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Peptide vaccines in early breast cancer

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ABSTRACT

The immune system plays a dual role of host-protecting and tumor-promoting, as elegantly expressed by the 'cancer immunoediting' hypothesis. Although breast cancer has not been traditionally considered to be immunogenic, recently there is accumulating and solid evidence on the association between immune system and breast cancer. To mount an effective anti-tumor response, host immunosurveillance must recognize tumor-specific epitopes, thus defining the antigenicity of a tumor. Neoantigens are mutant cancer peptides that arise as terminal products of the expression of somatic cancer mutations. Neoantigens and major histocompatibility complex (MHC) proteins present together to effector cells of the immune system. Neoantigen vaccines have shown promising results in inducing neoantigen-specific T-cell responses. Currently, cancer vaccines are under evaluation in breast cancer to avoid recurrences in patients at high risk despite optimal standard therapy. Given the promise of a very specific long-term antitumor immune response, the development of cancer vaccines continues is of great interest. Combinations of neoantigen vaccines and other immunotherapies are also studied to evade cancer immune escape.

1. Introduction

The immune system plays a dual role in cancer: it suppresses tumor growth by destroying cancer cells or impeding their outgrowth, but it also promotes tumor progression by selecting tumor cells more likely to survive in an immunocompetent host or by creating conditions within the tumor microenvironment that may facilitate tumor outgrowth. The immune system's dual roles of host-protecting and tumor-promoting is very elegantly expressed by the 'cancer immunoediting' hypothesis [1].

Although breast cancer has not been traditionally thought to be an immunogenic cancer type, there is accumulating and solid evidence on the association between tumor infiltrating lymphocytes (TILs) and breast cancer [2]. Furthermore, the success of immune checkpoint blockade in other tumor types, and very recently in a small subset of patients with triple negative breast cancer, has provided restorated excitement in the role of immunosurveillance in cancer progression [1,3,4].

Evading immune destruction is recognized as a new hallmark of cancer [5]. Host immunosurveillance must recognize tumor-specific epitopes to mount an effective anti-tumor response, thus defining the antigenicity of a tumor [6]. Neoantigens are mutant cancer peptides that arise as terminal products of the expression of somatic cancer mutations [7,8]. Neoantigens and major histocompatibility complex (MHC) proteins present together to effector cells of the immune system.

2. Breast cancer immunogenicity

2.1. The immunogenicity of breast cancer varies across subtypes

TILs reflect an individual immunological tumor response and may correlate with the neoantigenic tumor load. TILs frequency depends on the breast cancer subtype. Triple-negative breast cancer (TNBC) and HER2+ breast cancer show higher TILs levels as compared to estrogen receptor positive (ER+)/HER2-subtype, thus suggesting these subtypes are more immunogenic [9]. TILs are an independent prognostic factor in TNBC and HER2+ breast cancer, with higher TILs tumors showing better outcome [2]. In addition, TILs may predict sensitivity to systemic therapy with higher TILs levels associated with higher rates of pathological complete response (pCR) in patients treated with neoadjuvant therapy in both TNBC and HER2+ subtypes [2,9]. These data suggest that anti-

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tumor immunity plays a central role in influencing the biology of TNBC and HER2+ breast cancer. ER + breast cancer usually expresses lower TILs levels, and the correlation of TILs with outcome in this subtype is less obvious, thus suggesting that this subtype might be less immunogenic [10]. TILs levels may provide an estimate of tumor immunogenicity. However, the balance between active and suppressed immune responses is influenced by TIL composition [3,11].

The immune-oncology field is becoming increasingly relevant, as immunotherapeutics are being showing more and more satisfactory results.

3. Genomic determinants of immunogenicity and neoantigens

Increased mutational load, which means the load of somatic mutations per tumor, correlates with increased tumor antigenicity [12]. There are differences in mutational load across different cancer types and within different subtypes of the same cancer type. Generally, breast cancer has a significantly lower mutational load than other diseases, such as melanoma and lung cancer. Within breast cancer, there are significant differences in mutational load amongst subtypes [13]. Briefly, a higher mutational load is usually observed in ER-tumors than in ER + tumors [14], confirming differences in TILs described earlier. However, the correlation between mutational load and outcome is not fully understood yet.

In addition to mutational load, some mutational signatures display high mutational loads, and may be enriched for immunogenic mutations. In this context, immunogenicity may be enhanced [15,16]. For instance, germline mutations in BRCA1 and BRCA2 show a typical mutational signature, and correlate with high genomic instability and mutational loads [16,17].

