

Optimal Dose and Schedule of an HER-2/*neu* (E75) Peptide Vaccine to Prevent Breast Cancer Recurrence

From US Military Cancer Institute Clinical Trials Group Study I-01 and I-02

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BACKGROUND. E75, a HER-2/*neu*-derived peptide, was administered as a preventive vaccine with granulocyte-macrophage-colony-stimulating factor (GM-CSF) in disease-free lymph node-positive (NP) and lymph node-negative (NN) breast cancer (BCa) patients. The optimal biologic dose (OBD) was determined based on toxicity and immunologic response.

METHODS. Patients were vaccinated over 6 months (3, 4, or 6 times) with different doses of E75 plus GM-CSF. Toxicities were graded per National Cancer Institute Common Terminology Criteria. GM-CSF was reduced for significant toxicity. Immunologic response was measured by delayed type hypersensitivity test (DTH), and E75-specific CD8⁺ T-cells were quantified with human leukocyte antigen-A2:immunoglobulin G dimer and flow cytometry.

RESULTS. Ninety-nine patients (48 NP and 51 NN) were vaccinated in 7 dose groups. The OBD was 1000 µg E75 plus 250 µg GM-CSF monthly × 6. The optimal dose group (ODG, n = 29) experienced similar toxicities to the suboptimal dose group (SDG, n = 70), which was comprised of the remaining 6 groups. The ODG demonstrated a trend toward an increase in the average postvaccine dimer (0.87 ± 0.10% vs 0.67 ± 0.05%; *P* = .07), a significantly larger DTH response (21.5 ± 2.5 mm vs 11.3 ± 1.3 mm; *P* = .0002), and a trend toward decreased recurrences (3.4% vs 12.9%; *P* = .27). Compared with the SDG, the ODG had larger tumors (percentage ≥T2: 55% vs 23%; *P* = .004), more positive lymph nodes (percentage NP: 76% vs 37%; *P* = .001), and higher grade tumors (percentage grade 3: 52% vs 30%; *P* = .07), but a shorter median follow-up time (20 months vs 32 months; *P* < .001).

CONCLUSIONS. Compared with suboptimally dosed patients, the optimally dosed E75 vaccine in disease-free BCa patients had similar toxicity but enhanced HER-2/*neu*-specific immunity that may lead to decreased recurrences with additional follow-up. *Cancer* 2008;113:1666-75. Published 2008 by the American Cancer Society.*

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Our group, the Cancer Vaccine Development Program (CVDP), has focused on immunogenic peptides derived from the HER-2/*neu* protein to develop vaccine-based strategies to prevent recur-

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rence of epithelial cancers. Specifically, the CVDP has examined vaccination with immunogenic peptides such as E75, GP2, and AE37 from the HER-2/*neu* protein along with an immunoadjuvant, granulocyte-macrophage-colony-stimulating factor (GM-CSF), to prevent disease recurrence in patients with prostate and breast cancer (BCa).

HER-2/*neu* is a proto-oncogene in the epidermal growth factor receptor family of tyrosine kinase receptors and encodes for a transmembrane glycoprotein that is highly expressed in many epithelial derived cancers and that has been shown to be an immune-recognized tumor associated antigen.¹⁻⁴ HER-2/*neu* expression is variable and may be detected by immunohistochemistry (IHC) and fluorescent in situ hybridization (FISH). IHC detects overexpression of HER-2/*neu* protein and is reported on a semiquantitative scale of 0 to 3⁺ (0 indicates negative, 1⁺ indicates low expression, 2⁺ indicates intermediate expression, and 3⁺ indicates overexpression). FISH conversely detects amplification (excess copies) of the HER-2/*neu* gene, which is expressed as a ratio of HER-2/*neu* to chromosome 17 and interpreted as positive if FISH is ≥ 2.2 .⁵ The concurrence rate of IHC and FISH is approximately 90%.⁶ Trastuzumab is reserved for patients who are overexpressors of HER-2/*neu*, defined as IHC 3⁺ or FISH ≥ 2.2 . Because the mechanism of action for vaccines differs from monoclonal antibodies, the former does not necessarily require overexpression of the target protein. Therefore, HER-2/*neu* may be targeted by vaccines in patients who are either 1 to 2⁺ for IHC or <2 for FISH.

Several immunogenic peptides from HER-2/*neu* are recognized by cytotoxic T lymphocytes (CTLs).^{7,8} One immunogenic peptide, E75 (KIFGSLAFL, HER-2/*neu*; 369-377), a human leukocyte antigen (HLA)-A2- and HLA-A3-binding 9 amino acid peptide recognized by CTLs, has to our knowledge become the most studied HER-2/*neu*-derived peptide both in vitro and in vivo.⁹⁻¹³ E75 has been used as an anticancer vaccine in various forms, including a single-peptide vaccine combined with different immunoadjuvants,¹¹⁻¹³ loaded onto autologous dendritic cells and reinfused,¹⁴⁻¹⁶ and embedded in longer peptides capable of binding HLA class II molecules to recruit CD4 helper T-cells.^{17,18} Each of the above methods is safe and effective at stimulating E75-specific immunity. The CVDP has combined this E75 peptide with the immunoadjuvant GM-CSF to create a simple exportable vaccine for the prevention of BCa recurrence in high-risk patients clinically free of disease.

