Expert Opinion

AE37: a novel T-cell-eliciting vaccine for breast cancer

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Introduction: Immunotherapy, including vaccines targeting the human EGFR2 (HER-2/*neu*) protein, is an active area of investigation in combatting breast cancer. Several vaccines are currently undergoing clinical trials, most of which are $CD8^+$ T-cell-eliciting vaccines. AE37 is a promising primarily $CD4^+$ T-cell-eliciting HER-2/*neu* breast cancer vaccine currently in clinical trials.

Areas covered: This article reviews preclinical investigations as well as findings from completed and ongoing Phase I and Phase II clinical trials of the AE37 vaccine.

Expert opinion: Clinical trials have shown the AE37 vaccine to be safe and capable of generating peptide-specific, durable immune responses. This has been shown in patients with any level of HER-2/*neu* expression. Early clinical findings suggest there may be benefit to AE37 vaccination in preventing breast cancer recurrence.

Keywords: AE36 peptide, AE37 vaccine, breast cancer, cancer vaccines, Ii-Key hybrid, prostate cancer

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1. Background

Current treatment for breast cancer includes surgery, chemotherapy, radiation, and hormonal therapies. Newer therapies include immunotherapy, with myriad passive and active immunization strategies in use or under investigation [1]. The most commonly utilized immunological target is human EGFR2 (HER-2/*neu*), a tyrosine kinase receptor in the human EGFR family (erbB-2) [2]. The HER-2/*neu* proto-oncogene is expressed in 75 – 80% of breast cancers and overexpressed in 20 – 25% [3]. In addition to expression in breast cancer, HER-2/*neu* is overexpressed in 11% of ovarian cancers [4], 39% of prostate cancers [5], 7 – 34% of gastric cancers [6], 10 – 82% of pancreatic adenocarcinomas [7] and is expressed at some level in the majority of epithelial-derived cancers.

Immunological agents targeting HER-2/*neu* already available include trastuzumab, a monoclonal antibody targeting the extracellular portion of the HER-2/*neu* protein, with additional antibodies such as pertuzumab and TDM1, a trastuzumb-DM1 immunoconjugate drug and several breast cancer vaccines currently under investigation. The HER-2/*neu* protein is an attractive target for immunotherapy as it is well characterized, contains multiple immunogenic epitopes and promotes cancer cell growth. This review focuses on AE37, an example of a HER-2/*neu*-targeted vaccine (**Box 1**).

2. HER-2/*neu*-targeted immunotherapy

The first HER-2/*neu*-targeted immunotherapy was trastuzumab, a recombinant, humanized, monoclonal antibody which binds the juxtamembrane region of the

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Box 1. Drug summary.

Drug name	AE37
Phase	Currently in Phase II clinical trials
Indication	HER-2/*neu*-expressing breast cancer
Mechanism of action	Peptide vaccine which primarily stimulates $CD4^+$ helper T cells
Route of administration	Intradermal
Phase II trial	A multicenter, prospective, randomized, single-blinded Phase II clinical trial of the AE37 vaccine in disease-free breast cancer patients to prevent recurrence is currently enrolling patients (NCT00524277) [16]

extracellular domain of the HER-2/*neu* protein. Trastuzumab in the adjuvant setting has been shown to reduce breast cancer recurrence by 50% and improve survival by a third [8-10]. Currently, trastuzumab is reserved for breast cancer patients who overexpress the HER-2/*neu* protein, as determined by immunohistochemistry, or have amplification of the HER-2/*neu* gene.

The HER-2/*neu* protein is also an attractive target for breast cancer vaccines [11]. Vaccines targeting HER-2/*neu* may add additional therapeutic options for breast cancer patients with any level of HER-2/*neu* expression, and may confer even greater immunological and clinical benefit to those patients with lower levels of HER-2/*neu* expression [12]. Additionally, active immunotherapy with vaccination may allow the generation of long-term immunological protection from cancer without the need for repeat infusions required with monoclonal antibodies. Several breast cancer vaccines are currently under investigation, though none have yet been FDA approved.

