage colony-stimulating factor (GM-CSF) dose. Toxicities were monitored. Immunologic response was assessed ex vivo via the HLA-A2:immunoglobulin dimer assay to detect GP2-specific CD8<sup>+</sup> T cells (and E75-specific CD8<sup>+</sup> T cells to assess epitope spreading) and in vivo via delayed type hypersensitivity (DTH) reaction (medians/ranges). **RESULTS:** Eighteen patients were enrolled. All toxicities were grade  $\leq 2$ . Eight (88.9%) of 9 patients in the first 3 dose groups required GM-CSF dose reductions for local reactions  $\geq 100$  mm or grade  $\geq 2$  systemic toxicity. GM-CSF dose was reduced to 125  $\mu$ g for the final dose group. All patients responded immunologically ex vivo (GP2-specific CD8<sup>+</sup> T cells from prevaccination to maximum, 0.4% [0.0%-2.0%] to 1.1% [0.4%-3.6%],  $P < .001$ ) and in vivo (GP2 pre- to postvaccination DTH, 0 mm [0.0-19.5 mm] to 27.5 mm [0.0-114.5 mm],  $P < .001$ ). E75-specific CD8<sup>+</sup> T cells also increased in response to GP2 from prevaccination to maximum (0.8% [0.0%-2.41%] to 1.6% [0.86%-3.72%],  $P < .001$ ). **CONCLUSIONS:** The GP2 peptide vaccine appears safe and well tolerated with minimal local/systemic toxicity. GP2 elicited HER-2/*neu*-specific immune responses, including epitope spreading, in high-risk, lymph node-negative breast cancer patients. These findings support further investigation of the GP2 vaccine for the prevention of breast cancer recurrence. *Cancer* 2010;116:292-301. © 2010 American Cancer Society.

**KEYWORDS:** breast cancer, GP2, HER-2/*neu*, peptide, vaccine.

**Breast** cancer is the most common malignancy in women (excluding cancers of the skin), and is the second leading cause of cancer mortality among women.<sup>1</sup> HER-2/*neu* is a tumor-associated antigen that is overexpressed in approximately 25% to 30% of breast cancer patients and correlates with more aggressive tumor behavior.<sup>2,3</sup> Immunogenic peptides derived from HER-2/*neu* can induce peptide-specific cytotoxic T lymphocytes (CTLs) that recognize tumor cells expressing these peptides complexed with major histocompatibility complex class I molecules.<sup>4</sup> Two such peptides include E75 and GP2. Our group has previously tested and reported on the E75 peptide used as a preventive clinical vaccine in combination with granulocyte-macrophage colony-stimulating factor (GM-CSF) and found it to be safe and effective in raising HER-2/*neu* immunity. Furthermore, this immunity may confer a clinical benefit in the adjuvant setting.<sup>5</sup>

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E75 and GP2 are both 9 amino-acid peptides that are human leukocyte antigen (HLA)-A2 restricted and stimulate CTLs to recognize and lyse HER-2/*neu*-expressing cancer cells.<sup>6,7</sup> E75 is derived from the extracellular domain of the HER-2/*neu* protein (369-377:KIFGSLAFL), whereas GP2 is derived from the transmembrane portion of the HER-2/*neu* protein (654-662:IISAVVGIL). E75 has a high binding affinity for the HLA-A2 molecule and is considered the immunodominant peptide of the HER-2/*neu* protein, whereas GP2 has a low binding affinity and is considered a subdominant epitope.<sup>6</sup> Despite the low binding affinity of GP2 to HLA-A2, GP2 has demonstrated an ability to induce a CTL response similar in magnitude to E75, suggesting that GP2 is equally or perhaps more immunogenic.<sup>8</sup> Intra-antigenic epitope spreading, as evidenced by clonal expansion of GP2-specific CTLs in response to E75 vaccination, has also been observed by our group, further supporting GP2's *in vivo* immunogenicity.<sup>9,10</sup> Because of its potential strong immunogenicity and evidence of epitope spreading, GP2 is an attractive candidate for peptide vaccine trials.

Two small clinical trials have been performed previously with GP2. In 2000, Brossart et al reported that GP2- and E75-pulsed dendritic cells injected subcutaneously in advanced ovarian and breast cancer patients induced a peptide-specific CTL immune response.<sup>8</sup> In 2004, Dees and colleagues used GP2-pulsed dendritic cells administered intravenously in 10 metastatic breast cancer patients, revealing only mild toxicity, with 2 patients in the series achieving a partial response to therapy. The GP2 peptide in the latter trial also generated a significant quantity of dendritic cells from CD34<sup>+</sup> precursors.<sup>11</sup> In both of these trials, the GP2 peptide was loaded onto dendritic cells *ex vivo* and reinfused in the patients with an immunoadjuvant in metastatic patients. Our approach differs in that we have vaccinated disease-free breast cancer patients after completion of standard therapies with the GP2 peptide + GM-CSF immunoadjuvant in an intradermal fashion to prevent disease recurrence.

Here, we report the results of the first GP2 peptide vaccine trial in HLA-A2<sup>+</sup>, HER-2/*neu*<sup>+</sup>, disease-free, high-risk, lymph node-negative breast cancer patients to document safety, measure immunologic responses, and assess for epitope spreading.

## MATERIALS AND METHODS

### *Patient Characteristics and Clinical Protocol*

The trial was institutional review board approved and conducted at Walter Reed Army Medical Center under an investigational new drug application (BB-IND #11,730).

Patients were eligible if they had histologically confirmed lymph node-negative breast cancer that expressed all levels of HER-2/*neu* by standard immunohistochemistry (IHC 1-3+), had completed a standard course of surgery, chemotherapy, and radiation therapy (as required) within 1 month of enrollment, were HLA-A2<sup>+</sup>, and were immunocompetent based on skin tests with a panel of recall antigens (Mantoux test). Patients were considered immunocompetent if they reacted (>5 mm) to  $\geq 2$  antigens. After screening for eligibility criteria and proper counseling and consent, eligible patients were enrolled. Those patients on hormonal chemoprevention were continued on their specific regimen.