Our clinical trials have all been prevention trials. The E75 vaccine administered to high-risk prostate

cancer patients was found to be well tolerated and effective in eliciting an immune response against HER-2/*neu*-expressing cancer cells. Our study suggested that the vaccine may be useful as a preventive strategy against disease recurrence, if used before the prostate-specific antigen increases.¹⁹ When the E75 vaccine was initially evaluated in patients with lymph node-positive (NP) BCa, it was also shown to be safe and effective in eliciting a peptide-specific immune response in vivo and appeared to reduce the recurrence rate.²⁰ Our combined study has enrolled 186 previously treated, disease-free NP and lymph node-negative (NN) BCa patients who were vaccinated with E75 plus GM-CSF.²¹ The vaccine was found to be safe and effective in raising dose-dependent HER-2/*neu* immunity, as observed with CD8⁺ E75-specific T-cell clonal expansion and delayed type hypersensitivity (DTH) in HLA-A2-positive (HLA-A2⁺) and HLA-A3⁺ NP and NN BCa patients. More importantly, E75 reduced disease recurrence in disease-free, conventionally treated, high-risk BCa patients at a median follow-up of 20 months. However, this statistical finding did not extend beyond 26 months in the absence of booster inoculations.

In the combined BCa trial, there were 7 different dose and schedule groups in the vaccine arm of the study. Herein, we present the analysis of the toxicity and immune responses in these dose groups to determine an optimal biologic dose (OBD).

MATERIALS AND METHODS

Patient Characteristics and Clinical Protocol

The NP and NN trials were approved by the local institutional review boards and conducted at Walter Reed Army Medical Center in Washington, DC and the Joyce Murtha Breast Care Center in Windber, Pennsylvania under an investigational new drug application (BB-IND#9187). All patients had histologically confirmed BCa, and all had completed a standard course of surgery, chemotherapy, and radiotherapy (as required) before enrollment. Patients receiving hormonal therapy were continued on their specific regimen. After proper counseling and consent, the patients were enrolled and then HLA typed. The HLA-A2⁺ patients were vaccinated, because E75 binds primarily HLA-A2⁺, and the HLA-A2⁻ patients were followed prospectively as unvaccinated controls. Because 40% to 50% of the general population is HLA-A2⁺, the groups were approximately equal in number.²² During the trials, we determined that E75 can also bind to HLA-A3. This was based on binding affinity data from 2 commonly used HLA-peptide

binding algorithms: BIMAS (available at: http://bimas.dcrf.nih.gov/molbio/hla_bind/ accessed on March 1, 2008) and SYFPEITHI (available at: <http://www.syfpeithi.de/> accessed on March 1, 2008).^{22,23} In addition, preclinical evaluation demonstrated that E75-stimulated HLA-A3⁺ CTLs could lyse HLA-A3⁺ HER-2/*neu*-expressing cancer cells (unpublished data).

Vaccine

The E75 peptide was commercially produced in good manufacturing practices grade by NeomPS, Inc (San Diego, Calif). Peptide purity (>95%) was verified by high-performance liquid chromatography and mass spectrometry, and the amino acid content was determined by amino acid analysis. Lyophilized peptide was reconstituted in sterile saline at 100 µg, 500 µg, or 1000 µg in 0.5 mL. This peptide was mixed with GM-CSF (Berlex, Seattle, Wash) in 0.5 mL, and the 1.0-mL inoculation was split and administered intradermally at 2 sites 5 cm apart. All inoculations were given in the same extremity.

Overall Study Design

The first E75 trial enrolled immunocompetent, disease-free, NP BCa patients. HLA A2⁺ and A3⁺ BCa patients were enrolled into the vaccination arm.²¹ The study was designed as a 3-stage safety trial with escalating doses of peptide in the initial stage and dose optimization in the second stage, followed by schedule optimization in the third stage, as shown in Figure 1. Details of the vaccine series have been previously published.²⁰ Initially, a small group of patients³⁻⁶ received either 4 or 6 monthly inoculations with 100 µg, 500 µg, or 1000 µg of E75 peptide mixed with 250 µg of GM-CSF. Groups were ultimately expanded to determine and confirm optimal dosing in NP patients, accounting for the larger number of patients in the latter dose groups.

We also conducted an overlapping second trial with immunocompetent, disease-free, NN BCa patients. Patients with non-HER-2/*neu*-expressing tumors were allowed in this trial to determine the feasibility of vaccinating a presumably antigen-naive host. The NN trial was designed to further delineate optimal biologic dosing by varying the dosage of GM-CSF (125 µg vs 250 µg) and peptide (500 µg vs 1000 µg) and altering the inoculation schedule (3, 4, or 6 injections over 5 months) as seen in Figure 1.

Combining the NP and NN BCa patients, there were a total of 7 different dosing groups from the parallel trials (Table 1). A dosing group was numerically identified by µg E75 : µg GM-CSF : total number of doses (ie, 1000.250.6).