The E75 peptide, an HLA-A2/A3-restricted MHC class I epitope derived from the extracellular domain of the HER-2/*neu* protein (HER-2/*neu*: 369 – 377) has been studied mixed with GM-CSF immunoadjuvant as a vaccine administered in the adjuvant setting in both node-positive and node-negative breast cancer patients. The combined clinical trial results showed a recurrence rate of 5.6% in vaccinated patients compared with 14.2% in controls at a median follow up of 20 months ($p = 0.04$), but as immunity from this $CD8^+$ T-cell-eliciting vaccine waned, recurrence rates of 8.3 and 14.8%, respectively were identified at 26 months of follow up ($p = 0.15$) [13]. This initiated the addition of booster inoculations, which appear to not only sustain an effective peptide-specific immune response but also to be of clinical benefit [14]. A Phase III trial of the E75 vaccine is currently under development. GP2 is another HLA-A2-restricted MHC class I epitope of the HER-2/*neu* protein (HER-2/*neu*: 654 – 662) currently under investigation as a peptide vaccine. The GP2 vaccine (GP2+GM-CSF) has completed a Phase I trial [15] and patients are currently being enrolled in a Phase II trial (NCT00524277) [16] in the adjuvant setting in breast cancer patients.

While these $CD8^+$ T-cell-stimulating vaccines are effective at eliciting cytolytic activity directed against HER-2/*neu* expressing tumors, concern exists that a durable vaccine-specific immune response may require the use of a $CD4^+$ helper T cell epitope to establish a long-term memory response [17]. This has led to the strategy of immunization with a vaccine capable of primarily stimulating $CD4^+$ helper T cells, such as the AE37 vaccine. Disis and colleagues are conducting a Phase II trial utilizing 15 amino acid peptides from the intracellular domain from the HER-2/*neu* protein in patients with locally advanced or metastatic breast cancer (NCT00343109) [18]. These MHC class II epitopes have embedded MHC class I epitopes with the hope that vaccination will stimulate both $CD4^+$ and $CD8^+$ T cell responses. The longer peptides used as MHC class II epitopes thus can function as a polyepitope vaccine with both $CD4^+$ and $CD8^+$ T cell epitopes present, the combination allowing for more efficient immunization [17].

Other cancer vaccine strategies include whole-tumor-cell, whole-protein, long peptide or DNA vaccines. These approaches, which can provide a wider array of immunogenic antigens, allow antigen presentation consistent with the individual's HLA type. Inherent to the immune system antigen processing mechanism required for these vaccines, less antigenic material may be available for presentation to the immune system. In contrast, single-peptide vaccines allow for the concise presentation of adequate amounts of known dominant epitopes within tumor-associated antigens. This strategy allows for minimal immune processing, resulting in an efficient antigen delivery system. Other advantages of single-peptide vaccines are their inexpensive cost, ease of production and ease of administration in a community clinical setting.

3. AE37

AE36 is a HER2-derived peptide from the intracellular domain of the HER-2/*neu* protein (HER-2/*neu*: 776 – 790, Ac-GVGSPYVSRLLGICL-NH_2) which promiscuously binds to MHC class II molecules. Vaccination with the AE36 peptide has been shown to generate peptide-specific T cell immune responses in breast and ovarian cancer patients [19,20]. AE37 represents the AE36 peptide with the addition of the four amino acid Ii-Key peptide (LRMK) moiety from the Ii protein. The immunoregulatory Ii protein functions to protect the MHC class II molecule binding site during endoplasmic reticulum synthesis. The Ii protein is then degraded in a post-Golgi compartment, allowing the MHC class II molecule to bind its antigen for presentation on the cell surface. The first four amino acids of the Ii protein, termed the Ii-Key peptide, increase the binding potency of MHC class II epitopes up to 250-fold when covalently linked to the epitope [21]. Ii-Key/MHC class II epitope hybrids have further been shown to enable direct extracellular charging of the MHC class II molecule by binding to its allosteric site

and inducing a favorable conformational change in the epitope binding groove, thus bypassing usual intracellular antigen processing [22].

