

REVIEW

Vaccination against Her-2/neu, with focus on peptide-based vaccines

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Immunotherapy has been a milestone in combatting cancer, by complementing or even replacing classic treatments like surgery, chemotherapy, radiation, and anti-hormonal therapy. In 15%-30% of breast cancers, overexpression of the human epidermal growth factor receptor 2 (Her-2/neu) is associated with more aggressive tumor development. Passive immunization/immunotherapy with the recombinantly produced Her-2/neu-targeting monoclonal antibodies (mAbs) pertuzumab and trastuzumab has been shown to effectively treat breast cancer and lead to a significantly better prognosis. However, allergic and hypersensitivity reactions, cardiotoxicity, development of resistance, lack of immunological memory which results in continuous application over a long period, and cost-intensiveness are among the drawbacks associated with this treatment. Furthermore, intrinsic or acquired resistance is associated with the application of therapeutic mAbs, leading to the disease recurrence. Conversely, these drawbacks could be potentially overcome by vaccination, i.e. an active immunization/immunotherapy approach by activating the patient's own immune system to target cancer, along with inducing immunological memory. This review aims to summarize the main approaches investigated and undertaken for the production of Her-2/neu vaccine candidates, with the main focus on peptide-based vaccines and their evaluation in clinical settings.

Key words: cancer, Her-2/neu, vaccination, T-cell peptide vaccine, B-cell peptide vaccine/mimotopes

INTRODUCTION

One of the leading causes of death among women is breast cancer, including the molecular sub-type with overexpressed Her-2/neu (ErbB2). Her-2/neu, a 185-kDa transmembrane protein, is a family member of the human epidermal growth factor receptors (EGFR), also including Her-1 (EGFR, ErbB1), Her-3 (ErbB3), and Her-4 (ErbB4).¹ The amplification of Her-2/neu was first observed in 1985 where the human mammary carcinoma cells MAC117 were shown to have 5- to 10-fold overexpression of the receptor.² The role of the Her-2/neu proto-oncogene in the pathogenesis and progression of human breast cancer was shown in 1987.³ Her-2/neu overexpression has been observed in 15%-30% of breast cancer patients⁴⁻⁶ resulting in a 100- to 200-fold increased concentration of the Her-2/neu protein

in tumor versus normal tissue.⁷ Furthermore, the overexpression of the receptor correlates with poor clinical outcomes resulting in an aggressive tumor phenotype and reduced survival.⁸⁻¹¹ Therefore, due to the association of Her-2/neu overexpression with enhanced tumor progression, as well as weaker response to traditional chemotherapy, and consequently poor prognosis, the receptor has been an attractive target as a tumor-associated antigen (TAA) for cancer therapy.¹²⁻¹⁵ Anticancer immunotherapies fall into the two categories of 'passive' or 'active' immunotherapy¹⁶; the emergence of both has been facilitated by the discovery of TAAs. By possessing an intrinsic antineoplastic activity, the application of therapeutic, tumor-targeting monoclonal antibodies (mAbs) is generally considered the passive form of immunotherapy.^{17,18} Conversely, anticancer vaccines under the category of active immunotherapy induce the engagement of the host immune system and the formation of immunological memory.^{19,20}

BIOLOGICAL BACKGROUND

Immune dysregulation and dysfunction result in cancer progression and therefore the aim of cancer immunotherapy is to restore a specific anti-tumor immune response. In systems biology, the Her-2/neu pathway, or Her network, has been described as a complex and robust biological network.^{21,22} Unlike the other members, no

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ligand has been identified for Her-2/neu, i.e. an orphan receptor. The ligand binding to the extracellular domain (ECD) of Her-1, Her-3, and Her-4 results in the formation of catalytically active homo- and heterodimers. Her-2/neu, which is in an open position and naturally ready for dimerization, is a preferred partner for dimerization.²³ Activation of the Her network as a result of the dimerization leads to auto-phosphorylation in C-terminal tyrosines of the receptors' intracellular domain (ICD) and activation and recruitment of cytoplasmic signal transducers, such as Ras-Raf-MEK-MAPKs, phosphatidylinositol-3 kinase (PI3K)-Akt-ribosomal S6 kinase, and the signal transducers and activators of transcription.²³ These consequently regulate cellular processes such as proliferation, differentiation, motility, adhesion, protection from apoptosis, and transformation.^{23,24} Over the past decades, due to the importance of the ECD and ICD of Her-2/neu, there has been remarkable progress in the regimens developed for the treatment of Her-2/neu-positive breast cancer.²⁵⁻²⁷

Trastuzumab, the first anti-Her-2/neu humanized mAb developed in 1990, interferes with Her-2/neu signaling via several mechanisms, including inhibition of ligand-independent dimerization, receptor internalization and/or degradation, inhibition of the PI3K-AKT signaling pathway, and antibody-dependent cellular cytotoxicity (ADCC).^{28,29} The addition of trastuzumab to chemotherapy was initially found to improve overall survival (OS) in women with Her-2/neu-positive metastatic breast cancer,³⁰ and 1 year of treatment with trastuzumab after adjuvant chemotherapy was shown to significantly improve disease-free survival (DFS) among women with Her-2/neu-positive breast cancer.³¹ Therefore, trastuzumab has become a standard-of-care treatment of both metastatic and early-stage Her-2/neu-positive breast cancers for more than a decade. Pertuzumab, the second anti-Her-2/neu humanized mAb, was approved by the Food and Drug Administration in 2012 for the treatment of Her-2/neu-positive metastatic breast cancer.³² Pertuzumab, which has a different binding site than trastuzumab, binds to the dimerization domain 2 of Her-2/neu resulting in the inhibition of ligand-induced Her-2/neu heterodimerization.^{32,33} The combination of trastuzumab and pertuzumab has been shown to synergistically inhibit tumor growth in both *in vitro* and *in vivo* preclinical models.^{34,35} In the phase III CLEOPATRA trial, the combination of pertuzumab plus trastuzumab and docetaxel, compared with placebo plus trastuzumab and docetaxel, prolonged median OS by 15.7 months without any increase in the risk of cardiac toxicity.³⁶ The improvement in clinical outcomes with the addition of pertuzumab to trastuzumab is seen as a result of its complementary mechanism of action to trastuzumab, resulting in maximal blockade of the Her-2/neu oncogenic pathway.³²