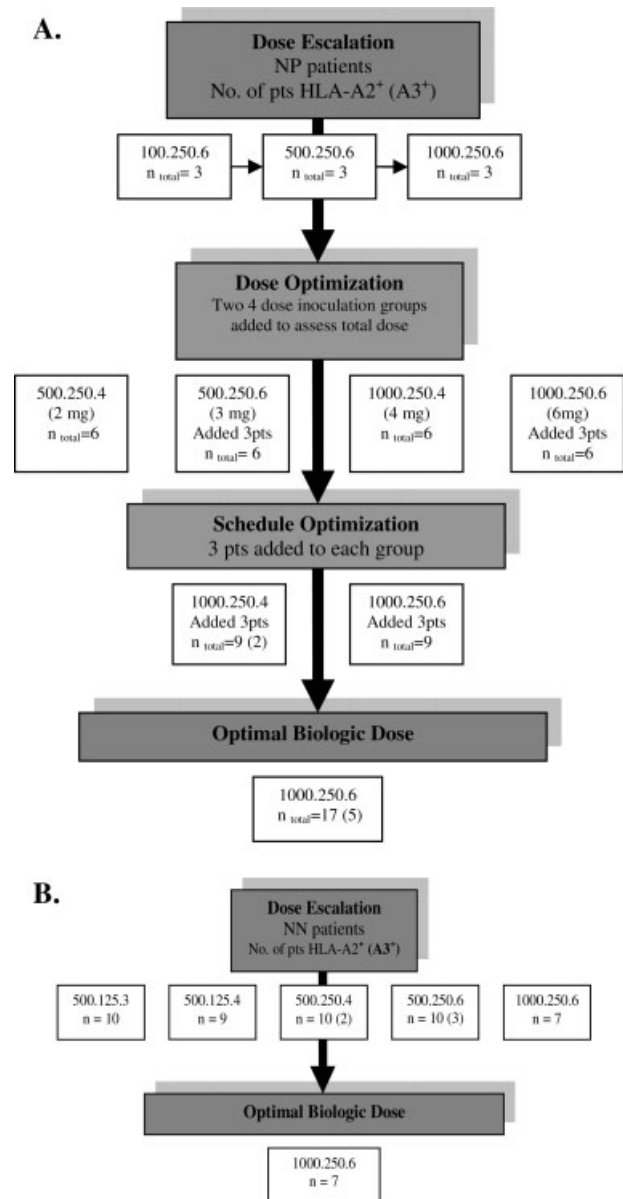


FIGURE 1. The diagram represents all lymph node-positive (NP) and lymph node-negative (NN) breast cancer (BCa) patients (pts) in the combined E75 trials (n = 99 patients). All immunocompetent, disease-free BCa patients who were vaccinated were either human leukocyte antigen (HLA)-A2-positive (HLA-A2⁺) or -A3⁺. (A) Forty-eight NP BCa patients were enrolled in the initial trial and evaluated for optimal dosing of the E75 peptide. (B) Fifty-one NN BCa patients were enrolled in the subsequent study to further delineate optimal biologic dosing.

Toxicity

Patients were observed for 1 hour after vaccination for immediate hypersensitivity and returned 48 to 72 hours later to have their injection sites measured and to be questioned regarding toxicities. Toxicities were graded by the National Cancer Institute Com-

TABLE 1
The 7 Different Combined Dosing Groups

Patient Group*	No. of Patients HLA-A2 ⁺ (A3 ⁺)	Peptide Dosage, μg	GM-CSF Dosage, μg	Months Vaccinated
100.250.6	3	100	250	0, 1, 2, 3, 4, and 5
500.125.3	10	500	125	0, 1, and 5
500.125.4	9	500	125	0, 1, 2, and 5
500.250.4	16 (2)	500	250	0, 1, 2, and 5
500.250.6	16 (3)	500	250	0, 1, 2, 3, 4, and 5
1000.250.4	9 (2)	1000	250	0, 1, 2, and 5
1000.250.6	24 (5)	1000	250	0, 1, 2, 3, 4, and 5
Total (HLA-A2 ⁺ + A3 ⁺)	99			

HLA indicates human leukocyte antigen; +, positive; GM-CSF, granulocyte-macrophage–colony-stimulating factor.

*Dosing groups were numerically identified as μg of E75 : μg of GM-CSF : total number of doses.

mon Terminology Criteria for Adverse Events (version 3.0) and reported on a scale from 0 to 5. Progression from 1 dose group to the next occurred only if no significant toxicity occurred in the lower dose group. Patient-specific results are reported based on maximal local and systemic toxicity occurring during the series.