Preclinical investigation revealed that mice immunized with the Ii-Key/AE36 hybrid had enhanced proliferation of native peptide recognizing $CD4^+$ T cells, increased IFN-γ release, as well as enhancement of cytotoxic T cell antitumor activity [23]. Moreover, patient-derived $CD4^+$ T cells primed with the Ii-Key/AE36 hybrid provided a significantly stronger helper effect to autologous $CD8^+$ T cells specific for a HER-2/*neu* cytotoxic T cell epitope, as illustrated by either IFN-γ spot ELISA (ELISPOT) assays or specific autologous tumor cell lysis [24]. These preclinical findings have led to clinical trials of the AE37 peptide vaccine in breast and prostate cancer.

4. Phase I clinical trial of AE37 in breast cancer

Our group has evaluated the AE37 vaccine in breast cancer patients (n = 15) in the adjuvant setting in a Phase I dose escalation trial to prove safety and to define optimal vaccine dosing [25]. Immunocompetent node-negative breast cancer patients were enrolled upon completion of standard-of-care therapy to include surgery, chemotherapy and radiation (as required). Patients were enrolled with any level of HER-2/*neu* expression (immunohistochemistry (IHC) 1+, 2 + or 3+ or fluorescence *in situ* hybridization (FISH) > 1.2); only one-third of the cohort over-expressed HER-2/*neu* (IHC 3+ or FISH > 2.2).

In this trial, the AE37 peptide was mixed with the immunoadjuvant GM-CSF and given together as a series of six monthly intradermal inoculations. Patients with local reactions greater than 100 mm^2 had the GM-CSF dose reduced by 50% in subsequent inoculations. This GM-CSF dosing strategy was developed from our earlier experiences with GM-CSF [26]. Five dosing groups of three patients each were studied (**Table 1**).

Toxicity to the vaccine was graded using the National Cancer Institute Common Terminology Criteria for Adverse Events v3.0. *In vitro* immunological responses were monitored by measuring peptide-specific T cell proliferation using the [^{3}H]-thymidine incorporation assay pre-vaccination, and at one and six months after completing the vaccination series. *In vivo* immunological response was assessed using a delayed-type hypersensitivity (DTH) reaction to the hybrid peptide AE37 pre-vaccination and one month after completion of the vaccination series.

The vaccine was safe and well-tolerated [25]. Local toxicities consisted of expected injection site reactions such as erythema, edema, induration and pruritis. The maximum local toxicities were grade 1 for 40% of patients and grade 2 for 60%. All patients in the second cohort experienced grade 2 local toxicities. Because each patient in the second cohort required multiple GM-CSF dose reductions, the third cohort was not initially given GM-CSF. In this group, the GM-CSF dose was added and increased as needed to produce a local reaction in subsequent inoculations. One patient from this cohort was given GM-CSF with 30 μg given at the fourth inoculation and increased to 125 μg by the sixth. In the final two cohorts, the AE37 dose was held constant while the GM-CSF doses of 125 μg (fourth cohort) and 30 μg (fifth cohort) were varied. No patients in the fifth cohort experienced grade 2 local toxicities, suggesting an optimal dose of GM-CSF of between 62.5 and 125 μg. Maximum systemic toxicities were grade 0 in 13%, grade 1 in 73% (fatigue, nausea, myalgias, rhinitis, diarrhea, headache and cough) and grade 2 in 13% (joint pain and stiffness). Only two patients experienced grade 2 systemic toxicities – one patient each from the second and third dosing cohorts. Overall, 47% of patients in the study underwent dose reductions for either grade 2 systemic toxicity or local reactions greater than 100 mm^2.

The vaccine-induced peptide-specific immune responses were measured both *in vitro* and *in vivo*. Proliferative responses to both AE36 and AE37 were significantly increased from baseline upon completion of the vaccination series, an increase that was sustained six months after completion of the series (**Table 2**). In analyzing proliferative responses by dose group, the 500 μg peptide/125 μg GM-CSF dose group had optimal immunological response. *In vivo* immunological responses measured by DTH reactions to the AE37 peptide were significantly increased from baseline for the combined group as well as for each dose group (**Table 2**). DTH reactions were more robust for peptide doses of 500 μg or greater with GM-CSF doses greater than 30 μg. Notably, the AE37 peptide was sufficiently potent to induce significant increases in both *in vivo* and *in vitro* immune responses in those patients who were not given the immunoadjuvant GM-CSF. Based on these immunological data, the vaccine dose of 500 μg AE37 and 125 μg GM-CSF was determined to be the optimal dose for use in the subsequent Phase II trial.