High mutational load likely mirrors higher probability of harboring immunogenic neoantigens. Many studies have described T cell reactivity to cancer-specific neoantigens [8,18–20].

Neoantigens are tumor-specific antigens resulting from somatic DNA alterations. Usually, neoantigens have a high-predicted binding affinity to MHC molecules.

Neoantigens are extremely important in predicting the efficacy of checkpoint blockade [21–24]. A meta-analysis of six tumor types (including breast cancer) examined the effects of mutational load and demonstrated that neoantigens correlate with improved overall survival, regardless of clinicopathological prognostic factors [25]. Patients with high neoantigen load were those with higher expression of checkpoints PD1 and CTLA4, thus emphasizing that these patients may be the ideal candidates for immunecheckpoint blockade. Neoantigen load generally reflects mutational load [26]. Hence, it is reasonable to hypothesize that ER-tumors may harbor more neoantigens than ER + tumors. Moreover, neoantigen clonality has to be considered. Reduced neoantigen intratumoural heterogeneity seems to be associated with better survival and increased sensitivity to immunecheckpoint blockade, thus suggesting that clonal neoantigens may be more important than subclonal neoantigens [27].

Prediction of neoantigens is difficult but useful to evaluate the probability of a tumor-specific epitope to bind to antigen presenting MHC class I molecules at high affinity [19,24,28,29]. Since only a trivial proportion (4–6%) of predicted neoantigens proves T cell response in functional studies, testing candidate neoantigens for T cell reactivity from patients' own TILs is necessary to define a true neoantigen [26]. Such a process is constantly evolving and has generated new interest in generating personalized vaccines. The mutational landscape of breast cancer is complex as demonstrated by genomic analyses [15].

Cancer neoantigen identification has been made possible by advances in next-generation sequencing (NGS) technologies, which allow fast and cost-effective comparisons between tumor and normal sequences [30]. Some somatic mutations detected by DNA sequencing determine altered amino acid sequences, which have to be effectively translated and then processed into short peptide fragments and presented on the cell surface in the context of MHC molecules to be recognized by the immune system. Antigen processing and presentation is a complex and multi-step process that can impact neoantigen presentation [31]. Antigen processing varies across MHC class I and class II molecules. MHC class I molecules have 8–10 amino acid peptides created endogenously or acquired by specialized antigen presenting cells. MHC class II molecules have 11–20 amino acids peptides resulted from exogenous proteins. The expression of the somatic mutation at the protein level, the processing of the mutant protein into an appropriate peptide for presentation, the binding affinity of the mutant peptide to the patient's autologous MHC molecules and the affinity of the mutant peptide/MHC complex to the TCR of responding T cells are all factors that may influence the likelihood of a somatic mutation of creating a neoantigen. However, the majority of somatic mutations detected by NGS do not result in real neoantigens [32].

Adequate prediction and prioritization of neoantigen candidates is challenging because of the huge amount of somatic mutations, and the intrinsic polymorphism of human MHC molecules. Several computational tools have been developed to predict peptide–MHC binding [33–38]. The perfect neoantigen prediction approach should evaluate proteasomal cleavage, MHC binding, TCR recognition, to assess the potential immunogenicity of neoantigen candidates.

4. Clinical trials in breast cancer

The progresses in neoantigen determination have led to increased interest in developing precision immunotherapy, such as personalized vaccines, which appear to be a feasible therapeutic strategy [39].

Cancer vaccines targeting neoantigens have created great interest, due to the potential advantages of targeting protein sequences not present in normal tissue including diminished central immune tolerance, and better safety profile. Cancer vaccines aim at eliciting robust antitumor immune responses.

There are several clinical trials with personalized vaccines for patients with breast cancer (Table 1), in the neoadjuvant and adjuvant settings.

In high-risk breast cancers, including TNBC subtype, cancer vaccines could play a role in preventing relapse. Many trials are currently ongoing and results are still pending.