Despite the tremendous success with its anti-tumor effect, immunotherapy by mAbs requires continuous application over a long period, and the half-life of the mAbs limits the duration of therapy, resulting in only temporary disease control, particularly once the tumor has

metastasized.³⁷⁻⁴⁴ Furthermore, the development of resistance to treatments with mAbs,⁴⁵ immune-related adverse events, and hypersensitivity,^{46,47} which may be as a result of the mAbs administered doses to ensure their immediate bioavailability and potency^{48,49} and cardiotoxicity,⁵⁰ are among the drawbacks of treatments with mAbs. Unlike mAbs or chemotherapy, TAA-based vaccines which induce the immune system to produce patients' own antibodies toward the antigen may prevent the development or reduce the intensity of hypersensitivity reactions, can induce prolonged activation of the immune system, immunological memory, and potentially result in more efficient cancer control. Further advantages of vaccines include their less-frequent and easier administration and their relatively safer nature than chemotherapy.⁵¹

ANTI-HER-2/NEU VACCINES UNDER DEVELOPMENT

Different approaches have been undertaken to construct anti-Her-2/neu vaccines. As depicted in Figure 1A, these approaches include, B- or T-cell peptide-based vaccines, matured dendritic cells (DCs) loaded with TAA or tumor-specific antigens (TSA), which, as specialized antigen-presenting cells, possess a key role in the initiation and regulation of innate and adaptive immune responses by sensitizing antigen-specific CD4- and CD8-positive T cells,⁵²⁻⁵⁴ or DNA vaccination using plasmids delivering genes encoding the TAA or TSA to harness the immune system and elicit or augment the adaptive immune response against the respective tumor.⁵⁵⁻⁵⁷ This review focuses on peptide-based vaccine candidates targeting Her-2/neu (Figure 1A; Table 1).

T-cell peptide vaccines

Therapeutic peptide-based vaccines can reduce tumor growth by generating tumor-specific CD8 cytotoxic T lymphocytes (CTLs) or CD4 helper T lymphocytes. Activation of T cells is achieved by both antigen-specific signaling, i.e. interaction between T-cell receptor and the antigen in the context of human leukocyte antigen (HLA) classes I and II of the antigen-presenting cells (APCs), to form a peptide-HLA complex, and CD28 co-stimulation, i.e. interaction between co-stimulatory receptors like CD28 with CD80 or CD86 on the APC.^{20,58} It was proposed that Her-2/neu may serve as a TAA since the processing of overexpressed Her-2/neu protein would theoretically result in an increased peptide supply which could occupy a significant number of major histocompatibility complex (MHC) molecules in competition with other peptides.⁷

The 9-mer peptide E75, derived from the ECD of Her-2/neu (amino acids 369-377), is among the most studied peptide vaccine candidates against Her-2/neu.⁵⁹ The peptide was identified as one of the common immunogenic epitopes of Her-2/neu that were recognized by CD3⁺ CD4⁺ CD8⁺ ovarian-specific CTL lines, and is based on the anchor motif of the HLA-A2 allele⁷ as well as HLA-A3 allele.⁶⁰

GP2, an MHC class I, nine-amino acid-long peptide (IISAVVGIL), is a fragment (amino acids 654-662) of the