### *Vaccine*

The GP2 peptide (HER-2/*neu*, 654-662) was commercially produced in accordance with federal guidelines for good manufacturing practices by NeoMPS Inc. (San Diego, Calif). Peptide purity (>90%) was verified by high-performance liquid chromatography and mass spectrometry, and the amino acid content was determined by amino acid analysis. Sterility, endotoxin (limulus amoebocyte lysate test), and general safety testing was carried out by the manufacturer. Lyophilized peptide was reconstituted in sterile saline at the following concentrations: 100  $\mu$ g/0.5 mL, 500  $\mu$ g/0.5 mL, and 1 mg/0.5 mL. The GP2 peptide was mixed with GM-CSF (Berlex, Seattle, Wash) at 250  $\mu$ g/0.5 mL, and the 1.0-mL inoculation was split and given intradermally at 2 sites 5 cm apart in the same extremity.

### *Vaccine Series*

The study was designed and conducted as a dose escalation safety trial to determine the safety, immunogenicity, and optimal best dose of the GP2 peptide in combination with the adjuvant GM-CSF. The optimal best dose was defined as the minimum dose of the vaccine and adjuvant that gives the best *in vivo* and *ex vivo* immunologic response and limits toxicity. Three patients were assigned to each of the first 3 dose groups receiving 6 monthly inoculations of GP2 and 250  $\mu$ g of GM-CSF. Dose groups are listed as GP2 peptide ( $\mu$ g):GM-CSF ( $\mu$ g):No. of inoculations, and included: 100:250:6, 500:250:6, and 1000:250:6. GM-CSF was reduced by 50% if patients developed a local reaction measuring  $\geq 100$  mm or grade  $\geq 2$  systemic toxicities. In the last group, GM-CSF was reduced so that these 9 patients received 500:125:6.

### *Toxicity*

Patients were observed 1 hour postvaccination for immediate hypersensitivity and returned 48 to 72 hours later to

have injection sites measured and be questioned in regard to local/systemic toxicities. Toxicities were graded using the National Cancer Institute Common Terminology Criteria for Adverse Events version 3.0. Progression from 1 dose group to the next occurred only in the absence of dose-limiting toxicities, defined as any hypersensitivity reaction or 2 patients within a dose group developing grade  $\geq 3$  toxicity.

### Peripheral Blood Mononuclear Cell Isolation and Cultures

Blood was drawn before each vaccination and at 1 (post-vaccination) and 6 months (long-term) after vaccine series completion. Fifty milliliters of blood was drawn, and peripheral blood mononuclear cells (PBMCs) were isolated. PBMCs were washed and resuspended in culture medium and used as a source of lymphocytes as described previously.<sup>12</sup>

### HLA-A2:Immunoglobulin Dimer Assay

The presence of GP2-specific CD8<sup>+</sup> T cells in freshly isolated PBMCs from patients was assessed directly *ex vivo* by the dimer assay at baseline, before each successive vaccination, and at 1 and 6 months after completion of the vaccination series.<sup>13</sup> Briefly, the HLA-A2:immunoglobulin (Ig) dimer (PharMingen, San Diego, Calif) was loaded with the GP2, E75, or control peptide (E37, folate-binding protein, 25-33:RIAWARTEL) by incubating 1  $\mu$ g of dimer with an excess (5  $\mu$ g) of peptide and 0.5  $\mu$ g of  $\beta_2$ -microglobulin (Sigma, St. Louis, Mo) at 37°C overnight, then stored at 4°C until used. PBMCs were washed and resuspended in PharMingen Stain Buffer, added at  $5 \times 10^5$  cells/100  $\mu$ L/tube in 5-mL round-bottom polystyrene tubes (Becton Dickinson, Mountain View, Calif), and stained with the loaded dimers and antibodies. In each patient, the level of GP2-specific and E75-specific CD8<sup>+</sup> cells was determined in response to each successive vaccination, and postinoculation levels were compared with preinoculation levels.

### Delayed Type Hypersensitivity

Delayed type hypersensitivity (DTH) reactions to the GP2 peptide were performed before and after the vaccination series. Intradermal injections, on the back or extremity (opposite side from vaccination), using 100  $\mu$ g of GP2 (without GM-CSF) in 0.5 mL saline, were compared with an equal volume control inoculum of saline. DTH reactions were measured in 2 dimensions at 48 to 72

**Table 1.** Patient Demographics, Prognostic Factors, and Treatment Profiles

Characteristics	GP2 Patients, n=18, No. (%)
<b>Age, y</b>	
Median	47
Range	32-68
<b>Race</b>	
White	14 (77.8)
Black	2 (11.1)
Other	2 (11.1)
<b>Tumor size</b>	
T2-T4	7 (38.9)
<b>Histological grade</b>	
Grade 3	7 (38.9)
HER-2/ <i>neu</i> IHC 3 <sup>+</sup> or FISH <sup>+</sup>	6 (33.3)
Hormone receptor negative	8 (44.4)
No chemotherapy	6 (33.3)
No XRT	6 (33.3)
Hormonal therapy	9 (50.0)

IHC indicates immunohistochemistry; FISH, fluorescent in situ hybridization; XRT, external-beam radiation therapy.

hours using the sensitive ballpoint pen method and reported as the orthogonal mean.<sup>14</sup>

### Statistical Analysis

*P* values for clinicopathological factors were calculated using Wilcoxon, Fisher exact test, or chi-square as appropriate. *P* values for comparing pre- and postvaccination DTH and dimer assays were calculated using Wilcoxon. Differences were considered significant when *P* < .05.