Given that AE37 is a MHC class II peptide capable of inducing robust proliferation of peptide-specific T cells, concern exists that this stimulation of $CD4^+$ T cells could also stimulate regulatory T cells (T_{regs}) which are commonly identified by the $CD4^+CD25^+FOXP3^+$ phenotype. T_{regs} suppress immune responses and have been demonstrated to be elevated in breast cancer patients and are associated with a poor prognosis [27]. Importantly, in this Phase I study, CD4+CD25+FOXP3+ Tregs decreased after vaccination with the AE37 vaccine [28].

5. Phase I clinical trial of AE37 in prostate cancer

In addition to breast cancer, the HER-2/*neu* protein is also overexpressed in many prostate cancers. This has prompted a Phase I trial evaluating the safety and immune responses of the AE37 vaccine in prostate cancer [29]. A total of 32 immunocompetent patients with castration-sensitive or castration-refractory prostate cancer with any level of HER-2/*neu* expression were enrolled. Fourteen (44%) of the patients had

Table 1. AE37 Phase I breast cancer trial dosing cohorts.

Cohort number	Number of patients	AE37 dose (μg)	GM-CSF dose (μg)	Number of monthly inoculations
1	3	100	250	6
2	3	500	250	6
3	3	1000	0*	6
4	3	500	125	6
5	3	500	30	6

*One patient in the third cohort required addition of GM-CSF for low vaccine reactivity. In this patient, 30 μg of GM-CSF was added at the fourth inoculation with a final GM-CSF dose of 125μg.

Table 2. Mean peptide-specific immune responses from the AE37 Phase I breast cancer trial.

Dose group	AE37 proliferation [^{3}H thymidine incorporation (cpm)]			DTH (mm)	
	Pre	Post	Long term	Pre	Post
100:250	62	1288	6078	6.8	46.7
500:250	0	12450	16169	2.8	77.5
1000:0	0	8264	7149	8.3	65.7
500:125	109	6674	10467	0	75.3
500:30	0	3461	2309	0	14.7

Dose groups are shown as AE37 dose (μg):GM-CSF dose (μg).
Post-vaccination responses (proliferation and delayed type hypersensitivity (DTH)) are significantly increased from pre-vaccination values in all dose groups. Long-term proliferation responses were significantly increased from baseline for all 500 μg peptide dose groups.

metastatic disease with three patients discontinuing the vaccination series due to rapid disease progression. Based on results from the AE37 Phase I breast cancer trial [25], vaccinations were given as six monthly intradermal inoculations at a vaccine dose of 500 μg AE37 and 125 μg GM-CSF. Dose reductions were performed in a similar manner for large local reactions (> 100 mm^2). Toxicity was graded using the National Cancer Institute Common Terminology Criteria for Adverse Events v3.0. *In vitro* immunological responses were monitored by using the IFN-γ ELISPOT assay pre-vaccination, before each inoculation, and at one and six months after completing the vaccination series. *In vivo* immunological response was measured with the DTH reaction to the native peptide AE36 pre-vaccination and at one and six months after completing the vaccination series. $CD4^+CD25^{high}CD127^{low/-}$ T_{regs}, serum TGF-β and serum HER-2/*neu* extracellular domain levels were also measured. Downregulation of the IL-7 receptor (CD127) is an alternative phenotypic description of regulatory T cells [30].