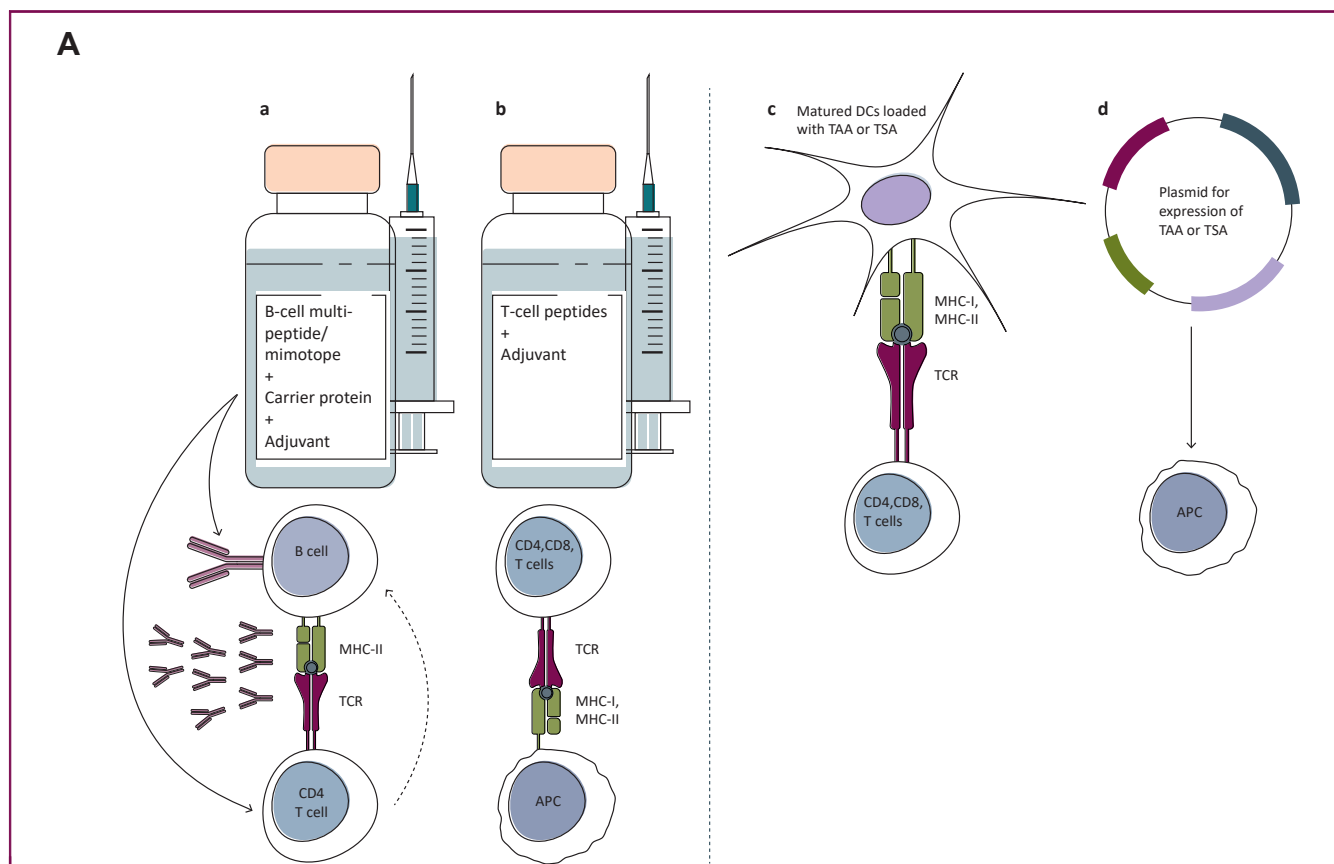


Figure 1. (A) A diagram depicting different types of cancer vaccines against Her-2/neu. B-cell peptides representing the binding epitope of anti-Her-2/neu therapeutic monoclonal antibodies (mAbs), directly activate B cells for the production of Abs, corresponding to the therapeutic mAbs. The presence of T-cell epitopes in the carrier protein results in stimulation of T cells, which in turn further activates B cells for the production of antibodies. T-cell-based (b) and dendritic-cell (DC)-based (c) vaccines bind to either CD4+ or CD8+ T cells and result in their stimulation. DNA-based vaccines are also aimed to stimulate the T cells after being processed by APC (d). (B) A diagram depicting the development of the two generations of our Her-2/neu vaccine with the different examined formulations, and their evaluation in preclinical and clinical studies.^{68,70,72,73,76,77} The extracellular domain (ECD) of Her-2/neu was initially subjected to computer-aided algorithms and resulted in the identification of one B-cell peptide (P4) on the ECD-III and two additional B-cell peptides (P6 and P7) on the ECD-IV. These single peptides were the basis for the different indicated formulations of our Her-2/neu vaccine's first generation, leading to the vaccine HER-Vaxx. The second generation of our vaccine candidate comprises the HER-Vaxx vaccine, formulated for co-application with pertuzumab and/or immune checkpoint inhibitors, or in combination with the mimotopes (B-cell epitopes) of pertuzumab and/or relevant immune checkpoint inhibitors.

APC, antigen-presenting cells; MHC-I/II, major histocompatibility complex I/II; TAA, tumor-associated antigen; TCR, T-cell receptor; TSA, tumor-specific antigen.

transmembrane domain of Her-2/neu which binds to the HLA-A2 molecule and activates CTLs.^{61,62}

AE37, an MHC class II peptide, activates CD4 T helper cells and is a modified version of the naturally occurring AE36 wild-type peptide (Her-2/neu, amino acids 776-790) derived from the ICD of Her-2/neu.⁶³ AE37 was generated by linking AE36 to li-Key (LRMK), a portion of the MHC class II-associated invariant chain (li protein), to enhance the peptide's potency in activating CD4⁺ T cells. The li-Key hybrid AE37, generated by linking LRMK to the known Her-2/neu MHC class II epitope (amino acids 776-790), has been shown to generate robust, long-lasting Her-2/neu-specific immune responses both in patients with breast cancer and prostate cancer.^{64,65}

B-cell peptide vaccines

The tremendous success of passive immunotherapy with trastuzumab has paved the way for more interest in the identification of B-cell peptides/binding epitope of mAbs for use as anti-Her-2/neu vaccine antigens.⁶⁶ This was further

reinforced by the proven efficacy of the dozens of additional approved therapeutic mAbs.⁶⁷ Vaccines including B-cell peptides induce an immune response specific to the TAA or TSA and, unlike T-cell peptides, B-cell peptides are not restricted to a specific HLA, advantageously allowing a broader application across all HLA types.²⁰ This becomes, in particular, important regarding the reduction of expression of MHC-I molecules as tumor evasion mechanisms.²⁰

In line with this approach, our group has been working on developing B-cell peptide-based Her-2/neu vaccines, formulated differently with regard to their immunogenicity, adjuvanticity, and their evaluation in several preclinical and clinical studies, as depicted in Figure 1B and described below.

By the means of computer algorithms, as the first generation of our vaccine, we identified three single peptides representing B-cell epitopes located in the ECD-III (P4; amino acids 378-394) and ECD-IV (P6 and P7; amino acids 545-560 and 610-623, respectively).⁶⁸ Immunization with the single peptides, conjugated to tetanus toxoid and mixed