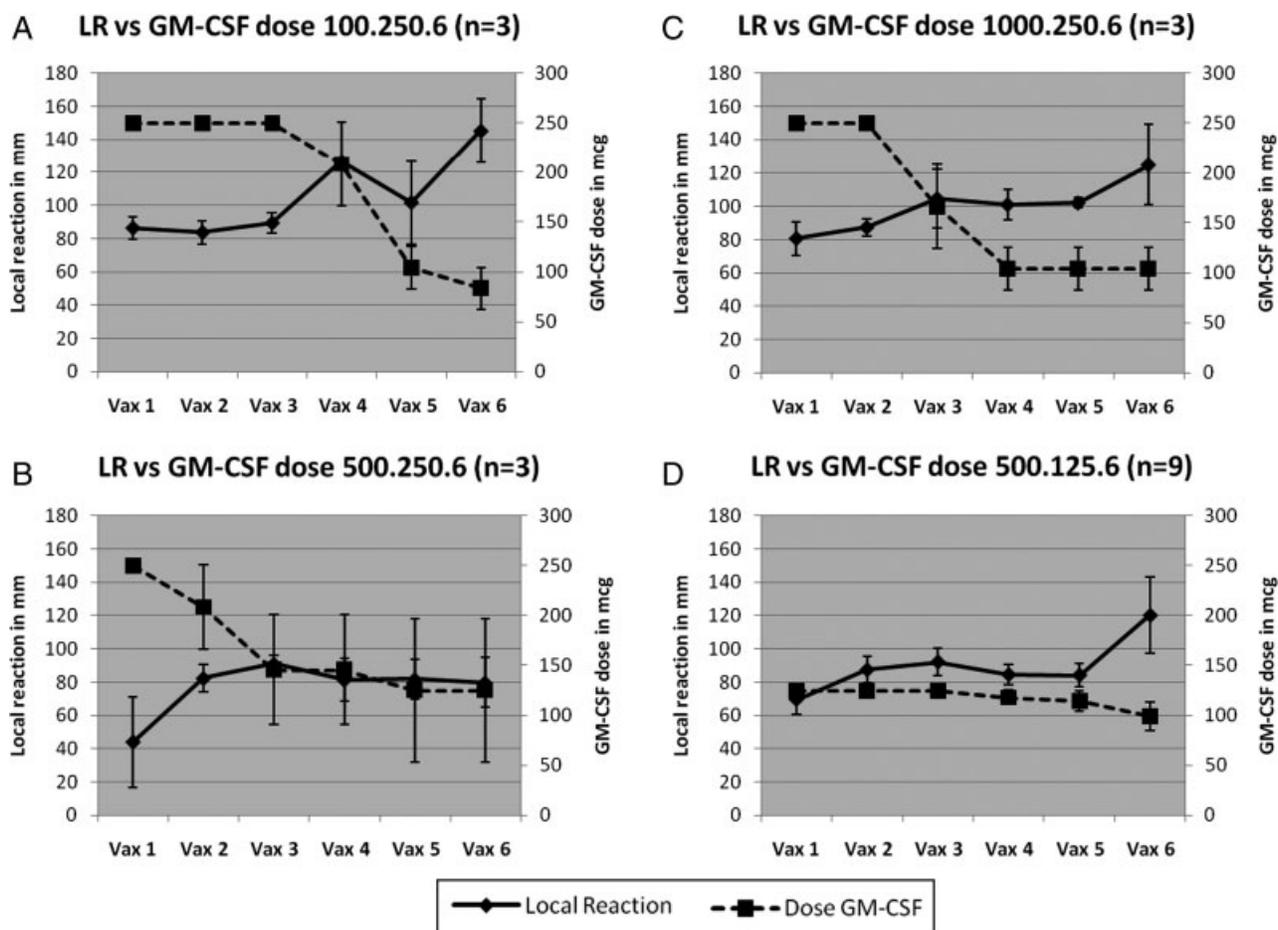
## RESULTS

### Patients

We enrolled and vaccinated 18 disease-free, lymph node-negative breast cancer patients with all levels of HER-2/*neu* expression (IHC 1-3+). No patients withdrew from this study or were lost to follow-up. Patient demographics, prognostic factors, and treatment profiles are presented in Table 1.

### Dose Groups

This dose escalation trial used an increasing GP2 peptide dose (100  $\mu$ g, 500  $\mu$ g, and 1000  $\mu$ g) with 250  $\mu$ g of GM-CSF and 6 monthly intradermal inoculations for the first 3 dose groups (abbreviated as GP2 peptide [ $\mu$ g]:GM-CSF [ $\mu$ g]:No. of inoculations: 100:250:6, 500:250:6, and 1000:250:6). The GM-CSF was reduced by 50% if patients developed a local reaction measuring  $\geq 100$  mm or grade  $\geq 2$  systemic toxicities. Eight (89%) of the first 9



**Figure 1.** Mean local reactions (LR) versus granulocyte-macrophage colony-stimulating factor (GM-CSF) dose by dose groups are shown. Patients were vaccinated with peptide and GM-CSF in 4 dose groups. (A) The 100- $\mu$ g peptide/250- $\mu$ g GM-CSF dose group is shown. (B) The 500- $\mu$ g peptide/250- $\mu$ g GM-CSF dose group is shown. (C) The 1000- $\mu$ g peptide/250- $\mu$ g GM-CSF dose group is shown. (D) The 500- $\mu$ g peptide/125- $\mu$ g GM-CSF dose group is shown. Local reactions were measured in millimeters (solid lines). A local reaction  $\geq 100$  mm induration necessitated a 50% reduction in GM-CSF dose (dashed lines). There were no peptide dose reductions. Vax indicates vaccination.