Peripheral Blood Mononuclear Cell Isolation and Cultures

Blood was drawn before each vaccination and at 1 month (postvaccine) and 6 months (long-term) after the completion of the vaccine series. 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 previously described.²¹

HLA-A2: Immunoglobulin Dimer Assay

The presence of E75-specific CD8⁺ CTLs in freshly isolated PBMCs from patients was directly assessed by using the dimer assay as previously described.²⁴ Briefly, the HLA-A2:immunoglobulin dimer (PharMingen, San Diego, Calif) was loaded with the E75 or control peptide (E37, folate binding protein; 25-33, RIAWARTELE) by incubating 1 μg of dimer with an excess (5 μg) of peptide and 0.5 μg of β₂-microglobulin (Sigma Chemical Company, St. Louis, Mo) at 37°C overnight, and then stored at 4°C until used. PBMCs were washed and resuspended in PharMingen Stain Buffer (PharMingen), added at 5 × 10⁵ cells/100 μL/tube in 5-mL round-bottom polystyrene tubes (Becton Dickinson, Mountain View, Calif), stained with the peptide-loaded dimers and antibodies, and then analyzed by flow cytometry. In each patient, the level of E75-specific CTL was determined in response

to each successive vaccination and is reported as a percentage of total CD8⁺ population. All postinoculation measurements were averaged for each patient and compared with their preinoculation levels.

Delayed Type Hypersensitivity

In both trials, a DTH reaction was assessed with 100 μg of E75 in 0.5 mL of normal saline (without GM-CSF) and 0.5 mL of normal saline as a volume control 1 month after the completion of the vaccine series as described previously. The DTH reaction was measured in 2 dimensions at 48 to 72 hours by using the sensitive ballpoint-pen method, reported as the orthogonal mean, and compared with control.²⁵ Patients in the NN group also underwent DTH testing before vaccination.

Clinical Recurrences

All patients were observed for clinical recurrence per standard cancer screening as dictated by the patient's primary oncologist. A patient was considered to have recurrent disease if proven by biopsy or if treated for disease recurrence by the primary oncology team.

Statistical Analysis

P values for clinicopathologic factors were calculated using the Wilcoxon Chi square rank or Fisher exact test. *P* values for comparing dosing groups with regard to toxicity and immunologic response were calculated using the Wilcoxon signed rank or Student *t* test. Statistical significance is defined as *P* < .05.

RESULTS

Vaccination Dosing Groups

In the combined E75 vaccine trial, 99 immunocompetent, disease-free NP and NN BCa patients were enrolled, assigned to 1 of 7 vaccination groups (Table 1), and completed the inoculation series.

Comparing Toxicity per Dosing Group

The maximal toxicity experienced by each patient during the series was recorded as previously described. The proportion of patients within a dosing group experiencing a specific grade of toxicity was compared to assess dose-related toxicity. Greater than 60% of maximal local toxicity within each group manifested as grade 1 reactions, with the remaining being grade 2 reactions. When groups were compared, there was no obvious trend toward proportional increases in local grade 2 reactions with increasing doses of E75 (Fig. 2A). The maximum systemic toxicity for all dose groups manifested as grade 1 reactions in 70.7%. Evaluating the individual dose

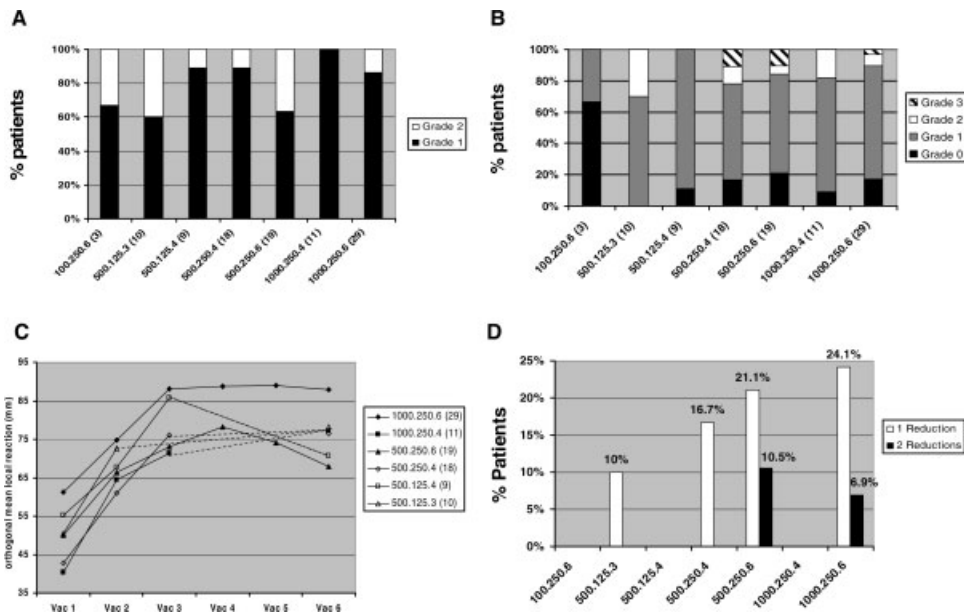


FIGURE 2. A comparison of toxicity and granulocyte-macrophage–colony-stimulating factor (GM-CSF) reductions per dose group. (A) Maximum local toxicity by dose group is shown. Greater than 60% of maximal local toxicities within each group manifested as grade 1 reactions. No proportional increases of grade 2 reactions were identified with increasing cumulative doses of E75. (B) Maximum systemic toxicity by dose group is shown. The maximum systemic toxicity for all dosing groups manifested as grade 1 reactions (70.7%). No proportional increases in grade 2 or 3 reactions were identified with increasing cumulative doses of E75. (C) Average local reaction over the course of vaccination (Vac) by dose group is shown, with greatest response noted within the 1000.250.6 group. (D) Patients requiring GM-CSF reduction are shown by dose group. Overall GM-CSF reductions were required in 19.2% of all vaccinated patients. Dose groups 500.250.6 and 1000.250.6 required reductions in 31.3% of the patients; reductions were required in 7.8% of the remaining 5 groups.

groups for systemic toxicity demonstrated no trend toward greater proportions of grade 2 or 3 reactions with increasing E75 (Fig. 2B). There were no grade 4 or 5 toxicities noted in the current study.