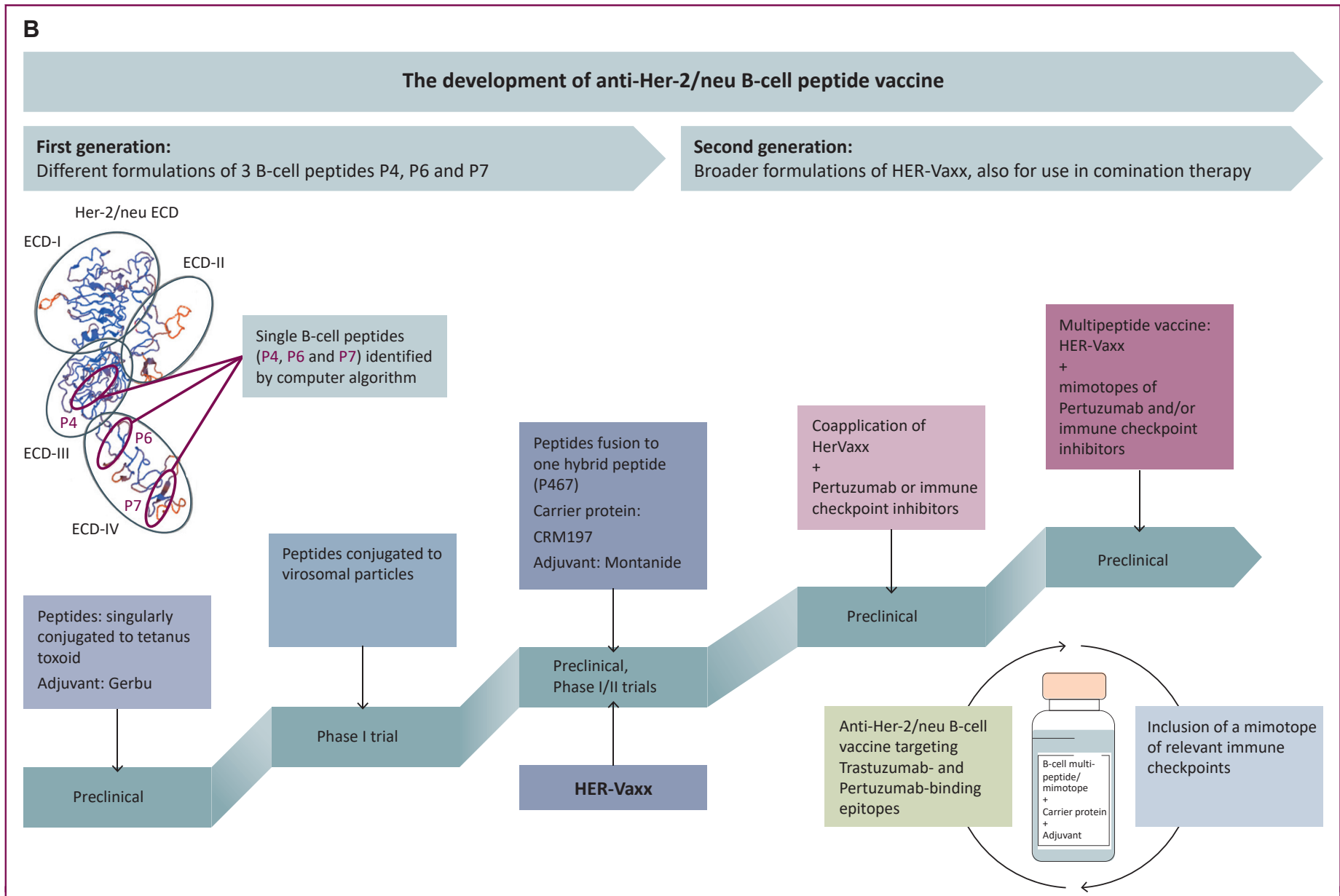


Figure 1. (Continued).

Table 1. Developed or under development anti-Her-2/neu vaccines

Platform	Vaccine		Reference
	Peptide	Characteristics	
T-cell peptide vaccines	E75	CD8, MHC class I	59
	GP2	Extracellular domain of Her-2/neu (amino acids 369-377)	62
		CD8, MHC class I (HLA-A2- and A3-restricted)	
	AE37	Transmembrane domain of Her-2/neu (amino acids 654-662)	63
B-cell peptide vaccines		CD4, MHC class II	
		Intracellular domain of Her-2/neu (amino acids 776-790)	
	HER-Vaxx	Three fused single peptides from the extracellular domain of Her-2/neu: PESFDGDPASNTAPLQPRVLQGLPREYVNRHSLPYMPIWKFPDEEGAC	68,73
	JTMP	A 42-mer peptide as pertuzumab's binding epitope on Her-2/neu (amino acids 260-301)	Reported here
Liposome-based vaccine with B-cell peptides	B-Vaxx	Trastuzumab-binding epitope (amino acids 597-626) and pertuzumab-binding epitope (amino acids 266-296)	82,83
		Liposome-based vaccine comprising spatially separated B-cell epitope of the Her-2/neu targeted by pertuzumab, and ovalbumin peptide OVA	85

MHC, major histocompatibility complex.

together with Gerbu, a veterinary adjuvant based on the immunomodulator glycopeptide from the cell wall of the bacterium *Lactobacillus bulgaricus*,⁶⁹ led to induction of antibodies with the capacity in inhibiting tumor growth *in vitro*, as shown by proliferation assays and complement-dependent and antibody-dependent cell cytotoxicity assays.⁶⁸ In a breast cancer mouse model with an activated *c-neu* oncogene, immunization with the aforementioned formulation was shown to delay tumor onset and reduce tumor growth progression. Moreover, co-application of the vaccine with IL-12 resulted in a Th1-polarized immune response with elevated Her-2/neu-specific IgG levels and increased *in vitro* production of interferon- γ by splenocytes.⁷⁰ For clinical evaluation, the single peptides were conjugated to virosomes, possessing an intrinsic adjuvant activity,⁷¹ and examined in a phase I study for evaluating the safety and immunogenicity in breast cancer patients in the advanced stage of the disease.⁷² While the study showed good immunogenicity as well as an excellent safety profile,⁷² drawbacks with regard to solubility and limited stability after coupling the single peptides to virosomes were associated with the formulation. This consequently led to the development of an improved formulation,⁷³ comprising the three single peptides fused into the hybrid peptide P467 (PESFDGDPASNTAPLQPRVLQGLPREYVNRHSLPYMPIWKFPDEEGAC), conjugated to the carrier protein CRM197 (CRM; cross-linking materials) and administered in conjunction with the Th1/Th2-driving adjuvant Montanide. As an enzymatically inactive and nontoxic (toxoid) form of diphtheria toxin,⁷⁴ CRM197 rapidly activates CD4⁺ T cells with a heterogeneous Th1 and Th2 cytokine profile and consequently activates B cells and regulates the quantity of the induced antibodies,⁷⁵ justifying its use with our vaccine. In preclinical evaluations, the hybrid peptide was shown to retain the B-cell epitopes of the single peptides with a strong capacity to induce long-lasting antibody responses. Additionally, the fusion of the single peptides had further generated a T-cell immunodominant epitope in the hybrid peptide, inducing cellular responses as shown by T-cell proliferation of splenocytes from mice.⁷³ This formulated vaccine, i.e. HER-Vaxx (Figure 1B), was evaluated *in vivo* for

its anti-tumor effect, showing that active immunization with the vaccine significantly inhibits tumor growth in our mouse syngeneic model with solid tumors following engraftment of BALB/c mammary carcinoma cells expressing human Her-2/neu (D2F2/E2).⁷⁶ Applying the mouse model, we have also shown that the combination of HER-Vaxx with immune checkpoint (PD-1) blockade enhanced the vaccine's anti-tumor effect.⁷⁶ As described below, HER-Vaxx has been evaluated in phase Ib⁷⁷ and phase II trials⁷⁸ involving patients with Her-2/neu-overexpressing metastatic or advanced adenocarcinoma of the stomach or gastroesophageal junction.