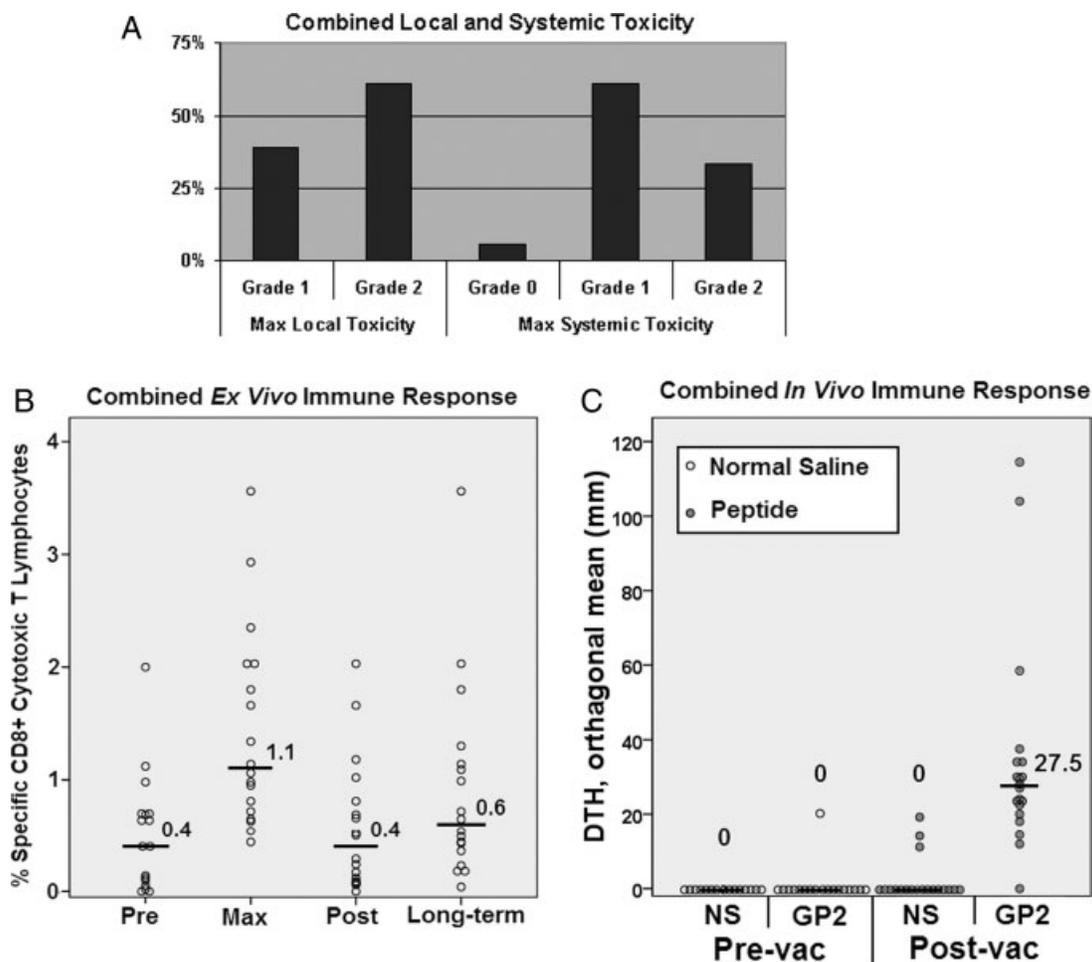
patients required GM-CSF dose reductions because of robust local reactions. Because of the number of dose reductions required, the starting dose of GM-CSF was reduced from 250  $\mu$ g to 125  $\mu$ g per inoculation for the fourth and final group of 9 patients (500:125:6). Only 2 (22%) of the 9 patients in the final dose group required a further GM-CSF dose reduction. No peptide dose reductions were required for the vaccination series. Figure 1 depicts the mean local reactions versus the mean GM-CSF dose for each dose group. Local reactions in the final dose group fluctuated less throughout the vaccination series using a GM-CSF starting dose of 125  $\mu$ g per inoculation.

#### Combined Dosing Group

There were no grade 3-5 toxicities among the 18 patients receiving a total of 108 doses of GP2 + GM-CSF. Among

all patients, the maximum local toxicity occurring during the entire series was grade 1 in 38.9% of the patients and grade 2 in 61.1%. The maximum systemic toxicity during the series was grade 0 in 5.6% of the patients, grade 1 in 61.1%, and grade 2 in 33.3%. The most common local reactions included erythema and induration (100% of patients), pruritis (25%), and inflammation (23%). The most common systemic reactions were grade 1 fatigue (40%) and grade 1 arthralgia/myalgia (15%). Overall combined local and systemic toxicity rates are noted in Figure 2A.

The GP2 + GM-CSF vaccine was capable of eliciting an immune response both ex vivo and in vivo. Ex vivo immune response was assessed via HLA-A2:Ig dimer assay to detect the percentage of circulating GP2-specific CD8<sup>+</sup> T cells. GP2-specific CTLs are reported as the



**Figure 2.** Toxicity and immunologic responses of all patients enrolled in the GP2 phase 1 trial are shown: (A) toxicity—no patients experience grade 3-5 local or systemic toxicities; (B) ex vivo immune response—percentage specific CD8<sup>+</sup> T cells statistically significantly increased from prevaccination (Pre) to maximum (Max) ( $P = .0005$ ); (C) in vivo immune response—GP2 delayed type hypersensitivity (DTH) statistically significantly increased from pre- to postvaccination (Post) ( $P = .000004$ ). A normal saline (NS) control is also shown for comparison.

median (range) percentage of the total circulating CD8<sup>+</sup> population. Time points analyzed include prevaccination (0.4% [0.0%-2.0%]), 1 month after completion of all inoculations (0.4% [0.0%-2.0%]), maximum value during series (1.1% [0.4%-3.6%]), and 6 months after completion of all inoculations (long-term, 0.6% [0.04%-3.6%]). Whereas a statistically significant increase occurred in patients when comparing prevaccination versus maximum E75-specific CTL levels ( $P = .0005$ ), no significant increase was seen comparing pre- versus postvaccination or long-term levels ( $P = .8$  and  $P = .1$ , respectively) (Fig. 2B).