The local inoculation site reactions were monitored over the course of the vaccination series and compared per dose group (Fig. 2C). If a patient experienced a grade 2 systemic toxicity, or the 2 inoculation sites merged and measured >100 mm, the GM-CSF was reduced by 50%. GM-CSF reductions were required more frequently in patients who received 6 doses of 250 µg of GM-CSF. Dosage reductions of GM-CSF were required overall in 19.2% of vaccinated patients, with dosing groups 500.250.6 and 1000.250.6 requiring reductions in 31.3% of the patients for the 2 groups, as compared with 7.8% for the remaining 5 groups (Fig. 2D). It is important to note that there was no dose-limiting toxicity of the peptide noted and thus no maximum tolerated dose was determined.

Comparing Immunologic Response per Dosing Group

Immunologic response was measured in vitro by analysis of E75-specific CTL using the dimer assay, heretofore referred to as the dimer level. The lowest dose group, 100.250.6, had only 3 patients, with data

missing for 1 patient who was excluded because of a paucity of data. Dimer levels were assessed prevaccination, 1 month after each inoculation (postvaccination: an average of 3 to 6 time points depending on dosing schedule), and 6 months after completion of the vaccination series (long-term). The percentage of E75-specific CTLs significantly increased from prevaccination to maximum in each group ($P \leq .05$). When comparing groups, there was a significant difference in the predimer levels for the 500.125.4 group, which was lower compared with all groups ($P \leq .05$) except 500.250.6 and 500.250.4 groups, which were lower than 1000.250.6 ($P = .01$). The only significant difference in the maximum dimer was the lower level in the 500.250.4 group compared with the 1000.250.4 group ($P = .04$). The postvaccination dimer trended toward increasing levels from the least to the greatest cumulative dose groups except for a significantly lower response in 500.125.3 compared with 500.250.6 ($P = .03$) and 1000.250.6 ($P = .004$) (Fig. 3A). The latter dose group appeared to have the most consistent results. Subsequently, long-term data were analyzed and suggest that the 4 inoculation series may be better for long-term maintenance of E75-specific CTLs; however, this finding will require further validation.

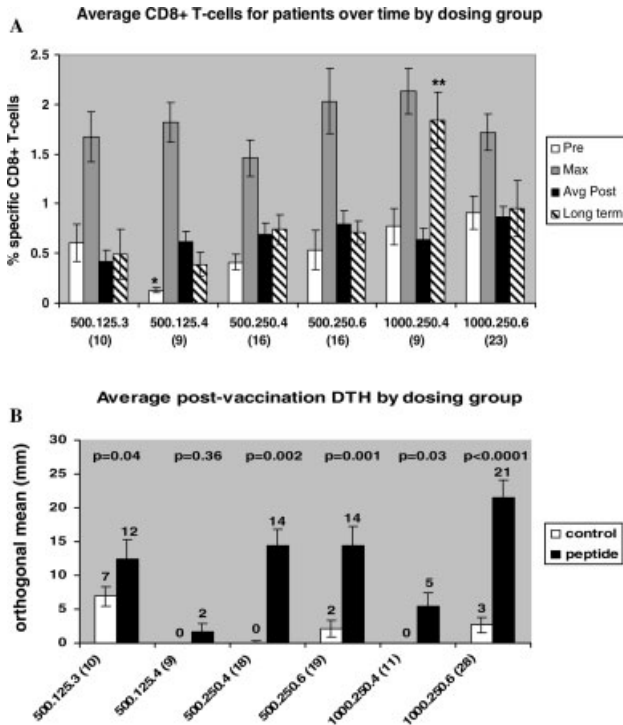


FIGURE 3. Dimer assay and delayed-type hypersensitivity test (DTH) are shown per dosing group. (A) Percentage of E75-specific cytotoxic T-lymphocytes (CTLs) identified prevaccine (Pre), at maximum (Max), postvaccine (Avg Post), and at 6 months (Long term) after the last vaccination are shown. The percentage of E75-specific CTLs significantly increased from prevaccine to maximum ($P \leq .05$). $*P \leq .05$ when compared with all prevaccine E75-specific CTL levels in each dosing group except for the 500.250.6 dosing group. $**P \leq .05$ when compared with all long-term E75-specific CTL levels in each dosing group. (B) Postvaccination delayed type hypersensitivity (DTH) response between the control and peptide (E75) is shown within each dosing group, with the greatest DTH response to the peptide noted in the 1000.250.6 group (21 ± 2.5 mm). Statistical analysis is provided.

