EXPERT REVIEWS

Comparison of different HER2/*neu* vaccines in adjuvant breast cancer trials: implications for dosing of peptide vaccines

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We have performed multiple adjuvant clinical trials using immunogenic peptides from the HER2/*neu* protein (AE37/E75/GP2) plus (GM-CSF) given intradermally to breast cancer patients. Four trials were performed with similar dose-escalation design with increasing doses of peptide (AE37/E75/GP2) and varying amounts of GM-CSF. Dose reductions (DRs) were made for significant local and/or systemic toxicity by decreasing GM-CSF for subsequent inoculations. *Ex vivo* and *in vivo* immunologic responses were used to compare groups. Of 132 patients, 39 required DR (30 for robust local reactions [DR-L]). DR patients, particularly DR-L, had greater immune responses both *ex vivo* and *in vivo*. Postvaccine delayed-type hypersensitivity in DR-L patients compared with all others was larger for E75 ($p = 0.001$), AE37 ($p = 0.077$) and GP2 ($p = 0.076$). All three peptide vaccines were safe and well-tolerated. These findings have led to a clinically relevant optimal vaccine dosing strategy, which may be applicable to other peptide-based cancer vaccines.

Keywords: AE37 peptide • dose reduction • E75 peptide • GM-CSF • GP2 peptide • HER2/*neu* peptide vaccine

Multimodal treatment for breast cancer includes surgery, chemotherapy, radiation, hormonal therapy and, for a select group, immunotherapy. Despite aggressive treatment regimens, the American Cancer Society projected that 40,930 women died from breast cancer in 2008 [101]. As a result, novel approaches continue to be sought that include more broadly applicable forms of immunotherapy, like cancer vaccines. A plethora of vaccine strategies exist, including modified whole-tumor cell vaccines, antigen-loaded autologous dendritic cells, DNA vaccines, and protein or peptide vaccines combined with various immunoadjuvants [1–6].

There are numerous advantages of vaccines that utilize immunogenic peptides. Peptide vaccines are chemically stable, do not include pathogens, are devoid of oncogenic potential, and are easily constructed, manufactured and administered. Peptide vaccines are also immunogenic, modifiable and combinable. In addition, peptide vaccines have minimal systemic toxicity and offer the potential for prolonged immunity, which is easily monitored. Just as there are advantages, there are also disadvantages. The most prominent of these are HLA restriction and lack of antigenic diversity. Both of these issues limit the universal application of single-peptide vaccines. To overcome these limitations, HLA-specific peptides from multiple antigens may be required. From a research perspective, peptide vaccines still offer the cleanest system to study the immune response to the delivered epitopes. This precise monitoring allows for the study of how to best dose and deliver peptide vaccines to optimize immunologic and clinical responses [7]. Finally, cancer vaccines may have limited clinical tumor response in metastatic cancer [8]; however, our vaccine trials have been performed in the adjuvant setting.

The Cancer Vaccine Development Program (CVDP) has performed multiple Phase I/II clinical trials using various immunogenic peptides from the HER2/*neu* protein. The E75 (amino acids [aa]: 369–377) and GP2 (aa: 654–662) peptides are both HLA-A2/A3^{+}-restricted, and the hybrid AE37 (aa: 776–790 + Ii-key modification of a four-amino acid [LRMK] addition that increases potency) peptide is a promiscuous

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HLA class II binder [9–11]. These peptides are located on the extracellular domain (E75), transmembrane portion (GP2), and intracellular domain (AE37) of the HER2/*neu* protein. We have attempted to determine the optimal dosing strategy by varying the number of inoculations, the amount of peptide, and the amount of the immunoadjuvant granulocyte-macrophage colony-stimulating factor (GM-CSF), in our trials. All of these vaccines have been well-tolerated systemically, but immunologically active to the point of necessitating dose reductions (DRs). Our strategy has been to serially reduce GM-CSF, and then peptide in the absence of an immunoadjuvant, on subsequent inoculations for robust local reactions or systemic toxicity.

In this article, we have analyzed and compared the toxicities and immunologic responses to the vaccines in our trials. Specifically, we have assessed patients requiring DRs and compared them with other vaccinated patients not requiring DRs (NDR). The purpose is to determine the immunologic and clinical significance of DR for either systemic toxicity (DR-S) or large local reactions (DR-L), and if DR should bear on the method of dosing HER2/*neu* peptide vaccines. All patients requiring DRs (DR-L and DR-S) were initially grouped together; however, the number of DR-S patients (nine of 39 total DR patients) was too small to make any meaningful comparisons alone.

Trials, vaccines, toxicities & monitoring

Patient characteristics & clinical protocol

E75 node-positive (NP) (I-01) and node-negative (NN) (I-02) Phase I/II trials, an AE37 (I-03) Phase I trial, and a GP2 (I-04) Phase I trial were approved by local Institutional Review Boards and conducted under IND # BB-9187, IND # BB-12229 and IND # BB-11730, respectively, filed with the US FDA. All trials were conducted at Walter Reed Army Medical Center (Washington DC, USA), and the E75 NP/NN Phase I/II trials were also conducted at the Joyce Murtha Breast Care Center (Windber, PA, USA). All patients had confirmed NN or NP (only E75) breast cancer, and had completed standard therapy before enrollment with hormonal therapy continued if applicable. After proper counseling, and after informed consent was obtained, disease-free breast cancer patients were enrolled and immunocompetency tested (Mantoux test reaction >5 mm to two or more antigens). All patients were HLA typed (both E75 and GP2 are HLA-A2$^+$/A3$^+$-restricted), and all patients receiving either the E75 or GP2 peptide vaccine were either HLA-A2$^+$ or HLA-A3$^+$.