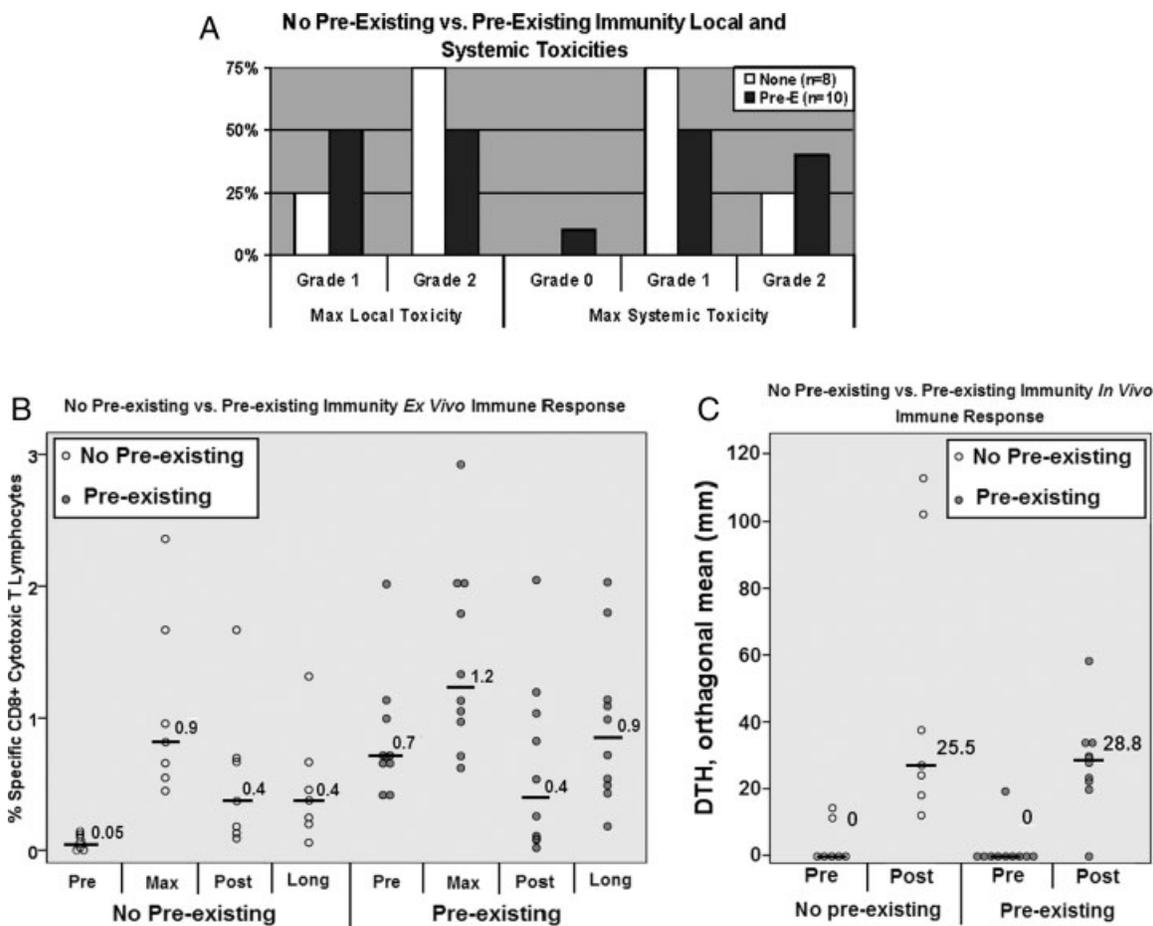
The vaccine's in vivo effectiveness was analyzed via pre- and postvaccination series DTH responses using GP2 (without GM-CSF) as well as a saline volume con-

trol. A statistically significant increase was noted in pre- versus postvaccination DTH responses to the GP2 peptide (0 mm [0.0-19.5 mm] vs 27.5 mm [0-114.5 mm],  $P = .000004$ ) (Fig. 2C).

To better elucidate the immunologic response to the GP2 vaccine, 2 different subset analyses were performed: response based on the presence of pre-existing GP2-specific immunity and response based on dose of GM-CSF. These are provided below.

#### **Pre-existing Versus No Pre-existing Immunity**

As previously defined for the dimer assay, pre-existing immunity is a peptide-specific dimer level  $\geq 0.3\%$ .<sup>5</sup> Ten (56%) patients had dimer levels consistent with pre-existing immunity to GP2, and 8 (44%) patients had no pre-



**Figure 3.** Toxicity and immunologic responses of patients enrolled in the GP2 phase 1 trial comparing no pre-existing immunity (predimer <0.03) versus pre-existing immunity (Pre-E) (predimer >0.03) are shown: (A) toxicity—toxicities were slightly increased in the patients without pre-existing immunity, albeit not significantly; (B) ex vivo immune response—patients without pre-existing immunity showed statistically significant increases in prevaccination (Pre) to maximum (Max), pre- to postvaccination (Post), and prevaccination to long-term (Long) percentage specific CD8<sup>+</sup> T-cells ( $P = .0003$ ,  $P = .004$ , and  $P = .004$ , respectively) in response to vaccination; (C) in vivo immune response—both pre- to postvaccination delayed type hypersensitivity (DTH) responses statistically increased (none,  $P = .0005$ ; Pre-E,  $P = .0004$ ). No statistical difference between postvaccination DTH responses was noted ( $P =$  not significant).