There was a significant difference in the postvaccination DTH response between the control and peptide (E75) within each dosing group except for the 500.125.4 group. When comparing dose groups, the largest DTH response was observed in the 1000.250.6 group (21.5 ± 2.5 mm) (Fig. 3B).

Determining the Optimal Biologic Dose

No maximum tolerated dose of E75 was reached in the dose escalation portions of the trials, and 1000.250.6 was the highest dose tested. As defined in the protocol, the 1000.250.6 schedule was deemed to be the optimal biologic dose because patients experienced the same minimal toxicity profile as the other groups, and a superior immunologic response was observed in vivo when compared with the other groups. This dosing schedule did not result in an

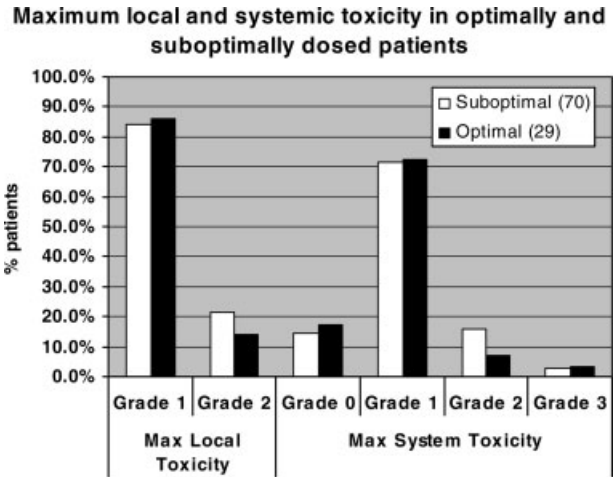


FIGURE 4. Maximum local and systemic reactions per optimal dose group (ODG) versus suboptimal dose group (SDG) are shown. The ODG was the 1000.250.6 dose group ($n = 29$ patients), and the SDG was the 6 remaining groups combined ($n = 70$ patients). No significant differences were identified with regard to local (grade 1 and 2: $P = .58$) or systemic toxicity (grade 0: $P = 1$; grade 1: $P = .64$; grade 2: $P = .72$; and grade 3: $P = 1$).

increase in local or systemic toxicity compared with the other groups (Figs. 2A and 2B). Although it was well tolerated, 31% of patients in this dose group did require a GM-CSF reduction (Fig. 2D). There was a trend toward a higher mean postvaccine dimer level with this dose group (Fig. 3A). Finally, postvaccination DTH was significantly larger in this dose group (Fig. 3B).

Comparing Toxicity Between Optimally and Suboptimally Dosed Patients

To validate the OBD, those patients receiving the OBD were compared with all others. The ODG, 1000.250.6, had 29 patients, and the SDG, comprised of the remaining 6 groups, contained 70 patients. Comparison of the ODG with the SDG demonstrated no significant difference with regard to local or systemic toxicity (Fig. 4).

Comparing Immunogenic Response Between Optimally and Suboptimally Dosed Patients

Further comparison of the ODG with the SDG demonstrated a significant difference in the average prevaccination dimer ($0.91 \pm 0.13\%$ vs $0.54 \pm 0.11\%$; $P = .03$). Although there was no significant difference between the average maximum dimer levels for the ODG compared with the SDG, the ODG demonstrated a trend toward an increase in the average postvaccination dimer ($0.87 \pm 0.10\%$ vs $0.67 \pm 0.05\%$; $P = .07$). However, there was no difference in the