We have recently established a platform for the identification of mimotopes (B-cell peptides) of therapeutic mAbs.⁷⁶ Toward the construction of a multi-peptide vaccine targeting the binding epitopes of trastuzumab and pertuzumab (Figure 1B), we aimed to identify the binding epitope of pertuzumab by the application of the mimotope platform. This was supported following *in vivo* evaluation of the anti-tumor effect of HER-Vaxx in combination with trastuzumab or pertuzumab in the aforementioned syngeneic solid tumor mouse model.⁷⁶ As shown in Figure 2A, the combination of HER-Vaxx with trastuzumab did not enhance the vaccine's anti-tumor effect, which is attributed to the fact that HER-Vaxx comprises the binding-site epitope of trastuzumab and therefore no synergistic effect was observed. However, in combination with pertuzumab, a significantly enhanced anti-tumor effect of HER-Vaxx, when compared with the vaccine alone, was shown (Figure 2A).

By applying our established platform for identification of mimotopes of therapeutic mAbs,⁷⁶ a 42-mer peptide (HSGICELHCPALVTYNTDTFESMPNPEGRYTFGASCVTACPY), located at the positions 260-301 on Her-2/neu, was designed. Based on solid phase-based inhibition ELISA assay, the mimotope was shown to significantly and dose-dependently inhibit the mAb's binding to recombinant Her-2/neu (Supplementary Figure S1, available at <https://doi.org/10.1016/j.esmoop.2021.100361>). Furthermore, using the breast cancer cell line SKBR-3, endogenously expressing high levels of Her-2/neu as well as Her-3,^{79,80} mimotope-specific mouse IgG antibodies were shown to modulate the phosphorylation of

intracellular Her-2/neu as well the phosphorylation of the protein kinase Akt, at the positions 308 and 473, in a similar manner as pertuzumab (Supplementary Figure S2, available at <https://doi.org/10.1016/j.esmoop.2021.100361>); the heterodimerization of Her-2/neu and Her-3 is considered to be the realm of oncologic signaling through the potent activation of the Akt signaling pathway.⁸¹

By employing the syngeneic solid tumor mouse model, we have shown that active immunization with the mimotope induces an anti-tumor effect, reflected by a significant tumor growth reduction compared with the corresponding control mice. Additionally, the level of the anti-tumor effect mediated by the mimotope-induced Abs was shown to be comparable with that following passive immunization of mice with pertuzumab (Figure 2B).

In an established mouse model for Her-2/neu-expressing lung metastases, reflecting the metastatic stage of the disease and the adjuvant setting in the clinic, active immunization with the mimotope has shown a significant capacity in preventing lung metastases (unpublished data). Furthermore, the potential enhancement of our multi-peptide vaccine's anti-tumor effect when combined with relevant immune checkpoint inhibitors or their respective mimotopes is currently being investigated in this model.

By the means of X-ray structures of the Her-2/neu—trastuzumab and Her-2/neu—pertuzumab complexes, another research group identified the rationally designed B-cell peptides as pertuzumab's binding epitope (amino acids 266-296, overlapping with the sequence of our pertuzumab's mimotope JTMP), and trastuzumab's binding epitope (amino acids 597-626).^{82,83} The peptides are individually fused at their N-terminus, by a linker consisting of GPSL, to a measles virus fusion protein (MVF), a carrier protein representing a 'promiscuous' T helper cell epitope. This allows MCH class II-mediated activation of multiple T helper cells, leading to increased production of also tumor peptide-specific Ab production by B cells.⁸⁴ A vaccine, B-Vaxx, consisting of a mixture of the two chimeric constructs in combination with a potent adjuvant nor-muramyl dipeptide (n-MDP) and emulsified in Montanide ISA 720, was formulated.⁸⁴

In a recent study, the use of a liposomal system for the delivery of B-cell peptide of Her-2/neu was also investigated.⁸⁵ The liposomal system was composed of a spatially separated B-cell epitope of the Her-2/neu ECD targeted by pertuzumab, and ovalbumin peptide OVA (amino acids 323-339), to provide non-cognate T-cell support.⁸⁵ Immunization of mice with the liposome-based vaccine was shown to induce significant humoral responses, specific for the Her-2/neu peptide, *in vivo*. The generated antibodies were shown to induce cell death *in vitro* using the mouse mammary carcinoma cell line TUBO overexpressing rat Her-2.⁸⁵

COMPLETED OR CURRENT ONGOING CLINICAL TRIALS—T-CELL PEPTIDE VACCINES

E75, GP2, AE37, multi-peptide

The T-cell peptide-based vaccine E75, otherwise known as nelipecimut-S or NeuVAX (Galena Biopharma/SELLAS Life

Sciences Group, Inc., New York, NY), has so far been the most extensively studied vaccine in several clinical trials (Table 2). The earliest phase I pilot study involved patients with breast or ovarian cancer that expressed HLA-A2 and Her-2/neu, or epithelial mucin MUC1, which is aberrantly overexpressed in various cancers in human epithelia.⁸⁶ Autologous DCs were pulsed with E75 or with MUC1-derived peptides and used for the vaccination which was well tolerated with no side effects.⁸⁷ The vaccination was also shown to induce the production of peptide-specific CTLs which lasted for >6 months.⁸⁷

In two follow-up paired sets of early phase I clinical trials (NCT00841399 and NCT00854789; Table 2), Her-2/neu-expressing breast cancer patients were vaccinated with E75 and the vaccine adjuvant granulocyte—macrophage colony-stimulating factor (GM-CSF). The vaccine was shown to be safe, and an 89.7% DFS in the vaccinated group versus 80.2% in the control group were observed, indicating the vaccine's clinical efficacy.⁸⁸ In a phase IIb combination trial (NCT01570036), the two arms of trastuzumab plus nelipecimut-S or trastuzumab plus GM-CSF were evaluated.⁸⁹ The primary outcome in this study was a 24-month DFS, and a 36-month DFS, safety, and immunologic responses were the study's secondary outcomes. Based on the results, no clinicopathologic differences between groups were observed and the concurrent treatment with trastuzumab and the vaccine was shown to be safe compared to the control group. No significant difference in the DFS at a median follow-up of 25.7 months was found between the vaccinated and the control groups. The results of this study indicated the safety of both nelipecimut-S and GM-CSF in combination with trastuzumab, findings that warranted further investigation in a phase III randomized trial.