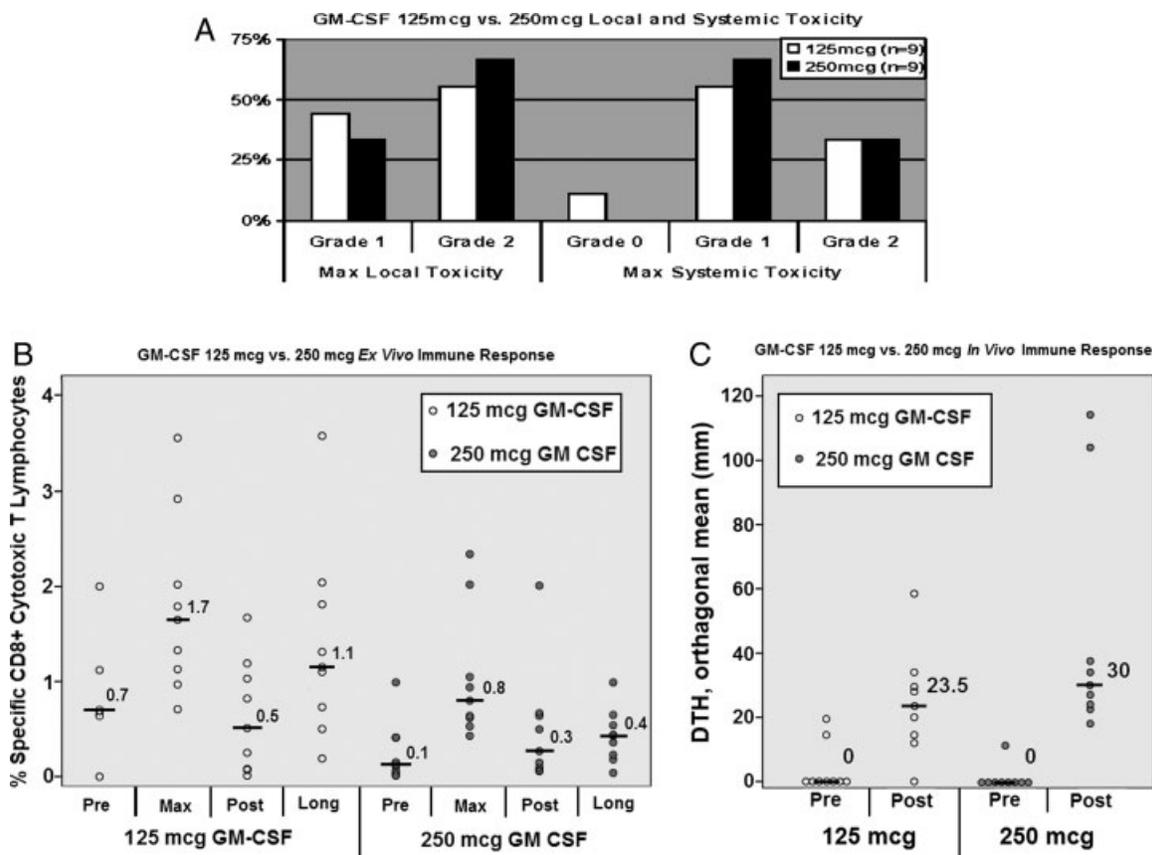
existing immunity. There was a statistical difference between the 2 groups' prevaccination GP2 dimer levels (pre-existing = 0.7% [0.4%-2.0%] vs no pre-existing = 0.05% [0.0%-0.1%],  $P = .00003$ ).

Patients without pre-existing immunity had slightly increased local reactions, with slightly higher local toxicities compared with the group with pre-existing immunity; however, this was not statistically significant (Fig. 3A).

Ex vivo and in vivo immune responses were observed in both groups, but were more robust in the group of patients without pre-existing immunity. GP2 dimer levels from the group without pre-existing immunity were prevaccination versus maximum, 0.05% (0.0%-0.1%) versus 0.9% (0.4%-2.4%),  $P = .0003$ ; prevaccina-

tion versus postvaccination, 0.05% (0.0%-0.1%) versus 0.4% (0.08%-1.7%),  $P = .004$ , and prevaccination versus long-term, 0.05% (0.0%-0.1%) versus 0.4% (0.04%-3.6%),  $P = .004$ . In the 10 patients with pre-existing immunity, the CTL response to vaccination was prevaccination versus maximum, 0.7% (0.4%-2.0%) versus 1.2% (0.6%-2.9%),  $P = .03$ ; prevaccination versus postvaccination, 0.7% (0.4%-2.0%) vs 0.4% (0.0%-2.0%),  $P = .4$ ; and prevaccination versus long-term, 0.7% (0.4%-2.0%) vs 0.9% (0.2%-2.0%),  $P = .6$  (Fig. 3B).

When comparing the groups' in vivo immune responses, both groups had statistically significant increases in their prevaccination versus postvaccination DTH responses: no pre-existing immunity = 0.0 mm



**Figure 4.** Toxicity and immunologic responses of patients enrolled in the GP2 phase 1 trial comparing granulocyte-macrophage colony-stimulating factor (GM-CSF) dose 125  $\mu$ g versus 250  $\mu$ g are shown: (A) toxicity—toxicities were slightly increased in the GM-CSF 250  $\mu$ g patients, albeit not significantly; (B) ex vivo immune response—the GM-CSF 125  $\mu$ g and 250  $\mu$ g prevaccination (Pre) to maximum (Max) percentage specific CD8<sup>+</sup> T cells both statistically increased ( $P = .005$  and  $.001$ , respectively), and GM-CSF 250  $\mu$ g prevaccination to long-term (Long) approached statistical significance ( $P = .08$ ); (C) in vivo immune response—GM-CSF 125  $\mu$ g and 250  $\mu$ g prevaccination to postvaccination (Post) delayed type hypersensitivity (DTH) statistically increased ( $P = .003$  and  $P = .00004$ , respectively). There was no statistical significance between GM-CSF 125- $\mu$ g postvaccination DTH and GM-CSF 250- $\mu$ g postvaccination DTH ( $P = .1$ ).