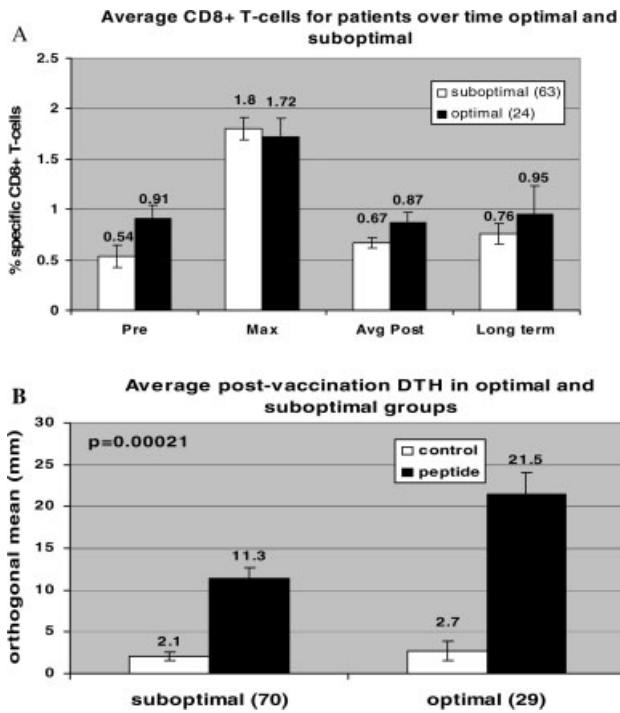


FIGURE 5. Dimer assay and delayed-type hypersensitivity test (DTH) per optimal dose group (ODG) versus suboptimal dose group (SDG) are shown. (A) A significant difference in the ODG versus the SDG was noted in the average (Avg) prevaccine (Pre) CD8-positive (CD8⁺) E75-specific T-cell levels ($0.91 \pm 0.13\%$ vs $0.54 \pm 0.11\%$; $P = .03$). No significant difference was noted between the average maximum (Max) CD8⁺ E75-specific T-cell levels. The ODG demonstrated a trend toward an increase in the average of monthly postvaccination (Avg Post) percentage of CD8⁺ E75-specific T cells ($0.87 \pm 0.10\%$ vs $0.67 \pm 0.05\%$; $P = .07$). No difference was noted in the average long-term CD8⁺ E75-specific T-cell levels between groups at 6 months. (B) Orthogonal mean DTH response between the ODG versus SDG demonstrated no difference with regard to the control inoculum (3.0 ± 1.1 mm vs 2.0 ± 0.5 mm). DTH response to the peptide was found to be significantly elevated in the ODG versus the SDG (21.5 ± 2.5 mm vs 11.3 ± 1.3 mm; $P = .00021$).

average long-term dimer levels noted between the groups at 6 months (Fig. 5A).

The average DTH response to the saline control was identical in ODG and SDG patients. Both groups demonstrated significantly larger DTH when comparing their saline control DTH with their E75 DTH (ODG, 2.7 ± 1.1 mm vs 21.5 ± 2.5 mm [$P = <.0001$]; and SDG, 2.1 ± 0.5 mm vs 11.3 ± 1.3 mm [$P = <.0001$]). The DTH response to E75 was significantly larger in the ODG compared with the SDG (21.5 ± 2.5 mm vs 11.3 ± 1.3 mm; $P = .0002$) (Fig. 5B).

Comparing Clinical Recurrence Between ODG and SDG

At a median of 30 months follow-up in the current trial, 10 recurrences have been identified within the

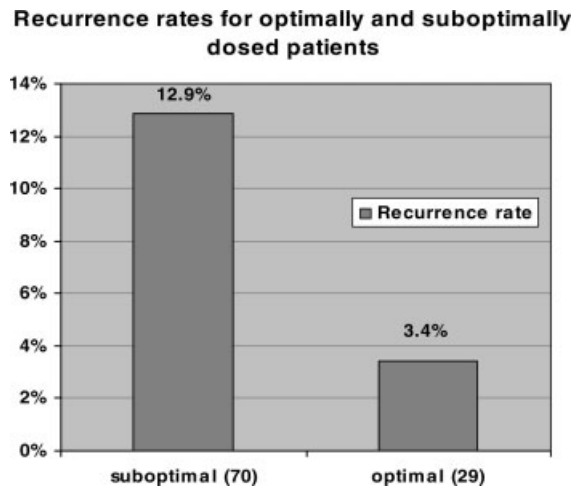


FIGURE 6. Despite a substantially shorter follow-up in the optimal dose group (ODG) compared with the suboptimal dose group, patients in the ODG were younger, had more aggressive disease, and demonstrated a proportionally lower rate of recurrence ($P = .27$).

TABLE 2
Clinicopathologic Factors and Treatment Profiles for the Suboptimal and Optimal Dose Groups

	Suboptimal (n = 70)	Optimal (n = 29)	P
Age, y	60 (31-78)	57 (28-72)	.05
Tumor $\geq T2$	22.9% (16/70)	55.2% (16/29)	.004
Lymph node positive	37.1% (26/70)	75.9% (22/29)	.001
Grade 3	30% (21/70)	51.7% (15/29)	.07
HER-2/ <i>neu</i> overexpression	27.7% (18/65)	22.2% (6/27)	.8
ER ⁻ /PR ⁻	34.8% (24/69)	24.1% (7/29)	.35
Chemotherapy	67.1% (47/70)	93.1% (27/29)	.01
Radiation therapy	74.3% (52/70)	72.4% (21/29)	1
Hormonal therapy	61.4% (43/70)	75.8% (22/29)	.2
Trastuzumab	4.3% (3/70)	17.2% (5/29)	.05
Median follow-up, mo	32 (8-60)	20 (3-60)	<.001

ER indicates estrogen receptor; -, negative; PR, progesterone receptor.

99 patients, with only 1 of these disease recurrences reported within the ODG (recurrence rate of 3.4% in the ODG vs 12.9% in the SDG), as shown in Figure 6. At the time of last follow-up, this decreased recurrence rate had not yet reached statistical significance ($P = .3$), and there were notable differences in the groups. As shown in Table 2, the ODG has a shorter median follow-up and a greater use of trastuzumab; however, the ODG had more aggressive disease. Compared with the SDG, patients in the ODG were younger and had larger tumors, a larger proportion of NP patients, and a trend toward higher-grade tumors. There was no difference in HER-2/*neu* overexpression or hormone receptor expression noted

between the 2 groups. Overall, the ODG had higher American Joint Committee on Cancer staging.