All systemic toxicity was grade 0 or 1 (mild fever) with local toxicity occurring as grade 1 (injection site pain, swelling, itching) or grade 2 (induration with blistering). Six patients had grade 2 local toxicities, and three patients required dose reductions. AE36 and AE37 peptide-specific responses with vaccination were observed in the IFN-γ ELISPOT assay as well as in peptide-specific $CD4^+$ and $CD8^+$ T cell populations, with increases peaking midway through the vaccination series (**Table 3**). Of the vaccinated patients 72 % responded with augmented immunity to AE37 while 66 % experienced augmented immunity to AE36. Long-term AE36 immunity (six months after completing the vaccination series) was observed to be equivalent to peak immunity levels. Where the Phase I trial in breast cancer patients evaluated DTH responses to the hybrid peptide AE37, this trial evaluated *in vivo* immunological responses by DTH responses to the native peptide AE36. Post-vaccination and long-term DTH responses to AE36 were significantly increased from baseline following vaccination. Median diameters of DTH responses were 11.5 mm (range 0.5 – 38.75) post-vaccination and 10.0 mm (range 0.5 – 45.0 mm) long-term compared with a median value of 0.0 mm (range 0.0 – 8.0 mm) at pre-vaccination. Monitoring of other immune-related parameters revealed that $CD4^+CD25^{high}CD127^{low/-}$ T_{regs} cells were decreased six months after vaccination from pre-vaccination levels. Serum level of the T-cell suppressing cytokine TGF-β were also decreased long-term after vaccination and the percentage decrease in TGF-β levels correlated with the percent decrease in $CD127^{low/-}$ T_{regs}. Finally, post-vaccination serum levels of the extracellular domain of the HER-2/*neu* protein decreased from pre-vaccination levels.

In analyzing vaccine benefit for different patient groups, the authors found that patients with metastatic disease had smaller *in vivo* responses to vaccination than patients with non-metastatic disease. This finding may be of importance as the AE37 vaccine is currently being investigated in disease-free breast cancer patients in the adjuvant setting as a therapy to prevent disease recurrence. Additionally, they found that the *in vitro* immune responses were significantly enhanced in prostate cancer patients with low levels of HER-2/*neu* (IHC 1+ or 2+) expression. This supports findings from the E75 vaccine trials [12], and is encouraging given that trastuzumab is currently reserved for HER-2/*neu* overexpressors. These findings have prompted design of further clinical trials evaluating the role of active and passive immunotherapy in HER-2/*neu* low-expressors.

Table 3. Median peptide-specific immune responses from the AE37 prostate cancer Phase I trial.

	Peptide specific T cell frequency		$CD4^+$ T cell population		$CD8^+$ T cell population	
	Prevaccine	Maximal response	Prevaccine	Maximal response	Prevaccine	Maximal response
AE36	1:83,333	1:19,231	< 0.0001%	0.017%	< 0.0001%	0.085%
AE37	1:33,333	1:11,765	< 0.0001%	0.025%	< 0.0001%	0.074%

Median AE36 and AE37 specific T cell frequencies as measured in the ELISPOT assay increased with vaccination ($p < 0.0001$). Intracellular IFN-γ staining demonstrated increases in the median percentage of $CD4^+$ and $CD8^+$ T cell populations specific for AE36 and AE37 ($p < 0.005$).

6. Phase II clinical trial of AE37 in breast cancer

Based on the encouraging results from the Phase I trial, we are conducting a multi-center prospective, randomized, single-blinded Phase II trial evaluating the AE37 vaccine in breast cancer (NCT00524277) [16]. The primary endpoint is disease recurrence. The trial is enrolling node-positive or high-risk node-negative breast cancer patients. High-risk node-negative is defined as: tumor size greater than 2 cm, poor differentiation, presence of lymphovascular invasion, negative for both estrogen and progesterone receptors or HER-2/*neu* overexpression defined as IHC 3+ or FISH either amplified or greater than 2.2. Patients are enrolled after completion of standard breast cancer treatments including surgery, chemotherapy, immunotherapy with trastuzumab and radiation therapy as indicated. Patients on hormonal therapy are allowed to continue their regimen. Patients must be within one to six months of completion of primary breast cancer treatments and clinically and radiographically disease-free at enrollment. Importantly, patients with any detectable level of HER-2/*neu* expression (IHC 1+, 2+, or 3+ or FISH > 1.2) are eligible to participate in the study.

On enrollment, patients are randomized to receive either AE37 (500 μg) with GM-CSF (125 μg) or GM-CSF alone (125 μg). Vaccinations are given as six monthly intradermal inoculations. Prior to the first vaccination, patients are tested for DTH response to both the AE36 and AE37 peptides and blood samples are obtained to assess baseline immunologic parameters. Patients return 48 – 72 h after each inoculation for measurement of local reactions and recording of any adverse effects. Dose reductions of GM-CSF are carried out for local reactions > 100 mm^2 or local or systemic toxicities greater than grade 2. Additional blood samples are taken at the midpoint, and at one, six and twelve months after completion of the vaccination series. A post-vaccination DTH reaction to AE36 and AE37 is measured at one month after completion of the vaccination series.