A phase I clinical trial (NCT02348320) is currently recruiting patients with persistent disease after neoadjuvant chemotherapy for TNBC to evaluate the safety and immunogenicity of a personalized polyepitope DNA vaccine strategy. The personalized polyepitope DNA vaccines are formulated as naked plasmid DNA vaccines. The hypothesis of this study is that personalized polyepitope DNA vaccines will be safe for human administration and capable of generating measurable CD8 T cell responses to mutant tumor-specific antigens. The study aims at enrolling 30 patients, who will be treated by electroporation with 4 mg of a personalized polyepitope DNA vaccine at day 1, day 29 (+/1–7 days), and day 57 (±7 days) with at least 21 days between injection days. Each DNA vaccination with be 4 mg vaccine administered intramuscularly using a TriGrid electroporation device. Primary outcome of the study is the safety of the personalized polyepitope DNA vaccine strategy, measured by both clinical observation and laboratory evaluation. Assessment of plasmid DNA safety will include both clinical observation and laboratory evaluation. Safety will be closely

Table 1
Clinical trials with vaccines for early breast cancer.

Trials in the adjuvant setting						
Setting	Agent	NCT number	Status	Phase	Patients enrolled	Experimental treatment
Radically resected, HER2 low-intermediate, node positive BC	NeuVax™ (Nelipepimut-S or E75 with Leukine® [sargramostim, GM-CSF])	NCT01479244 (PRESENT)	C	III	758	NeuVax™ vs Leukine® alone once a month, for six consecutive months, and then every six months for a total of 36 months
Radically resected, low and intermediate HER2-expressing BC	NeuVax™	NCT01570036	ANR	II	300	Trastuzumab + NeuVax vaccine vs Trastuzumab + GM-CSF (sargramostim) for a total of 30 months
Radically resected, high-risk HER2 + BC (early-stage node positive disease)	allogeneic GM-CSF-secreting breast cancer vaccine	NCT00847171	C	II	13	Trastuzumab + Cyclophosphamide + allogeneic GM-CSF-secreting whole cell breast cancer vaccine
Radically resected, node positive or high risk HER2 positive BC	HER-2/neu peptide GP2 + GM-CSF or AE37 peptide + GM-CSF	NCT00524277	U	II	600	GP2 peptide + GM-CSF vaccine in HLA-A2+ patients vs GM-CSF alone in HLA-A2+ patients vs AE37 peptide + GM-CSF vaccine in HLA-A2- patients vs GM-CSF alone in HLA-A2- patients (given every 3–4 weeks up to 6 inoculations)
Radically resected, stage I/II/III TNBC	Vaccination with Folate Receptor Alpha Peptides (FRα vaccine) with GM-CSF	NCT02593227	ANR	II	80	Low dose FRα vaccine vs High dose FRα vaccine either alone or in combination with intravenous cyclophosphamide
Stage II/III HER2+ BC or stage IV HER2+ BC treated to NED or stable bone only disease	HER-2/neu peptide vaccine	NCT01355393	ANR	I/II	50	HER-2/neu peptide vaccine + rintatolimod vs HER-2/neu peptide vaccine and sargramostim vs HER-2/neu peptide vaccine + sargramostim + rintatolimod
Early stage, node positive, HER2 negative BC	pUMVC3-IGFBP2-HER2-IGF1R Plasmid DNA Vaccine (WOKVAC)	NCT02780401	R	I	30	WOKVAC with sargramostim every 28 days for up to 3 courses
Stage IIIA HER2 + BC or greater or recurrent stage IV HER2 + BC treated to NED	HER-2 pulsed Dendritic Cell Vaccine	NCT02063724	ANR	I	15	6 weekly HER-2 pulsed dendritic cell vaccines followed by 3 booster vaccines once every 3 months, within 1 year of completion of standard adjuvant therapy
Radically resected, stage II/III TNBC; Human Leukocyte Antigen (HLA)-A2+ Subjects	PVX-410	NCT02826434	R	I	20	6 bi-weekly PVX-410 with Durvalumab for 2 infusions
Radically resected, stage II or III BC	Autologous vaccination with lethally irradiated, autologous breast cancer cells engineered by adenoviral mediated gene transfer to secrete GM-CSF	NCT00880464	ANR	I	8	Vaccine administered on days 1, 8, 15, 29 and then every 2 weeks until the supply of vaccine runs out
Radically resected HER2+ BC	MVA-BN-HER2	NCT01152398	C	I	15	6 monthly vaccine injections after completion of standard adjuvant therapy
Radically resected HER2+ BC	Combination immunotherapy with HER2/Neu Peptide GP2 + GM-CSF Vaccine	NCT03014076	C	Ib	30	HLA-A2+/A3+ subjects receive GP2 + GM-CSF vaccine and trastuzumab; HLA-A2-/A3- patients receive only trastuzumab and are followed as controls
Radically resected stage II/III, HER2 + BC	HER-2/neu peptide vaccine	NCT01632332	C	I	22	HER-2/neu peptide vaccine every 28 days for up to 6 courses after completion of standard adjuvant treatment
Radically resected TNBC	IVAC_W_bre1_uID IVAC_W_bre1_uID/ IVAC_M_uID	NCT02316457 (TNBC-MERIT)	R	I	39	IVAC_W_bre1_uID vaccination vs IVAC_W_bre1_uID/IVAC_M_uID vaccination vs IVAC_W_bre1_uID vaccination + RBLTet.1
Radically resected, stage I/II/III TNBC	MUCI Peptide and Poly-ICLC Vaccine	NCT00986609	C	I (pilot study)	29	MUCI Peptide + Poly-ICLC Vaccine
TRIALS IN THE POST-NEOADJUVANT SETTING						
TNBC with residual disease post neoadjuvant therapy	FRα vaccine + sargramostim	NCT03012100	R	II	280	FRα vaccine + sargramostin + oral cyclophosphamide vs placebo + sargramostin + oral cyclophosphamide
HER2+ BC with residual disease post neoadjuvant therapy	NeuVax™	NCT02297698	R	II	100	Trastuzumab + NeuVax (for a total of 24 months) vs Trastuzumab + Sargramostim (for a total of 24 months)
HER2+ BC with residual disease post neoadjuvant therapy	Dendritic Cell (DC1) Vaccine/ pUMVC3-IGFBP2-HER2-IGF1R (WOKVAC Vaccine)	NCT03384914	R	II	110	DC1 vaccine vs WOKVAC vaccine administered for a total of 12 months after completion of standard treatment
TNBC with residual disease post neoadjuvant therapy	Neoantigen DNA Vaccine	NCT03199040	R	I	24	Neoantigen DNA Vaccine alone vs Neoantigen DNA Vaccine plus Durvalumab after completion of standard of care therapy
HER2 negative BC at high-risk of relapse (residual disease or node positive after neoadjuvant chemotherapy	Synthetic Multiple Antigenic Glycopeptide Displaying a Tri Tn Glycotop (MAG-Tn3) Plus AS15	NCT02364492	R	I	30	3 escalating doses of MAG-Tn3 in combination with a fixed dose of AS15 adjuvant.