The phase III trial (NCT01479244; Table 2), with nelipecimut-S as a single agent, involved 758 patients with early-stage, node-positive breast cancer with low to intermediate Her-2/neu expression. Patients received nelipecimut-S plus GM-CSF or GM-CSF alone, over the course of six intradermal injections followed by boosting every 6 months for 3 years. The interim analysis of the results demonstrated no overall difference in DFS between arms at 16 months' follow-up.⁹⁰ Based on the results from this phase III trial, combination studies were designed.⁹¹

In an ongoing phase II trial, patients with ductal carcinoma *in situ* of the breast (NCT02636582; Table 2) were treated either with nelipecimut-S or with sargramostim (the recombinant GM-CSF). The aim of this study, with pending results, is to evaluate the level of circulating immune response at 6 months after vaccination, as well as toxicity and safety.⁸⁸ In an additional ongoing randomized phase II trial (NCT02297698; Table 2),⁸⁸ patients with non-metastatic Her-2/neu overexpression (immunohistochemistry 3+) were vaccinated with nelipecimut-S + GM-CSF after completion of infusion with trastuzumab, or vaccinated only with GM-CSF and simultaneously treated with trastuzumab. Based on interim results, nelipecimut-S was shown to be well tolerated and no significant differences

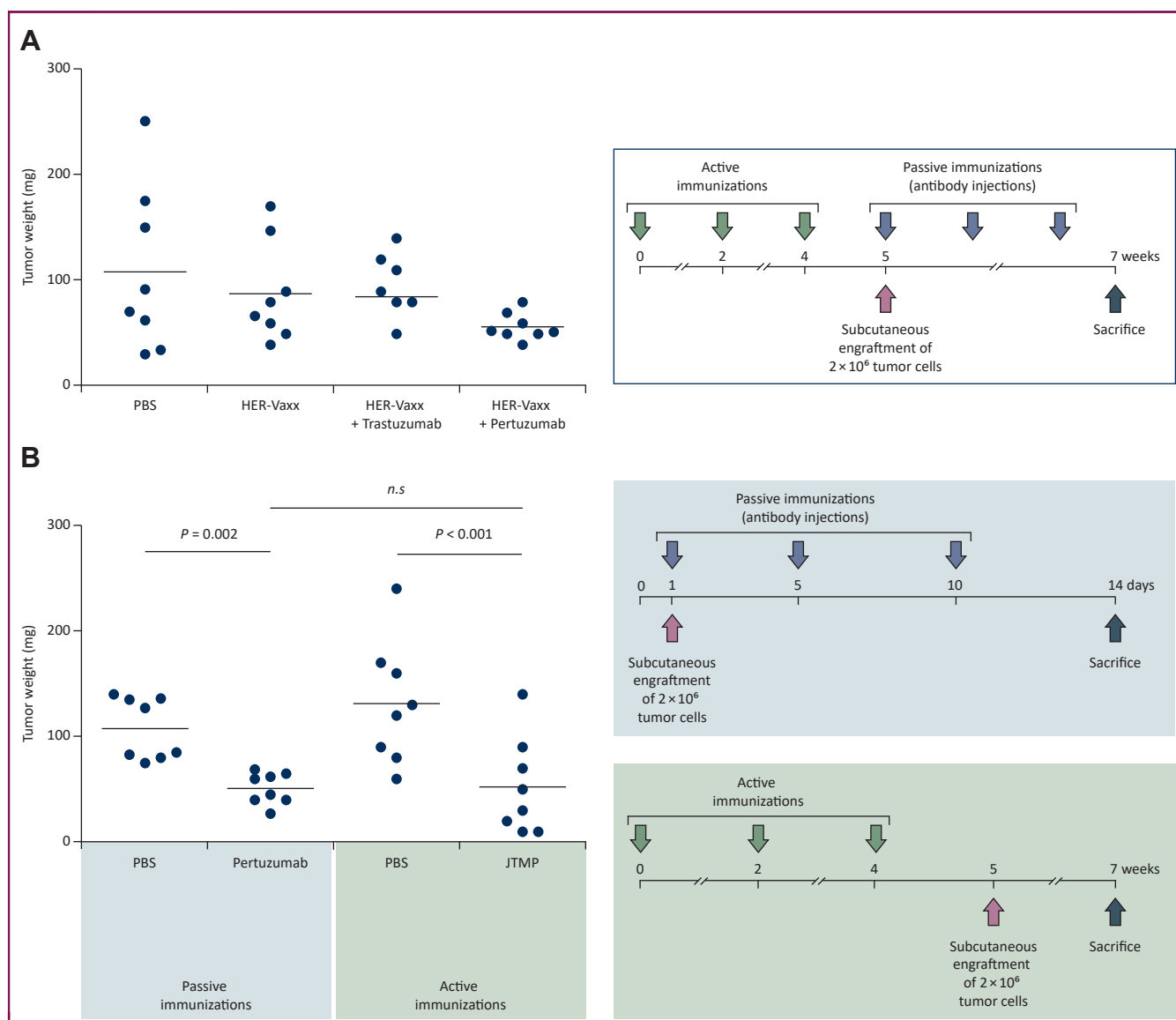


Figure 2. Evaluation of anti-tumor capacity by active or passive immunization with HER-Vaxx, Trastuzumab, Pertuzumab, or the mimotope of Pertuzumab (JTMP) in a syngeneic tumor mouse model.

(A) Female BALB/c mice (Charles River, Germany, 6–8 weeks of age at the time of delivery) were used in immunization experiments involving a syngeneic solid tumor mouse model (Tobias et al.⁷⁶). The experiment, representative of at least two experiments, consisted of four groups of mice ($n = 8$) subcutaneously injected with phosphate-buffered saline (PBS) (control mice), with HER-Vaxx alone or followed by intraperitoneal injection (100 μ g/dose) with either trastuzumab or pertuzumab, as depicted in the immunization schedule. Two weeks after the tumor cell grafting, mice were sacrificed, the tumors were excised, and their weight was measured. (B) Female BALB/c mice (Charles River, Germany, 6–8 weeks of age at the time of delivery) were used in immunization experiments involving a syngeneic solid tumor mouse model (Tobias et al.⁷⁶). The experiment, representative of at least two experiments, consisted of two settings. In the passive immunization setting, mice ($n = 8$) were either intraperitoneally injected with PBS or with pertuzumab (100 μ g/dose), as depicted in the immunization schedule. In the active immunization setting, mice ($n = 8$) were either subcutaneously injected with PBS or with JTMP, the mimotope of pertuzumab (50 μ g/dose), as depicted in the immunization schedule. Two weeks after the tumor cell grafting, mice were sacrificed, the tumors were excised, and their weight was measured. Tumor mass data were analyzed by a General Linear Model with a log link. Residuals were tested for normality by Kolmogorov–Smirnov tests and homogeneity of variances by the Brown–Forsythe test. Comparisons to controls and between active and passive immunization were done by linear contrasts. Significant differences are indicated by the respective P values. n.s., not significant.