Vaccines

The E75/GP2/AE37 peptides were produced under good manufacturing practices by NeoMPS Inc. (San Diego, CA, USA). Peptide purity (>95%) was verified, and the manufacturer carried out sterility, stability and safety testing. Peptides were manufactured as lyophilized peptide and reconstituted in 0.5 ml saline. Reconstituted peptides were mixed with 0.5 ml of GM-CSF (Berlex, Seattle, WA, USA). The 1.0 ml inoculation was split and given intradermally at two sites 5 cm apart in the same extremity.

Vaccination series

The studies were performed as dose-escalation trials to determine peptide and GM-CSF optimal dosing and number of inoculations. GM-CSF, or peptide in the absence of GM-CSF, DR of 50% were performed in subsequent inoculations for local reactions ≥100 mm (DR-L) or grade 2 systemic toxicities (DR-S). The cutoff of 100 mm was determined from experience (at ≥100 mm, the sites become confluent or local toxicity increases). Utilizing this cutoff, no patient has ever experienced skin disruption or ulceration in over 750 inoculations.

Toxicity

Patients were observed 1 h postvaccination for immediate hypersensitivity and 48–72 h later for injection site measurements and questioning in regards to local or systemic toxicities (NCI Common Terminology Criteria for Adverse Events v3.0).

Peripheral blood mononuclear cell isolation & cultures

Blood was drawn before each inoculation and at 1 (postvaccine) and 6 months (long-term) after vaccine series completion. A total of 40 ml of blood was drawn and peripheral blood mononuclear cells were isolated as previously described [12].

Ex vivo *immune monitoring*

Peripheral blood mononuclear cells were used for monitoring vaccine-specific *ex vivo* immune responses. CD8$^+$ peptide-specific T lymphocytes were assessed by HLA-A2:immunoglobulin dimer assay specific for E75 and GP2 peptides for each successive inoculation and average pre- and post-inoculation levels were calculated [13,14]. For AE37, proliferative activity of T lymphocytes was assessed using standard ^{3}H-thymidine incorporation assays and measured by thymidine incorporation, determined as counts per minute, performed in triplicate and averaged [11].

Delayed-type hypersensitivity

Delayed-type hypersensitivity (DTH) reactions were performed prior to vaccination, except for the E75 NP trial, and at 1 month after completion of vaccine series. Intradermal injections on the extremity (opposite the side of vaccination) of 100 µg of peptide (without GM-CSF) in 0.5 ml of saline were compared with an equal volume control inoculum of saline. DTH reactions were measured in two dimensions at 48–72 h using the sensitive ballpoint pen method (reported as orthogonal mean) [15].

Statistical analysis

The p-values for clinicopathological factors were calculated using Wilcoxon, Fisher's exact or χ^2 tests as appropriate. Calculation of p-values for comparing pre- and postvaccine dimer assays, proliferative assays and DTH reactions was performed using the Student's *t*-test, paired or unpaired, as appropriate.

Results of the AE37 Phase I trial

AE37 patients

AE37 was a Phase I dose-escalation trial with each group of three patients having varying amount of peptide and GM-CSF

with six inoculations. A total of 15 disease-free, NN breast cancer patients were vaccinated, and nine (60%) required DR, all for robust local reactions (≥100 mm) (Table 1). When comparing NDR patients to DR-L patients, there were no significant differences in demographics, prognostic factors, or treatment profiles (Table 2).

AE37 safety & immune response

Safety

There were no statistical differences in the maximum local or systemic toxicities experienced by NDR and DR-L patients (Figure 1A).

Ex vivo

Overall, DR-L patients, at each time point, had a statistically significant increase in proliferation as measured by ^{3}H-thymidine incorporation compared with baseline (Figure 1B). The NDR patients showed a statistically significant increase at the maximum value; although, postvaccination and long-term proliferation values were not statistically greater than baseline. When comparing the NDR versus DR-L groups, enhanced proliferation in the DR-L group is noted, but not statistically significant.

In vivo

The *in vivo* immune response to AE37 postvaccination was significantly increased compared with control (DR-L: p = 0.0003; NDR: p = 0.048) (Figure 1C). When comparing the postvaccination DTH responses between NDR and DR-L patients, DR-L patients had larger reactions; although, statistical significance was not achieved (p = 0.077).

Results of the GP2 Phase I trial

GP2 patients

GP2 was also a Phase I dose-escalation trial with each group of three patients receiving a varying amount of peptide and GM-CSF with six inoculations, and an additional six patients in the optimal dose group. A total of 18 disease-free, NN breast cancer patients were vaccinated, and 11 (61.1%) required DR for either local (DR-L=8) or systemic toxicity (DR-S = 3) (Table 1). Similar to AE37, GP2 patients had no significant differences in demographics, prognostic factors or treatment profiles when comparing NDR and DR patients (Table 2).

GP2 safety & immune response

Safety

Both groups, NDR and DR, were statistically comparable with regards to maximum local and systemic toxicities (Figure 2A).