(0.0-14.5 mm) vs 25.5 mm (12.0-114.5 mm),  $P = .0005$ ; pre-existing immunity = 0.0 mm (0.0-19.5 mm) versus 28.8 mm (0.0-58.5 mm),  $P = .0004$ .

Patients regardless of pre-existing immunity had similar postvaccination DTH responses: 25.5 mm (12.0-114.5 mm) versus 28.8 mm (0.0-58.5 mm) for no pre-existing versus existing immunity, respectively,  $P =$  not significant (Fig. 3C).

**GM-CSF 250  $\mu$ g Versus 125  $\mu$ g**

Analysis of the patients according to the 2 starting doses of GM-CSF was also performed. Both local and systemic toxicities were decreased in the final dose group of 125  $\mu$ g GM-CSF, albeit not statistically significantly (Fig. 4A).

CTL response to vaccination in the 250- $\mu$ g dose group (n = 9) was: prevaccination versus maximum,

0.1% (0.0%-1.0%) versus 0.8% (0.4%-2.4%),  $P = .001$ ; prevaccination versus postvaccination, 0.1% (0.0%-1.0%) versus 0.3% (0.08%-2.0%),  $P = .2$ ; and prevaccination versus long-term, 0.1% (0.0%-1.0%) versus 0.4% (0.04%-1.0%),  $P = .08$ . The CTL response in the 125- $\mu$ g dose group (n = 9) was: prevaccination versus maximum, 0.7% (0.0%-2.0%) versus 1.7% (0.7%-3.6%),  $P = .005$ ; prevaccination versus postvaccination, 0.7% (0.0%-2.0%) versus 0.5% (1.0%-1.7%),  $P = .8$ ; and prevaccination versus long-term, 0.7% (0.0%-2.0%) versus 1.1% (0.2%-3.6%),  $P = .2$  (Fig. 4B). Both the 250- $\mu$ g and 125- $\mu$ g GM-CSF groups had significant increases in prevaccination to maximum dimer response, and the 250- $\mu$ g group trended toward significance in the prevaccination versus postvaccination levels. This analysis may be confounded by the finding that only 33% (3 of 9) of the

patients from the 250- $\mu$ g group had pre-existing immunity, whereas 77.8% (7 of 9) patients from the 125- $\mu$ g group had pre-existing immunity.

For in vivo immune responses, all patients, regardless of GM-CSF dose, had a statistically significant increase in DTH response comparing pre- versus postvaccination measurements: 125  $\mu$ g = 0.0 mm (0.0-19.5 mm) to 23.5 mm (0.0-58.5 mm),  $P = .003$ , and 250  $\mu$ g = 0.0 mm (0.0-11.5 mm) to 30.0 mm (18.0-114.5 mm),  $P = .00004$ . Patients receiving 250  $\mu$ g of GM-CSF had a trend toward larger postvaccination DTH responses, although this was not statistically significant: 23.5 mm (0.0-58.5 mm) vs 30.0 mm (18.0-114.5 mm),  $P = .1$  (Fig. 4C).

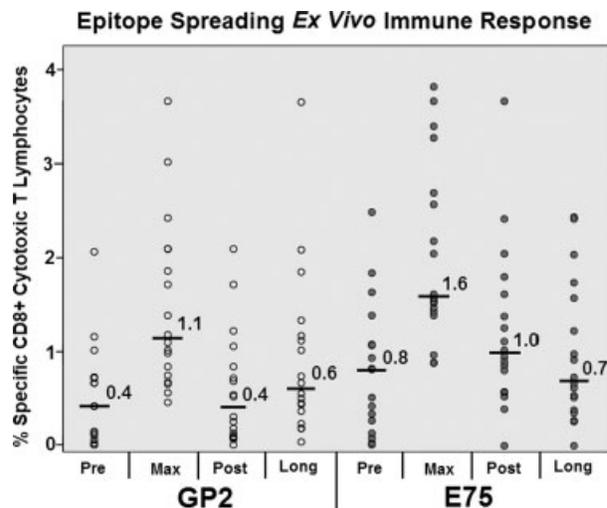
### Epitope Spreading

Lastly, evaluation for evidence of intra-antigenic epitope spreading in response to vaccination with GP2 + GM-CSF was performed. As stated earlier, measurement of both GP2-specific and E75-specific CTLs before, during, and after vaccination was performed. We observed that the percentage of E75-specific CTLs did rise significantly when we compared prevaccination versus maximum levels (0.8% [0.0%-2.4%] vs 1.6% [0.9%-3.7%],  $P = .0003$ ), and increased, but not significantly, from pre- to postvaccination (0.8% [0.0%-2.4%] vs 1.0% [0.0%-3.6%],  $P = .1$ ) and from prevaccination to long-term (0.8% [0.0%-2.4%] vs 0.7% [0.0%-2.4%],  $P = .5$ ) in response to vaccination with GP2 peptide (Fig. 5). Of note, these levels of E75-specific CTLs are similar in magnitude to primary vaccination with E75.

## DISCUSSION

This is the first phase 1 clinical trial of the HER-2/*neu*-derived GP2 peptide with the GM-CSF immunoadjuvant in disease-free breast cancer patients. The GP2 + GM-CSF vaccine is both safe and highly immunogenic. The immune responses, both ex vivo and in vivo, appear to be influenced by the presence or absence of GP2-specific immunity at the initiation of the inoculation series and by the GM-CSF dose used. In addition, GP2 vaccination efficiently results in intra-antigenic epitope spreading.