in side-effect profile or cardiac ejection fractions were observed between the two arms of the study.⁸⁸

In a phase I dose-escalation trial (Walter Reed Army Medical Center, Washington DC; drug application BB-IND #11,730) involving disease-free, lymph node-negative, HLA-A2-positive breast cancer patients, the peptide GP2 in combination with GM-CSF was found to be safe and well tolerated with minimal local/systemic toxicity (Table 2). GP2 elicited Her-2/neu-specific immune responses, including epitope spreading in high-risk, lymph node-negative breast

cancer patients.⁹² However, in a follow-up phase II trial (NCT00524277) involving clinically disease-free HLA-A2-positive patients with Her-2/neu-expressing tumors, the vaccinated group did not show a statistically significant difference in the rate of recurrence compared to the control group. The trial confirmed that the GP2 vaccine is safe, suggesting that the vaccination may have clinical activity, particularly in patients with Her-2/neu overexpression who received the full vaccine series.⁹³ In an additional phase Ib trial (NCT03014076) involving Her-2/neu-overexpressing

Table 2. Completed or current ongoing clinical trials with T- and B-cell peptide vaccines

T-cell peptide vaccines							
Examined vaccine	Phase	Adjuvant	Systemic intervention	Patients	Setting	NCT/trial's identifier	Reference
E75	I	GM-CSF	None	Breast cancer	Experimental (vaccine) versus no intervention (control)	NCT00841399	⁸⁸
	I	GM-CSF	None	Breast cancer	Experimental (vaccine) versus no intervention (control)	NCT00854789	⁸⁸
	II	GM-CSF	Trastuzumab	Breast cancer	Vaccine + trastuzumab versus trastuzumab + GM-CSF	NCT01570036	⁸⁹
	III	GM-CSF	None	Breast cancer (low to intermediate Her-2/neu expression)	Vaccine versus GM-CSF only	NCT01479244	⁹⁰
	II	GM-CSF	None	Breast cancer	Vaccine versus GM-CSF only	NCT02636582	⁸⁸
	II	GM-CSF	Trastuzumab	Breast cancer	Vaccine + trastuzumab versus trastuzumab + GM-CSF	NCT02297698	⁸⁸
GP2	I	GM-CSF	None	Breast cancer	Dose escalation	#11 730 ^a	⁹²
	II	GM-CSF	None	Breast cancer	Vaccine versus GM-CSF only	NCT00524277	⁹³
	Ib	GM-CSF	Trastuzumab	Breast cancer	Vaccine + trastuzumab versus trastuzumab	NCT03014076	⁹⁴
AE37	I	GM-CSF	None	Breast cancer	Dose escalation	#12 229 ^a	⁹⁵
	II	GM-CSF	None	Breast cancer	Vaccine versus GM-CSF only	NCT00524277	⁹⁶
HER2/neu peptide	I/II	Breast and ovarian cancers	Trastuzumab	Breast or ovarian cancer	Active, not recruiting	NCT00194714	⁹⁸
B-cell peptide vaccines							
Examined vaccine	Phase	Adjuvant	Systemic intervention	Patients	Setting	NCT/trial's identifier	Reference
HER-Vaxx (IMU-131)	Ib	Montanide	Chemotherapy	Her-2 ⁺ gastric cancer	Dose escalation		⁷⁷
	II	Montanide	Chemotherapy	Her-2 ⁺ gastric cancer	Vaccine + chemotherapy versus chemotherapy alone		Ongoing study
B-vaxx	I		None	Metastatic solid tumors	Dose escalation		¹⁰²
	II		None	Metastatic solid tumors (breast, ovarian and gastrointestinal cancers)	Extension trial		Ongoing study

GM-CSF, granulocyte-macrophage colony-stimulating factor.

^a Walter Reed Army Medical Center.

breast cancer patients, the combination of GP2 with trastuzumab was evaluated showing that the vaccine is safe and stimulates an immunologic response when given concurrently with trastuzumab.⁹⁴

In a phase I clinical trial (Walter Reed Army Medical Center, Washington, DC; drug application #12 229), the peptide AE37 in conjunction with GM-CSF was shown to be safe and well tolerated with minimal toxicity in breast cancer patients and induced Her-2/neu-specific immune response without the use of an immunoadjuvant⁹⁵ (Table 2). In a follow-up phase II adjuvant trial (NCT00524277) involving clinically disease-free node-positive and high-risk node-negative breast cancer patients with tumors showing any degree of Her-2/neu expression (immunohistochemistry 1+ to 3+), the efficacy of AE37 in conjunction with GM-CSF was evaluated.⁹⁶ Although no benefit of vaccination was observed, the study confirmed the safety of the vaccine and its clinical benefit in patients with low Her-2/neu-expressing tumors.⁹⁶

A vaccine consisting of putative Her-2/neu helper T-cell epitopes (369-384, 688-703, and 971-984) as well as HLA-A2-binding motifs (369-377, 689-697, and 971-979)⁹⁷ was recently examined in phase I and II clinical trials (NCT00194714) involving patients with stage IV Her-2/neu-positive breast cancer (Table 2). The safety and immunogenicity of combination therapy consisting of concurrent

vaccination and treatment with trastuzumab were the primary objectives of the trials, and the therapy was found to be associated with minimal toxicity and resulted in prolonged and robust antigen-specific immune responses in treated patients.⁹⁸