Ex vivo

DR patients, at each time point, had statistically significant increased percentage of GP2-specific T cells at maximum compared with baseline. NDR patients had higher levels of preexisting immunity (definition = GP2 peptide-specific dimer level ≥0.3%) (Figure 2B) [16], which makes this group more

Table 1. Breakdown of 132 patients receiving peptide vaccines by type of vaccine, dose groups and patients requiring dose reduction/dose reduction for large local reaction.

Trial	Total patients (n)	Total patients with DR, n (%)	Total patients with DR-L, n (%)	Dosing group†	Total patients per dosing group (n)	Patients with DR, n (%)
AE37	15	9 (60.0)	9 (60.0)	100:250:6	3	2 (66.7)
				500:250:6	3	3 (100)
				1000:0:6	3	1 (33.3)
				500:125:6	3	3 (100)
				500:30:6	3	0 (0)
GP2	18	11 (61.1)	8 (44.4)	100:250:6	3	3 (100)
				500:250:6	3	2 (66.7)
				1000:250:6	3	3 (100)
				500:125:6	9	3 (33.3)
E75	99	19 (19.2)	13 (13.1)	100:250:6	3	0 (0)
				500:125:3	10	1 (10)
				500:125:4	9	0 (0)
				500:250:4	18	3 (16.7)
				500:250:6	19	6 (31.6)
				1000:250:4	11	0 (0)
				1000:250:6	29	9 (31.0)

†Dosing group = peptide (μg):GM-CSF (μg):number of inoculations.
DR: Dose reduction; DR-L: Dose reduction for large local reaction.

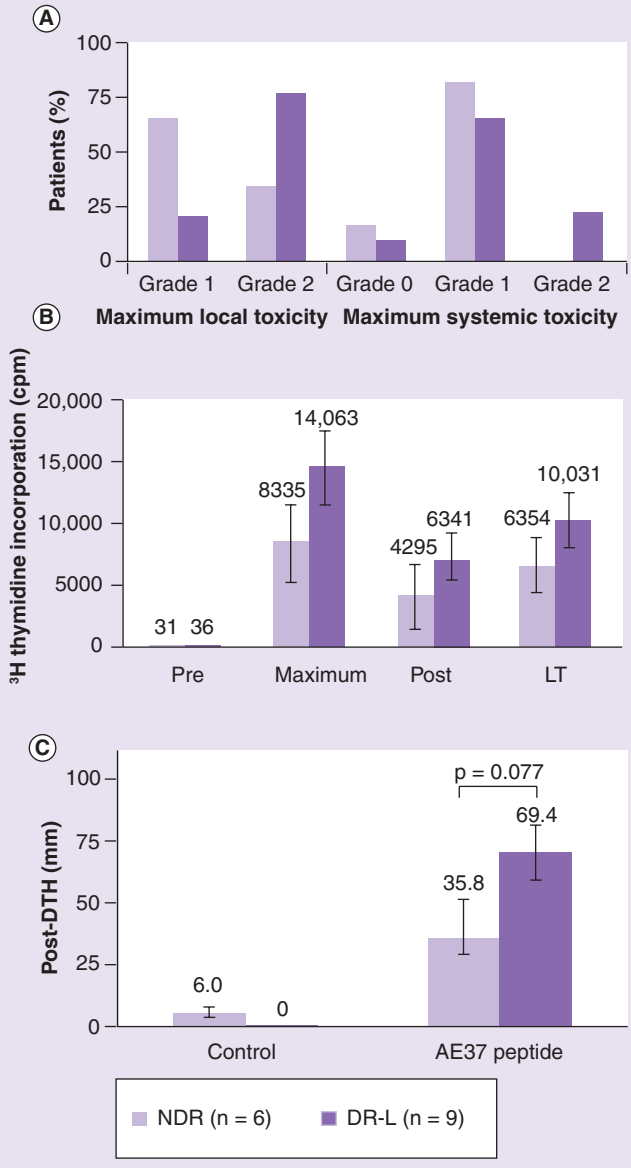

Figure 1. AE37 (n = 15) toxicity and immunologic responses. (A) Maximum toxicity. **(B)** *Ex vivo* immune response: DR-L had increased ^{3}H-thymidine incorporation (pre–maximum: p = 0.004; pre–post: p = 0.010; pre–LT: p = 0.002), NDR (pre–maximum: p = 0.035; pre–post: p = 0.056; pre–LT: p = 0.085). **(C)** *In vivo* immune response: control vs AE37 post-DTH (NDR: p = 0.048, DR-L: p = 0.0003). DR-L vs NDR for AE37-specific post-DTH (p = 0.077). Error bars represent ± standard error.
CPM: Counts per min; DR-L: Dose reduction for large local reaction; DTH: Delayed-type hypersensitivity; LT: Long term; NDR: Not requiring dose reduction.

challenging to interpret. For the NDR patients, the increase in GP2-specific T cells at maximum was not statistically significant (p = 0.084). The long-term *ex vivo* response was unchanged from baseline while the postvaccination response was decreased for NDR patients. When comparing NDR versus DR patients, there are more patients in the NDR group with pre-existing immunity (p = 0.023). The maximum, post and long-term differences between NDR and DR groups are not statistically different.

In vivo

The *in vivo* immune response to GP2 postvaccination was significantly larger compared with control (DR: p = 0.004; NDR: p = 0.002) (Figure 2C). Patients requiring DR for any reason had larger GP2-specific DTHs, but those were not statistically significant (p = 0.288).