As of January 2011, we have enrolled 206 patients [31]. With over 750 inoculations to date, the toxicity profiles between the two groups are similar (**Table 4**). In the adjuvant group, 70% have experienced grade 1 and 29% grade 2 local toxicity compared with 67% grade 1 and 33% grade 2 for the vaccine group. There was one patient who experienced a grade 3 local toxicity (pruritis) in the adjuvant group. Systemic toxicities in the adjuvant group have been grade 0 in 9%, grade 1 in 66% and grade 2 in 26%. For the vaccine group, 6% have had grade 0 systemic toxicities, 77% grade 1, 16% grade 2 and a single patient (1%) had a grade 3 reaction (myalgias, back and bone pain). The similarity of toxicity profiles between the vaccine and adjuvant groups suggest that much of the adverse effects result from GM-CSF.

We have monitored immunological responses in both the vaccine and GM-CSF-only adjuvant groups with AE36 and AE37 peptide-specific T cell proliferation using the [^{3}H]-thymidine incorporation assay as well as *in vivo* measurements by DTH reactions to both the AE36 and AE37 peptides. Vaccinated patients have exhibited statistically significant increases from baseline in both AE36 and AE37 proliferative responses at each measured time point, including maintenance of this response up to 12 months post-vaccination while there have been no proliferative changes for the adjuvant patients (**Table 5**). Vaccinated patients have also exhibited statistically significant increases in DTH response to AE36 (2.2 to 16.3 mm, $p < 0.001$) and AE37 (2.7 to 30.2 mm, $p < 0.001$) from pre- to post-vaccination while there has been no change in adjuvant patients. Similar to the breast cancer and prostate cancer Phase I trials of AE37, we have shown a decrease in $CD4^+CD25^{high}CD127^{low}$ regulatory T cells in vaccinated patients, with no change in adjuvant-treated patients [32]. Although preliminary, in the current Phase II trial we have observed an approximately 40% reduction in breast cancer recurrence at 17 months of median follow-up in patients treated with the AE37 peptide vaccine. Per trial design, patients are being followed for clinical recurrences and data regarding the efficacy of the vaccine will be reported after a median follow-up of 24 months.

7. Conclusion

These clinical trials of the AE37 vaccine administered with GM-CSF have shown it to be safe and well-tolerated with the ability to induce robust $CD4^+$ peptide-specific immune responses to both the native peptide AE36 and the hybrid peptide AE37. Induced *in vitro* and *in vivo* immune responses have been persistent, present as long as 12 months after completion of the vaccine series. At times, the AE37 vaccine was sufficiently immunogenic to induce responses even without

Table 4. Local and systemic toxicity profiles in AE37 Phase II trial.

	Vaccine	Adjuvant
Local toxicity		
Erythema/edema	36%	43%
Induration	34%	25%
Pruritis	29%	31%
Other (blistering, bruising, pain)	< 1%	< 1%
Systemic toxicity		
Fatigue/Malaise	39%	36%
Arthralgia	20%	8%
Headache	10%	13%
Bone pain	8%	8%
Fever/Chills	7%	13%
Myalgia	5%	8%
Back pain	5%	3%
Nausea/Vomiting	4%	9%
Diarrhea	1%	1%
Dizziness	1%	1%

Table 5. Peptide-specific immune responses in AE37 Phase II breast cancer trial.

	AE36 proliferation index		AE37 proliferation index	
Time Point	**Adjuvant**	**Vaccine**	**Adjuvant**	**Vaccine**
R0	1.03	0.95	1.05	0.98
R3	1.12	1.92	1.09	3.29
R6	1.09	2.12	1.14	3.38
RC6	1.00	2.18	1.17	3.64
RC12	1.16	1.80	1.47	3.68

Proliferation responses to AE36 and AE37 in the AE37 Phase II breast cancer trial are shown for prevaccination (R0), mid-series (R3), one month postvaccination (R6), six months post vaccination (RC6), and twelve months post vaccination (RC12). Vaccinated patients exhibited statistically significant increases in AE36 and AE37 proliferation from R0 at all measured time points. There were no statistically significant increases in adjuvant-only-treated patients.

the immunoadjuvant GM-CSF. Early findings suggest a clinical benefit of the AE37 vaccine in the prevention of breast cancer recurrence.