Toxicity was limited to large local reactions (which are desired and serve as a surrogate measure of in vivo response and vaccine immunogenicity) and mild systemic responses, many of which are known side effects of GM-CSF. There were no dose-limiting toxicities, and dose reductions in GM-CSF were sufficient to limit the local



**Figure 5.** Ex vivo immune response and epitope spreading in response to GP2 are shown. Median E75-specific CD8<sup>+</sup> T lymphocytes were measured in response to vaccination with GP2 peptide vaccine. Prevaccination (Pre) E75 dimer versus maximum (Max) (0.8% [0.0%-2.4%] vs 1.6% [0.9%-3.7%],  $P = .0003$ ), pre- versus postvaccination (Post) (0.8% [0.0%-2.4%] vs 1.0% [0.0%-3.6%],  $P = .1$ ), and prevaccination versus long-term (Long) (0.8% [0.0%-2.4%] vs 0.7% [0.0%-2.4%],  $P = .5$ ) were compared.

reactions encountered with serial inoculations to grade  $\leq 2$ . Overall, the vaccine combination was well tolerated.

The ex vivo immunogenicity of the vaccine was demonstrated, but primarily evident when performing subgroup analysis of the patients without pre-existing immunity. Patients without pre-existing immunity, as previously defined as peptide-specific dimer level  $\leq 0.3\%$ , achieved the greatest induction of a CTL response to GP2 vaccination. This response was uniform without regard to the dose of GP2 peptide. Patients with pre-existing immunity demonstrated a lesser CTL response relative to starting levels, which suggests either a level of tolerance to the endogenously expressed peptide, or a previously optimized endogenous immune response.

The in vivo immunogenicity of the GP2 + GM-CSF vaccine was demonstrated by an increase in the DTH reaction in response to the GP2 peptide (without GM-CSF) after the vaccination series. This difference in response reached statistical significance cumulatively and within each dose group. Also, patients receiving the 250- $\mu$ g GM-CSF dose trended toward a larger DTH response, but this finding was confounded by a larger percentage of patients with pre-existing immunity in the lower GM-CSF dose group. Therefore, it is unclear if the difference seen in the 250- $\mu$ g GM-CSF patients is because of the

adjuvant dose or lack of tolerance. Together, these DTH responses would indicate that in vivo immunity is maintained and/or augmented in all groups in response to vaccination.

GP2, initially described by Peoples et al, is a 9 amino acid peptide derived from the transmembrane portion of the HER-2/*neu* protein.<sup>7</sup> The peptide was isolated using tumor-associated lymphocytes from patients with breast and ovarian cancer, and later found to be shared among several epithelial malignancies including nonsmall cell lung cancer and pancreatic cancer.<sup>15-18</sup>

GP2 differs from E75 in that it has been termed a subdominant epitope of the HER-2/*neu* protein because of its relatively lower binding affinity for the HLA-A2 molecule.<sup>7,19</sup> E75, conversely, has a high affinity for HLA-A2 and has been identified as the immunodominant HER-2/*neu* epitope.<sup>6</sup> Interestingly, both peptides are capable of inducing similar percentages of peptide-specific CTLs, despite GP2 having a substantially lower binding affinity compared with E75. Possible explanations for this observation include that the GP2 peptide stimulates a different and perhaps more varied population of CD8<sup>+</sup> T cells. Kawano and colleagues have previously noted that variations in the E75 peptide alter affinity of the antigen for T-cell receptors and result in differing T-cell responses.<sup>20</sup> If GP2 is in fact generating a more diverse immune response, then vaccination with GP2 may prove more efficacious than with E75.

Previous studies of GP2 differed from this trial in that they used autologous dendritic cells pulsed with GP2 (and other peptides) ex vivo and reinjected subcutaneously or intravenously into patients with metastatic breast or ovarian cancer to induce a CTL response. The trials by Brossart et al were able to detect a peptide-specific (GP2 and E75) CTL response in vivo, and they too noted that both peptides showed a similar immune response despite known differences in HLA-A2 binding affinities.<sup>8</sup> Dees et al evaluated GP2-pulsed dendritic cells in metastatic breast cancer patients and were able to document clinically stable disease in 2 patients.<sup>11</sup> Importantly, these earlier trials detected evidence of intra-antigenic epitope spreading, a finding seen in our trials as well and an integral aspect of the peptide vaccine strategy.<sup>9</sup>

The observation of more robust DTH and local reactions along with greater relative CTL responses among the patients starting with higher GM-CSF doses suggests that the immunoadjuvant dose plays an important role in the immunogenicity and possibly the efficacy of HER-2/*neu* peptide vaccines. As previously reported,

larger doses of E75 + GM-CSF led to more robust DTH reactions and trends toward fewer recurrences, with improved survival in the patients who did recur.<sup>21</sup> In this trial with GP2, larger DTH responses were seen in patients without pre-existing immunity as well as patients receiving the higher GM-CSF dose. If DTH responses do in fact correlate with efficacy, this may lead to a change in the dosing strategy of this and other peptide vaccines. In comparison to our previous E75 trials, the average DTH reaction to GP2 was approximately twice the size of that induced by E75 with on average half the peptide dose. These findings further illustrate the immunogenicity of GP2 and underscore its potential clinical relevance.

In addition, our group has previously demonstrated a possible synergy between trastuzumab and GP2 peptide-stimulated CTLs ex vivo. Pretreatment of breast cancer cells with trastuzumab followed by incubation with GP2 peptide-induced CTLs resulted in enhanced cytotoxicity in 3 tumor cell lines compared with treatment with trastuzumab or GP2-specific CTLs alone. These results suggest that concurrent GP2 vaccination during trastuzumab therapy may be an intriguing combination immunotherapy.<sup>22</sup>

These findings, taken in total, have prompted our group to undertake a phase 2 trial of GP2 + GM-CSF to evaluate its efficacy in lymph node-positive and high-risk lymph node-negative, disease-free breast cancer patients, and a phase 1 trial of GP2 in combination with trastuzumab. Future directions include combining HER-2/*neu* peptides in an effort toward developing an efficient and potent multiepitope combination peptide vaccine that raises HER-2/*neu* immunity for the prevention of HER-2/*neu*-expressing cancers.

## CONFLICT OF INTEREST DISCLOSURES

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