COMPLETED OR CURRENT ONGOING CLINICAL TRIALS—B-CELL PEPTIDE VACCINES

HER-Vaxx

The vaccine was recently evaluated in a multicenter phase Ib clinical trial (NCT02795988) involving 14 patients with advanced and metastatic Her-2/neu-overexpressing gastroesophageal adenocarcinomas in three dose-escalated cohorts of 10, 30, and 50 µg, who were also treated with chemotherapy⁷⁷ (Table 2). Considering the immunosuppressive effect of cytotoxic chemotherapies on lymphocytes,⁹⁹ particularly B cells,¹⁰⁰ the first vaccination was initiated before the onset of chemotherapy.⁷⁷ This scheduling was shown to effectively prime the immune response and patients develop good immune responses and thus the vaccine is immunogenic enough for concomitant treatments with chemotherapy.⁷⁷ The results also indicated that all the patients who had received the highest dose of the vaccine mounted substantial Her-2/neu-specific IgG. In addition to the observed tolerability and safety of the vaccine at the

highest dose, the progression-free survival (PFS) in this cohort was prolonged to 450 days (Figure 3) and was shown to be correlated with vaccine-specific humoral and cellular responses.⁷⁷ Based on clinical laboratory findings along with vital signs, electrocardiograms, and physical examinations no dose-limiting toxicities were observed, indicating the vaccine's safety and good tolerability.⁷⁷ This is in particular a positive important observation, as the association of treatments with trastuzumab and cardiotoxicity poses challenges for both clinicians and patients.¹⁰¹ Based on the success of this evaluation, the highest dose (50 µg) was chosen for further evaluation of the vaccine in an ongoing phase II clinical trial.

This phase II trial has included patients with Her-2/neu-overexpressing metastatic or advanced adenocarcinoma of the stomach or gastroesophageal junction, randomized to those receiving HER-Vaxx plus standard chemotherapy (arm 1) or the standard chemotherapy alone (arm 2).⁷⁸ The interim analysis results have shown that the OS and the PFS rates are higher in patients who received HER-Vaxx plus chemotherapy, with hazard ratios of 0.418 (OS) and 0.532 (PFS), corresponding also with the overall response rates in patients who received the vaccine. Furthermore, patients who received the vaccine plus chemotherapy had more reduction in tumor size compared to the patients who were treated only with chemotherapy, correlating with the observed OS results.⁷⁸

Overall, the results of these trials have shown that HER-Vaxx is safe, as reflected by intensive preclinical toxicity studies in different species which showed no signs of toxicity and inflammation. Moreover, the vaccine is immunogenic, clinically efficacious, and leads to increased OS and PFS rates among vaccinated patients.

B-Vaxx

In a first-in-human phase I clinical trial (NCT01376505; Table 2) involving patients with metastatic solid tumors, the safety and optimal immunologic/biological dose of the vaccine were evaluated (Table 2). The results of the study indicated that the vaccine is well tolerated and able to generate a sustained anti-Her-2/neu immune response. Additionally, the patients' antibodies, generated in response to the vaccine, were shown to possess anti-tumor effects and trigger defense mechanisms *in vitro* (i.e. induction of ADCC and apoptosis, inhibition of proliferation, and phosphorylation).¹⁰²

CONCLUSIONS

In the past decades, the dramatically evolving concept of cancer immunotherapy has been a milestone in the treatment of several types of advanced cancers, including the application of mAbs targeting Her-2/neu-expressing tumors in various cancer entities. However, to overcome the disadvantages associated with such treatments, peptide-based vaccines are progressively becoming promising alternatives, considering the comprehensive involvement of the patient's immune system and also the easier and cheaper means for

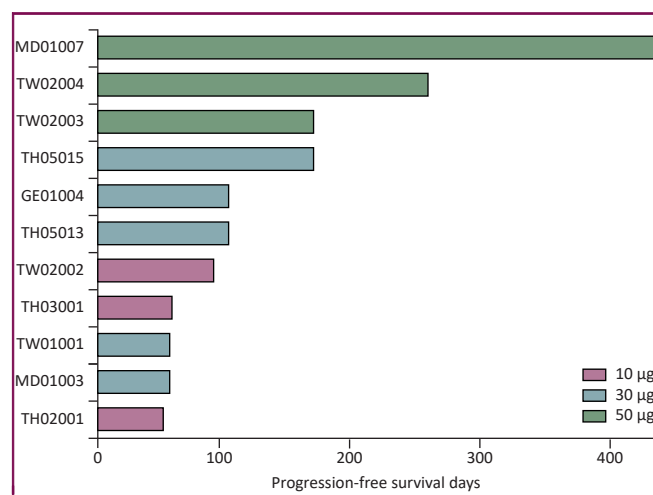


Figure 3. Progression-free survival (in days, swimmer plot per treatment group).

Progression-free survival in days is shown for each patient enrolled in the trial (NCT02795988), following the administration of HER-Vaxx at the indicated doses. With permission from the publishing journal *Clinical Cancer Research*.⁷⁷

their production. Supported by the successful approach of active immunization with HER-Vaxx, a new formulation of the vaccine, broadened to a B-cell-based multi-peptide vaccine including the mimotope of pertuzumab, is aimed for prevention of metastasis and tumor recurrence (i.e. adjuvant setting) (Manuscript in preparation). The breast tumor microenvironment (TME) has been shown to be an important and a long understudied barrier to the efficacy of therapeutic vaccines.¹⁰³ Therefore, combination strategies are being evaluated to add additional strategies for modulating the TME and killing the tumor cells, for example, by combining a tumor-specific cancer vaccine with immune checkpoint inhibitors.^{76,104-107} Our group has taken this concept of combination therapy one step forward and has recently identified the mimotope of the immune checkpoint PD-1 and has shown the enhanced anti-tumor effect of HER-Vaxx in combination with this mimotope^{76,107}; along this line, the combination of a PD-1 peptide (PD1-Vaxx) and B-Vaxx is planned to be evaluated in a clinical trial.¹⁰⁸ Preclinical studies by our group are also ongoing to evaluate the effect of vaccination in the prevention of metastasis development. Based on the results of the clinical trials reviewed here, new therapies against Her-2/neu-expressing cancers are on the horizon. Such immune system-based therapies may potentially pave the way for treatments overcoming the disadvantages of mAbs administration, which could be used as monotherapy, or in combination with the latter. Furthermore, these vaccination strategies will always be carried out in combination with other treatments such as chemotherapy or even with checkpoint inhibitors as described elsewhere,^{76,107} in regimens adapted to the type, stage, and progression phase of the tumor.

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DISCLOSURE

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