8. Expert opinion

Experience with the E75 vaccine has shown efficacy of this $CD8^+$ T-cell-eliciting vaccine in breast cancer patients in the adjuvant setting but with waning clinical response suggesting the lack of an immune response capable of generating memory. While this issue is being explored further through the investigation of booster inoculations [14], the potential of memory generation from a $CD4^+$ T-cell-eliciting vaccine offers an alternative approach to produce sustained immunity. The AE37 vaccine has been demonstrated to be safe and effective in eliciting a $CD4^+$ T cell response. Phase I clinical trials evaluating AE37+GM-CSF have demonstrated both *in vitro* and *in vivo* immune responses to vaccination, and these responses appear to be sustained, lasting up to 12 months after completion of the primary vaccination series. Further follow-up of patients vaccinated with AE37 is required to determine the durability of the $CD4^+$ T cell immune response. Breast cancer vaccine treatment strategies may ultimately evolve to multiple epitope vaccines capable of eliciting both $CD4^+$ and $CD8^+$ T cell responses.

The breast cancer Phase I trial demonstrated that the innovative Ii-Key hybrid technology has improved the potency of the AE37 vaccine to the point where it is capable of inducing immune responses even without immunoadjuvant. This is important as GM-CSF appears responsible for most adverse effects of vaccination. These studies have also shown the immune response generated from vaccination with the hybrid AE37 peptide has extended to the native peptide AE36.

While AE37 is thought of primarily as a $CD4^+$ T-cell-eliciting vaccine, preclinical work has shown the AE37 vaccine to be capable of enhancing the frequencies of IFN-γ-producing peripheral $CD8^+$ T lymphocytes in response to both AE37 and AE36. In addition to more efficient and potent MHC class II epitope delivery, the AE37 vaccine appears to enhance cytotoxic T cell immune responses. Embedded within the AE36 peptide are naturally-occurring $CD8^+$ cytotoxic T cell epitopes. In addition to directly charging MHC class II molecules, the AE37 peptide may also undergo antigen processing resulting in presentation of natural MHC class I epitopes to $CD8^+$ cytotoxic T cells. Furthermore, the addition of the Ii-Key peptide to AE36 may introduce MHC class I 'neo-epitopes' within the AE37 peptide.

Findings from the E75 vaccine trials have shown that HER-2/*neu* low-expressors may derive a greater immunological and clinical benefit from adjuvant vaccination against HER-2/*neu* epitopes. In the AE37 Phase I prostate cancer trial, HER-2/*neu* low-expressors were shown to have an enhanced immunologic response relative to HER-2/*neu* overexpressors. The relative response to vaccination may be further elucidated with results from the larger ongoing Phase II trial. Demonstrating a benefit for HER-2/*neu* low-expressors would add to the therapeutic options for these patients, as trastuzumab is currently reserved only for HER-2/*neu* overexpressors. These findings have prompted the design of further trials exploring the role of active and passive immunotherapy in patients with HER-2/*neu* low-expressing tumors.

Other interesting findings from these studies include the improved immunity achieved in prostate cancer patients without metastatic progression relative to those with metastatic disease. This finding is possibly related to the known immunosuppressive effects of cancer, with greater immunosuppression in those patients with more advanced disease. This may support the use of the AE37 vaccine as an adjuvant

treatment as is being evaluated in the ongoing Phase II trial. Regardless of disease stage, all three trials had the consistent finding of decreasing regulatory T cells in vaccinated patients. This is encouraging given the concern that stimulation of $CD4^+$ T cells may lead to undesired stimulation of the immunosuppressive regulatory T cells.

In summary, the available evidence shows the AE37 vaccine to be safe and well-tolerated with the ability to generate sustained robust *in vitro* and *in vivo* immune response in vaccinated breast and prostate cancer patients. While still early in clinical trial investigation, preclinical and early clinical trials of the AE37 show potential promise for this $CD4^+$ T-cell-eliciting vaccine in the adjuvant treatment of breast cancer.

Declaration of interest

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