Table 1 (*continued*)

Trials in the adjuvant setting						
Setting	Agent	NCT number	Status	Phase	Patients enrolled	Experimental treatment
TNBC with residual disease post neoadjuvant therapy	Personalized polyepitope DNA vaccine	NCT02348320	R	I	30	4 mg vaccine at day 1, 29, 57 administered using a TriGrid electroporation device
TNBC with residual disease post neoadjuvant therapy	Poly-ICLC Vaccine	NCT02427581	S	I	15	Poly-ICLC Vaccine on days 1, 4, 8, 15, 22, 50, 78
TRIALS IN THE NEOADJUVANT SETTING						
Stage II and III HER2 negative BC patients (luminal A and triple negative)	Autologous Dendritic Cell Vaccination	NCT01431196	C	II	29	Neoadjuvant chemotherapy (Dose dense EC x 4 → Docetaxel x 4) plus Autologous Dendritic Cell Vaccination
Stage II/III TNBC	P10s-PADRE with MONTANIDE™ ISA 51 VG [Carbohydrate Mimotope-based Vaccine]	NCT02938442	NYR	II	102	Standard neoadjuvant chemotherapy alone or in combination with P10s-PADRE vaccine.
Stage II/III TNBC	NANT NEOADJUVANT Triple-Negative Breast Cancer VACCINE	NCT03554109 (QUILT-3.057)	NYR	II	376	NANT NEOADJUVANT Triple-Negative Breast Cancer plus a combination of agents vs dose dense neoadjuvant AC → paclitaxel
Early ER+, HER2 negative BC	Mammaglobin-A DNA Vaccine	NCT02204098	R	I	60	Neoadjuvant endocrine therapy + Mammaglobin-A DNA Vaccine vs endocrine therapy alone
p53-Overexpressing stage III BC {adjuvant and neoadjuvant}	Autologous dendritic cell-adenovirus p53 vaccine	NCT00082641	ANR	I/II	24	Arm I (neoadjuvant): dose dense AC x 4 → paclitaxel x 4 plus a total of 4 vaccinations during and after neoadjuvant chemotherapy Arm II (Adjuvant): vaccination at 6, 8, 10, and 12 weeks after completion of radiotherapy.