DISCUSSION

In the E75 vaccine trial in disease-free BCa patients, the CVDP vaccinated 99 patients with 7 different dose schedules of the vaccine. Comparing these individual groups, there was no increased toxicity associated with increasing cumulative dosages of the peptide and no maximum tolerated peptide dosage was reached. GM-CSF was reduced in 19.2% of patients overall primarily for large local reactions rather than high-grade toxicity. There was a trend toward better *in vitro* and *in vivo* immunologic response with higher cumulative peptide dosages. The ODG was set at 1000.250.6, and it would appear to be safe and effective in raising HER-2/*neu*-specific immunity. In addition, those patients in the ODG had numerically fewer cases of disease recurrence despite the more aggressive disease noted within this group. However, the follow-up period was substantially shorter in the ODG, and further long-term follow-up will be required to determine the actual impact of the optimally dosed vaccine on recurrence and survival.

As described by the Cancer Vaccine Clinical Trial Working Group (CVCTWG), biologic agents used for cancer vaccines are safer than conventional cytotoxic drugs in the treatment of cancer.²⁶ The optimal dose of a cancer vaccine should generate a sufficient immunogenic response, which is unlikely to cause significant toxicity, to bring about the desired clinical outcome. By comparison, cytotoxic agents are dosed under the principle that maximizing dose should maximize efficacy, and thus determining the maximum tolerated dose for the cytotoxic agent is the goal of phase 1 trials. Because of the difference in safety and primary goal of therapy between biologic and cytotoxic agents, there should be a change in research oncology from the standard 3-phase trials for cytotoxic agents to the 2-phase trial for biologic agents composed of a proof-of-principle trial followed by an efficacy trial, as proposed by the CVCTWG.²⁶

Combining E75 with an immunoadjuvant is a simple and effective way to induce peptide-specific immunity. Both GM-CSF and incomplete Freund adjuvant have been used as immunoadjuvants with E75.^{11-13,27} GM-CSF appears to be a better peptide vaccine immunoadjuvant.²⁸ Most of the toxicity in the currently studied vaccine appeared to be related to the GM-CSF dose, which must be regulated. Differences in dosing of GM-CSF appear to be related to the

immunogenicity of the peptide with which it is combined, as reported in currently ongoing studies with other peptides derived from HER-2/*neu* at the CVDP. GP2 (Class I HER-2/*neu* peptide) dosing is more effective with 125 μ g of GM-CSF and AE37 (Class II HER-2/*neu* peptide) is more effective with 62.5 μ g of GM-CSF (unpublished data). There may also be individual sensitivities to GM-CSF. Although the strategy we used in our current trial was to start with the stated dose and then reduce it as needed, this approach may not be practical for large-scale use, and therefore it may be better to start with a lower dosage with a goal of no GM-CSF dosage reductions.

Multiple possibilities exist when attempting to dose and schedule a vaccine comprised of a peptide and an immunoadjuvant. Peptide vaccine trials in melanoma have used weekly administration of various multipolypeptide vaccines with differing doses of GM-CSF and other adjuvants, concurrent or delayed use of other cytokines, with or without peptide pulsed dendritic cells, and the use of intradermal as well as subcutaneous injections.²⁹⁻³¹ Disis et al. demonstrated that a peptide vaccine derived from the intracellular domain of HER-2/*neu* could be administered safely in breast and ovarian cancer patients at dosages ranging from 25 μ g to 900 μ g of peptide with 100 μ g of GM-CSF on a monthly basis for 6 months.³² Again, this points to a wide spectrum in the administration of peptide-based vaccines, indicating that an ideal regimen has not been identified and may also be peptide specific.^{33,34}

Many future questions will need to be addressed that center on vaccine dosing. To our knowledge, it is not known whether the most significant and most enduring immunologic response can be generated by cumulatively building the immune response with lower frequent dosages of the vaccine or by generating an initial robust immunologic response with a large dosage of vaccine followed by vaccine boosters to maintain immunity. Both strategies have their basis in research at the cellular level, but the answer lies in the eventual clinical outcome.

The desired effect of a cancer vaccine is to modify the clinical outcome of the patient population of interest. When choosing a dosing regimen for a preventive vaccine to be tested in a disease-free population, there are 2 potential study methods. First, patient groups could be given various doses of the vaccine and followed for clinical efficacy in the long term. This approach requires large patient numbers and is costly and labor intensive. Second, an immunologic response could be chosen as a surrogate marker for clinical response, allowing for a dose to be chosen in a relatively short period of time.

Immune response, as opposed to tumor response in metastatic patients, could be used in patients who are clinically free of disease. Patients could then be administered this potentially optimal dose, and clinical efficacy then evaluated in the long term. Unfortunately, to our knowledge, there currently is no immunologic response that has been demonstrated to be a good surrogate for clinical outcome. However, in this study, DTH reactions were found to be more robust in the ODG, and this group had a trend toward fewer clinical recurrences. These data suggest that the often overlooked DTH reaction should be considered as a surrogate marker.

In the current study, we demonstrated that a peptide, E75, derived from the HER-2/*neu* protein can be administered with an immunoadjuvant in serial inoculations at various doses with equivalent local and systemic toxicity and stimulate an immune response that can be measured at the cellular and systemic level. The immunologic response was compared with clinical outcome, and optimally dosed patients demonstrated a trend toward fewer recurrences despite more aggressive disease, albeit at a shorter follow-up. These results will need to be confirmed with longer follow-up of these patients and additional larger studies.

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