Dose-reduction cause

As expected, patients requiring DR-L had a higher maximum local toxicity (grade 2 maximum local toxicity DR-L vs others: p = 0.004) but no increased systemic toxicity (Figure 2D). Both DR-L and others (NDR + DR-S) had statistically increased maximum *ex vivo* responses. DR-L patients also had a statistically increased postvaccination *ex vivo* response, while the postvaccination response for other patients was decreased from baseline (Figure 2E). As stated earlier, this is difficult to assess owing to the large number of patients in the latter group with pre-existing immunity. When evaluating DR-L patients compared with all other patients, the postvaccination GP2-specific DTH responses were larger, but did not achieve statistical significance (p = 0.076) (Figure 2F).

Results of the E75 Phase I trial

E75 patients

E75 was a combined Phase I/II trial with varying amounts of peptide and GM-CSF, and varying numbers of inoculations (range: three to six). A total of 99 disease-free, NP and NN breast cancer patients were completely vaccinated, and 19 (19.2%) required DR for either local (DR-L = 13) or systemic toxicity (DR-S = 6) (Table 1). Unlike AE37 and GP2, the E75 trial had statistically more patients with HER2/*neu* immunohistochemistry 3+ or FISH >2.2 tumors in the NDR group of patients (p = 0.020). Aside from this, there were no differences noted in demographics or treatment profiles, when comparing NDR to DR patients (Table 2).

E75 safety & immune response

Safety

Dose-reduction patients had increased maximum local (grade 2; p = 0.048) but not systemic toxicities (grade 3; p = 0.353) when compared with NDR patients (Figure 3A).

Ex vivo

The 19 DR patients had a high level of pre-existing E75 immunity compared with the NDR patients (p = 0.034). DR patients had a significant increase in the percentage of E75-specific T cells at the maximum time point, but this was not seen at other time points (Figure 3B). In the NDR group, there was a similar significant increase at maximum, but also at the long-term time point. When comparing NDR and DR patients, it was noted that DR patients had a higher level of pre-existing E75

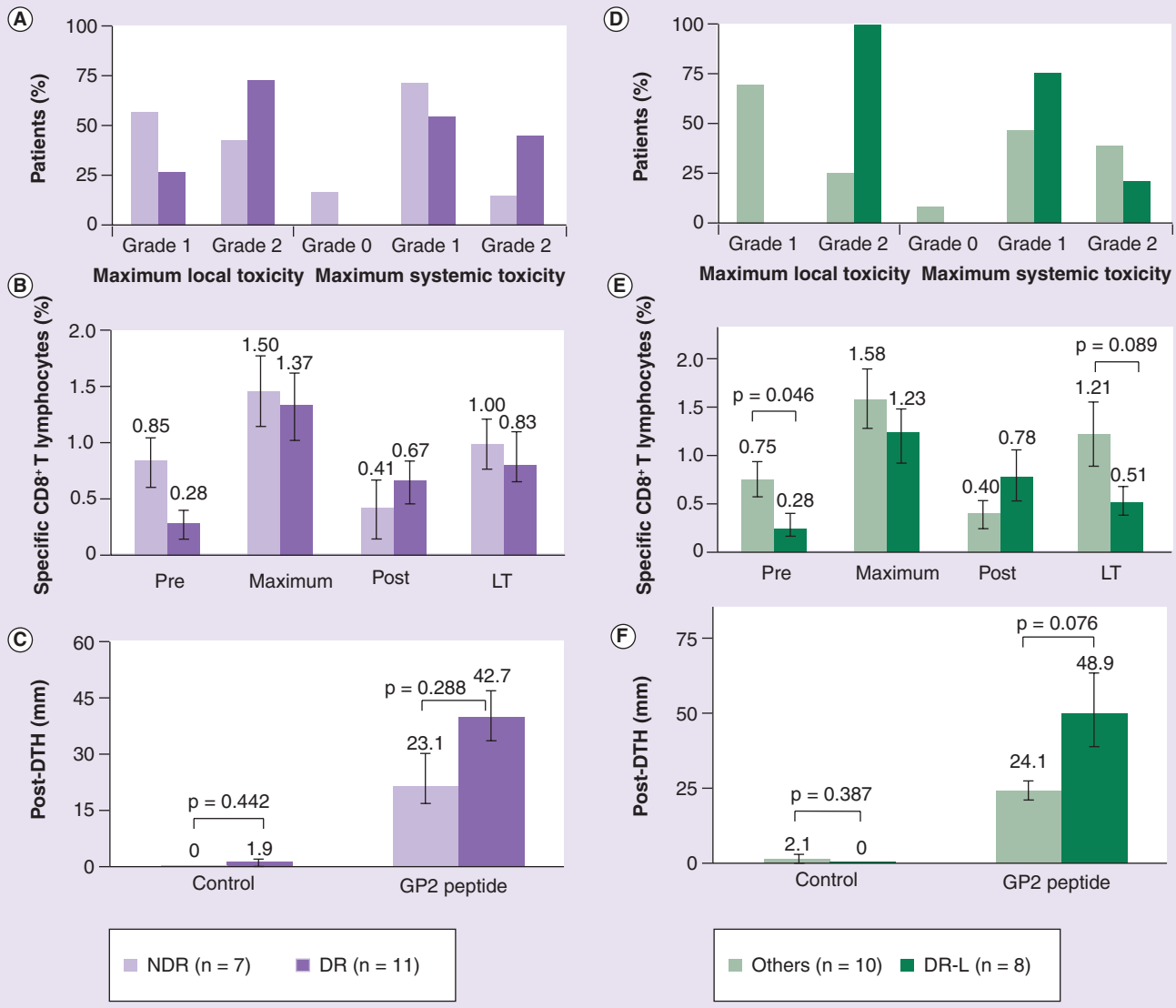

Figure 2. GP2 (n = 18) toxicity and immunologic responses. (A–C) NDR vs DR. **(A)** Maximum toxicity. **(B)** *Ex vivo* immune response: DR had increased maximum dimer levels (pre–maximum: p = 0.002; pre–post: p = 0.054; pre–LT: p = 0.109), NDR (pre–maximum: p = 0.084; pre–post: p = 0.016 [decrease]; pre–LT: p = 0.708). **(C)** *In vivo* immune response: control vs GP2 post-DTH (NDR: p = 0.002; DR: p = 0.004). **(D–F)** NDR + DR-S = others vs DR-L. **(D)** Maximum toxicity. **(E)** *Ex vivo* immune response: DR-L (pre–maximum: p = 0.004; pre–post: p = 0.03; pre–LT: p = 0.22), others (pre–maximum: p = 0.04; pre–post: p = 0.02 [decrease]; pre–LT: p = 0.53). **(F)** *In vivo* immune response: control vs GP2 post-DTH (NDR: p < 0.001; DR-L: p = 0.01). DR-L vs other for GP2-specific post-DTH (p = 0.076). Error bars represent ± standard error.
DR: Dose reduction; DR-L: Dose reduction for large local reaction; DTH: Delayed-type hypersensitivity; LT: Long term; NDR: Not requiring dose reduction.

immunity (p = 0.034). The maximum percentage of E75-specific T cells was larger for DR patients than NDR patients, but this difference did not achieve statistical significance (p = 0.088).

In vivo

The *in vivo* immune response to E75 postvaccination was significantly increased compared with control (DR and NDR: p < 0.0001). Patients requiring DR had statistically larger E75-specific DTH reactions compared with NDR patients (p = 0.002) (Figure 3C).

DR cause

DR-L patients had similar toxicity to all other patients (Figure 3D). DR-L patients had a high percentage of patients with E75 pre-existing immunity and the only significant increase seen was from baseline to maximum percentage in E75-specific T cells (p = 0.021). The other patients (NDR + DR-S) also had a statistically significant increase in E75-specific T cells at maximum (p < 0.0001). When comparing DR-L with all other patients, it is interesting to note that DR-L patients had

Table 2. Patient demographics, prognostic factors and treatment profiles.

	AE37			GP2			E75		
	NDR (n = 6)	*DR (n = 9)*	*p-value*	*NDR (n = 7)*	*DR (n = 11)*	*p-value*	*NDR (n = 80)*	*DR (n = 19)*	*p-value*
Median age, years (range)	61 (44–66)	54 (44–70)	0.089	51 (38–69)	49 (33–66)	0.274	55 (31–77)	59 (27–71)	0.241
Race (%)									
– White (%)	50.0	66.7	0.622	71.4	81.8	1	91.3	84.2	0.399
– Black (%)	50.0	22.2	0.329	0.0	9.1	1	2.5	10.5	0.165
– Latino (%)	0.0	11.1	1	14.3	0.0	0.389	2.5	0.0	1
– Asian (%)	0.0	0.0	1	14.3	9.1	1	3.8	5.3	0.580
Tumor size, T2–T4 (%)	16.7	0.0	0.400	42.9	45.5	1	30.0	36.8	0.589
Histological Grade III (%)	16.7	22.2	1	57.1	18.2	0.141	40.0	21.1	0.184
Node positive (%)	0.0	0.0	1	0.0	9.1	1	45.0	57.9	0.444
HER2/*neu* IHC 3+ / FISH >2.2 (%)	33.3	33.3	1	28.6	27.3	1	32.5	5.3	0.020†
Hormone receptor negative (%)	33.3	22.2	1	57.1	36.4	0.631	33.8	21.1	0.411
Chemotherapy (%)	33.3	11.1	0.525	57.1	72.7	0.627	75.0	73.7	NS
Radiation (%)	83.3	66.7	0.604	57.1	63.6	1	72.5	78.9	0.773
Hormonal therapy (%)	66.7	77.8	1	42.9	54.5	1	62.5	78.9	0.282
Trastuzumab (%)	16.7	0.0	1	14.3	18.2	1	10.0	0.0	0.347

†Denotes statistical significance.
DR: Dose reduction; FISH: Fluorescent *in situ* hybridization; IHC: Immunohistochemistry; NDR: Not requiring dose reduction; NS: Not significant.

a higher percentage of pre-existing immunity (p = 0.012) and larger maximum responses (p = 0.010) (Figure 3E). When evaluating *in vivo* immune responses, DR-L patients had statistically larger E75-specific postvaccination DTH reactions when compared with all others (p = 0.001) (Figure 3F). Figure 4 shows a comparison of *in vivo* immune responses to the three separate peptide vaccines.

Conclusion

This analysis is our first attempt to compare and contrast all three HER2/*neu* peptide vaccines tested in four clinical trials. Each peptide has been shown to be individually safe with no serious adverse events and less than 20% grade 2 systemic toxicities reported for over 750 inoculations in a total of 132 vaccinated patients. We have shown that all three peptides are capable of producing a significant immunologic response as measured both *ex vivo* (dimer assay or proliferation) and *in vivo* (DTH). Of particular note, patients who required DR, specifically for robust local reactions, in all of our peptide trials have evidence of heartier immune responses. Therefore, we believe that the optimal method of dosing HER2/*neu* peptide vaccines consists of a constant peptide dose and sufficient doses of GM-CSF (125–250 µg depending on the peptide immunogenicity) to generate a robust local reaction (>100 mm) and then serial reductions of the GM-CSF to prevent significant local toxicity.

Expert commentary

The three peptides used in our four clinical trials are similar but with unique differences. The AE37 peptide is the most individual as it is a HLA class II-binding peptide that stimulates CD4 T-helper cells and appears to have the ability to generate long-term peptide-specific immune responses. In addition, the AE37 peptide is coupled with the Ii-key modification that increases its potency. The four-amino acid addition (LRMK) is a sequence from the invariant chain that helps the peptide find and bind to the HLA class II molecule. The enhanced potency of this peptide is demonstrated in this analysis (Figure 4) showing the largest DTH reaction produced by AE37 vaccine. Lastly, AE37 is a promiscuous HLA class II binder resulting in broad applicability for this vaccine [17–20]. The GP2 and E75 peptides are similar in that both are HLA class I peptides that stimulate $CD8^+$ peptide-specific T lymphocytes and both are HLA-restricted. Fortunately, $HLA\text{-}A2^+/HLA\text{-}A3^+$ patients make up 60–75% of the population [21–23]. One difference between GP2 and E75 are their locations within the HER2/*neu* protein (transmembrane portion vs extracellular domain, respectively), which means that GP2 peptide cannot be reached via antibody and is not shed like the extracellular domain. Importantly, GP2 has a much lower binding affinity for HLA-A2; however, this peptide has been shown to have at least equivalent immunogenicity compared with E75 [24,25]. In this analysis, GP2 required more frequent DR, produced equivalent levels of *ex vivo* immunity and larger

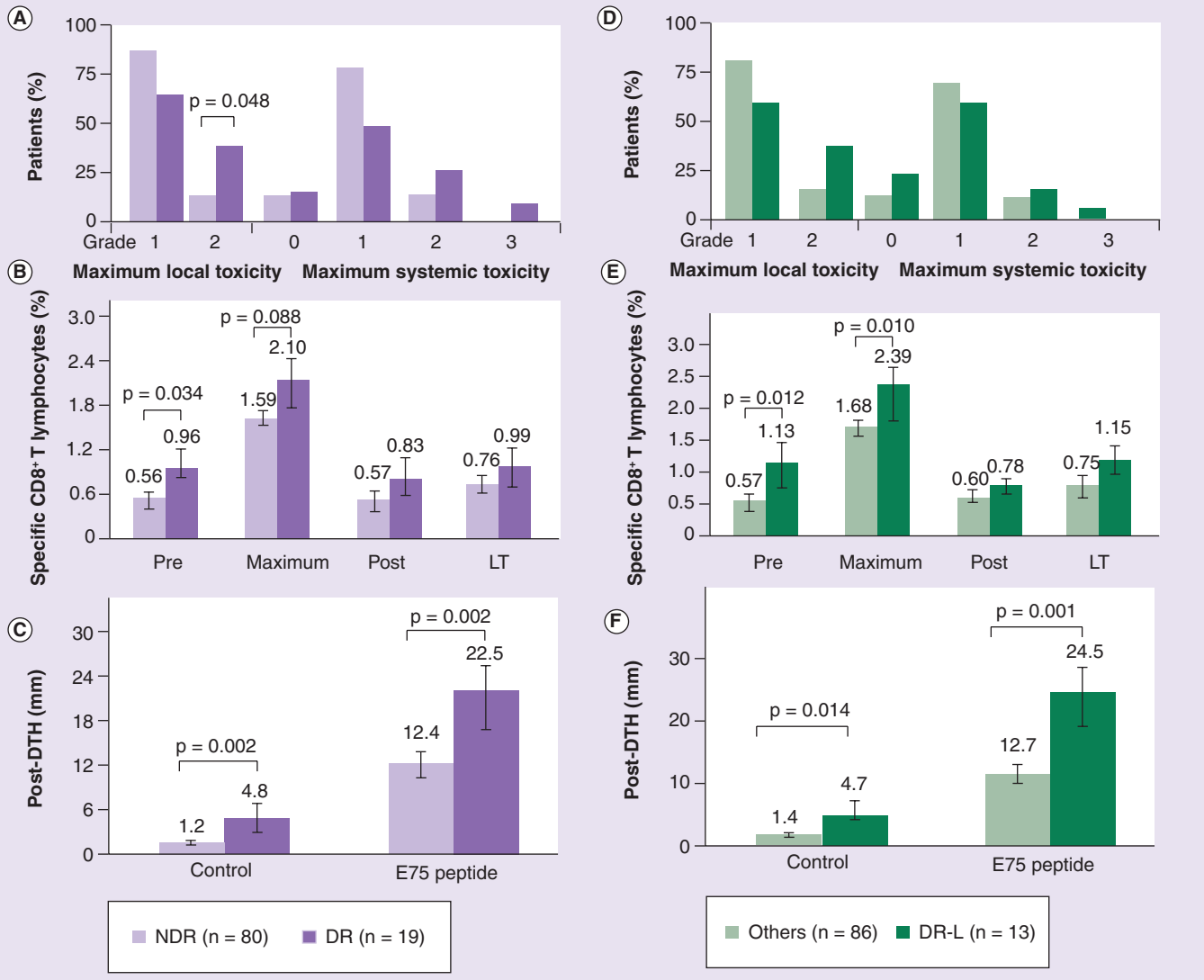

Figure 3. E75 (n = 99) toxicity/immunologic responses. (A–C) NDR vs DR. **(A)** Maximum toxicity. **(B)** *Ex vivo* immune response: DR pre-existing E75-specific immunity (p = 0.034). Both groups had increases in pre–maximum immune responses: DR (pre–maximum: p = 0.004; pre–post: p = 0.707, pre–LT: p = 0.663), NDR (pre–maximum: p < 0.0001; pre–post: p = 0.572; pre–LT: p = 0.049). **(C)** *In vivo* immune response: control vs E75-post-DTH (DR + NDR: p < 0.001). **(D–F)** NDR + DR-S = others vs DR-L. **(D)** Maximum toxicity. **(E)** *Ex vivo* immune response: Both groups had increases in pre–maximum immune responses. DR-L (pre–maximum: p = 0.021; pre–post: p = 0.44; pre–LT: p = 0.52), others (pre–maximum: p < 0.0001; pre–post: p = 0.44; pre–LT: p = 0.074). **(F)** *In vivo* immune response: control vs E75-specific post-DTH (DR-L and others: p < 0.001). DR-L vs other for E75-specific post-DTH (p = 0.001). Error bars represent ± standard error.
DR: Dose reduction; DR-L: Dose reduction for large local reaction; DR-S: Dose reduction for systemic toxicity; DTH: Delayed-type hypersensitivity; LT: Long term; NDR: not requiring dose reduction.

DTH reactions compared with E75. E75 peptide does have one major advantage over the other peptides; it is the most extensively studied HER2/*neu*-derived peptide to date, with not only safety and immunologic data, but also long-term clinical follow-up data suggesting a clinical benefit.

While our ultimate goal is to use a combination of peptides for a multi-epitope vaccine, it is interesting to note that the potency of immunologic response, especially that measured via DTH reactions, reveals that AE37 is greater than GP2, which is greater than E75 for both DR and NDR patients (Figure 4). In previous reports, DTH has been described as a very reliable method of monitoring immune response to cancer vaccines. Disis and colleagues have stated that tumor antigen-specific DTH responses correlate with better antigen-specific T-cell responses and, therefore, are a reflection of systemic immunization [25]. This poses the question: does a large DTH reaction correlate with clinical outcome? Reviewing all of the peptide results, it would appear that patients requiring DR due to larger local reactions developed greater postvaccine DTH reactions, and in E75 patients, these responses were associated with decreased recurrence and mortality (data not shown).

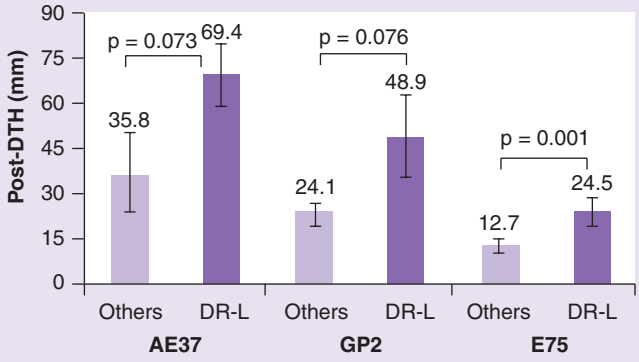

Figure 4. *In vivo* immune response comparing all three peptide vaccines. NDR + DR-S = others vs DR-L (AE37 = 69.4 ± 10.3 mm > GP2 = 48.9 ± 14.1 mm > E75 = 24.5 ± 4.1 mm). Error bars represent ± standard error.
DR-L: Dose reduction for large local reaction; DTH: Delayed-type hypersensitivity.

Of interest, in E75 peptide trials, there was a significant absence of patients with HER2/*neu* overexpression (immunohistochemistry 3+ or FISH >2.2) [26] among the 19 patients requiring DR. Given that 30% of NDR patients demonstrated HER2/*neu* overexpression (on par with known percentages) [27], the fact that only 5% of DR patients were HER2/*neu* over-expressors stands out. This finding suggests that patients with HER2/*neu* overexpression may have developed immunologic tolerance; therefore, patients with low expression may derive a greater benefit from the HER2/*neu* peptide vaccines as we have shown [28]. The lack of a disparity between the DR and NDR patients receiving the other two vaccines suggests that either these peptides are less tolerogenic *in vivo,* or that these peptide vaccines are sufficiently immunogenic to overcome the existing tolerance.

Our previous model for HER2/*neu* peptide immunization has been to slowly build a targeted immune response with emphasis on commercialization, so as not to require DRs for ease of administration [10,29]. However, it would appear from these analyses that the optimal method of dosing HER2/*neu* peptide vaccines is to administer the peptide in such a manner so as to produce large local reactions. This strategy would appear to have an immunologic and perhaps clinical benefit. Regarding the DR strategy for peptide vaccines co-administered with GM-CSF, there is no other significant data published to address this issue. One question has been how much of the local reaction is due to GM-CSF? This issue is being assessed in our AE37 and GP2 Phase II trials that have GM-CSF-only control arms. The preliminary data demonstrate that GM-CSF plays a significant role in the intensity of local reactions, suggesting the maintenance of higher doses of the immunoadjuvant for future trials. In conclusion, we propose that the optimal method of dosing HER2/*neu* peptide vaccines consists of a constant peptide dose and sufficient GM-CSF to generate robust local reactions and then serial reductions to limit toxicity. Based on our experience, we have set the goal orthogonal mean local reaction size as 100 mm measured via the sensitive ball point pen technique [14]. We recommend performing DRs of GM-CSF by 50% for subsequent inoculations after local reactions are larger than 100 mm. This method appears to be safe as none of our patients have experienced skin disruptions or ulcerations and it is generally well tolerated. The number of patients requiring DR for systemic toxicities was too small to adequately evaluate this group. However, we continue to perform DRs for both excessive local and systemic toxicities. Further data may develop regarding those patients undergoing DRs for systemic toxicities from our ongoing clinical trials. This dosing method embraces the paradigm shift towards individualized medicine where each patient receives tailored therapy dependent on their own immune system's ability to respond to the vaccine. Furthermore, we believe that this strategy may be applicable to other peptide-based cancer vaccines.

Five-year view

Clinical trials will continue exploring breast cancer vaccines. We believe that E75 will progress to a Phase III clinical trial with a US FDA concurrence from a Special Protocol Assessment. Two Phase II trials are underway assessing the ability of the AE37 and GP2 peptides combined with GM-CSF to prevent disease recurrence in high-risk breast cancer patients. Both of these trials are prospective randomized single-blinded trials comparing the peptide vaccines versus GM-CSF alone. Other approaches under investigation include combining epitopes (AE37 + GP2) or giving peptide vaccines in combination with trastuzumab (GP2 or E75). Clinical outcome data such as vaccine effects on recurrence and mortality will be elucidated with these ongoing trials. Dosing strategies also continue to be actively explored in ongoing trials to investigate the ultimate dosing strategy for both peptides and immunoadjuvants. Additional research in the use of booster vaccinations to prolong the impact of our vaccines is also underway and is especially important for the CD8-eliciting vaccines. Other epitopes may also emerge as promising immunological targets. The ultimate goal of our group is to develop a combinational multiple epitope breast cancer vaccine with broad patient applicability to be given to disease-free patients with the aim to prevent recurrences and decrease breast cancer mortality.

Authors' note

Article discusses data from the US Military Cancer Institute Clinical Trials Group Studies I-01, I-02, I-03 and I-04 .

Disclaimer

The opinions or assertions contained herein are the private views of the authors and are not to be construed as official or reflecting the views of the Department of the Army, the Department of the Navy, or the Department of Defense.

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Key issues

- Peptide cancer vaccines are stable, free of pathogens or oncological potential and are easy to construct, manufacture and administer. They offer the potential for prolonged immunity with minimal toxicity and are easily monitored.
- We have reported three clinical trials evaluating different breast cancer vaccines. These vaccines represent three different immunogenic peptides from the HER2/*neu* protein given intradermally with granulocyte macrophage colony-stimulating factor.
- AE37, a promiscuous HLA class II-binding peptide, is a hybrid of the AE36 peptide from the intracellular domain of the HER2/*neu* protein and a four amino acid Ii-key modification, which increases both HLA class II binding and potency. This CD4-eliciting vaccine has the potential for long-term peptide-specific immunity.
- Phase I clinical trials in disease-free node-negative breast cancer patients have shown that the AE37 vaccine is safe and immunogenic by both *ex vivo* (^{3}H-thymidine incorporation) and *in vivo* (delayed-type hypersensitivity reaction) measures.
- GP2 is a HLA class I-binding peptide that is HLA-A2$^+$/A3$^+$ restricted. The peptide is from the transmembrane domain of the HER2/*neu* protein. Despite lower HLA-binding affinities, the peptide has equivalent immunogenicity to the E75 peptide.
- Phase I clinical trials in disease-free node-negative breast cancer patients have shown GP2 to be safe and immunogenic by both *ex vivo* (HLA-A2:Ig dimer assay) and *in vivo* (delayed-type hypersensitivity reactions) measures.
- E75 is a HLA class I-binding peptide that is HLA-A2$^+$/A3$^+$ restricted. The peptide is from the extracellular domain of the HER2/*neu* protein and is the most extensively studied HER2/*neu* peptide.
- Phase I and II clinical trials in disease-free node-positive and node-negative breast cancer patients have shown E75 to be safe and immunogenic by both *ex vivo* (HLA-A2:Ig dimer assay) and *in vivo* (delayed-type hypersensitivity reaction) measures.
- Vaccinated patients with robust immunological responses (local or systemic) underwent dose reductions of immunoadjuvant (GM-CSF) or peptide (if no immunoadjuvant was given). Those dose-reduced patients had heartier immune responses than the non-dose-reduced patients, suggesting that an individualized dosing approach based on immune response be adopted.

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