ANR: Active, not recruiting; AC: doxorubicin + cyclophosphamide; BC: Breast Cancer; C: Completed; EC: Epirubicin + Ciclofosfamide; ER: Estrogen Receptor; NED: No evidence of Disease; NYR: Not yet recruiting; R: Recruiting; S: Suspended; TNBC: Triple Negative Breast Cancer; U: unknown.

monitored after injection with eight or more clinical and laboratory assessments in the first 24 weeks of the trial. Local and systemic signs and symptoms, laboratory evaluations, adverse and serious adverse events will be assessed following vaccination.

In a phase I clinical trial (NCT01532960), a vaccine consisting of 9 MHC class I-restricted breast cancer-associated peptides (from MAGE-A1, -A3, and -A10, CEA, NY-ESO-1, and HER2 proteins), combined with a TLR3 agonist, poly-ICLC, along with a helper peptide derived from tetanus toxoid, was evaluated in 11 early breast cancer patients after completion of their standard adjuvant treatment (except for endocrine therapy in specific cases). Almost all patients experienced injection site reaction/induration, flu-like symptoms and fatigue. The TLR3 adjuvant, poly-ICLC, plus helper peptide mixture provided a modest immune stimulation: $CD8^+$ T cell responses to the vaccine were assessed by stimulated interferon gamma ELIspot assays and were observed in 4 out of 11 patients [40].

NeuVax™ is the vaccine at the most advanced stage of development [41]. It is a major histocompatibility complex (MHC) class I vaccine that consists of the HER2-derived peptide E75 (nelipepimut-S) combined with the immunoadjuvant granulocyte macrophage colony-stimulating factor (GM-CSF).

Two early-phase clinical trials evaluated the safety and efficacy of E75 + GM-CSF administered in the adjuvant setting. The first study was a standard dose escalation study, which enrolled patients with node-positive breast cancer. The second study was a dose and schedule optimization trial, which enrolled high-risk node-negative patients. Both trials included patients whose tumors expressed any degree of HER2. All patients had completed standard-of-care therapy and were disease free upon enrollment. Patients on endocrine treatment continued on that therapy. At the time of enrollment, HLA-A2 status was determined for each patient, and those who were HLA-A2+ were vaccinated, while those who were HLA-A2- were followed prospectively as controls. Patients received escalating doses of E75 with GM-CSF immunoadjuvant every month for 4 or 6 months. The vaccine stimulated an antigen-specific immune response and was well tolerated at all dose levels, with negligible toxicity [42]. Hence, the development moved to phase II trials.

The trials enrolled 195 patients, 100 with node-positive, and 95 with node-negative disease. These patients were followed for 60 months, and the primary endpoint was disease-free survival (DFS). Among the 187 evaluable patients, 108 received the vaccine and 79 did not but were followed as controls. The two groups were well matched, except for the higher percentage of ER-patients in the vaccinated group [43]. The vaccine was well tolerated, with mild toxicities. Local toxicities, usually injection site erythema and pruritus, were of grade 1 (83.3%) and grade 2 (16.7%). Systemic toxicities, most frequently bone pain, flu-like symptoms, and fatigue, were trivial. Only 1.9% of patients experienced grade 3 toxicities, and no greater than grade 3 toxicities were observed. The 5-year DFS was 89.7% in vaccinated patients and 80.2% in unvaccinated controls ($p = 0.08$), with a 48% reduction in relative risk of recurrence with the vaccine [43]. The optimal biologic dose was the maximal dose administered (1000 μg E75 with 250 μg GM-CSF).

Patients with low-HER2-expressing tumors (IHC 1 + or 2+) had the strongest immune responses [44], and derived benefit from vaccination, with a 5-year DFS of 88.1% in vaccinated patients vs 77.5% in unvaccinated controls ($p = 0.16$), with a relative risk reduction of 48% [43].

Among patients who benefited the most from vaccination, there were the 37 patients who received the optimal biologic dose (5-year DFS 94.6% vs 80.2% in unvaccinated controls, $p = 0.05$) [43], and those patients with grade 1 or 2 breast cancer (5-year DFS rate 96.7% in low-grade vaccinated patients vs 80.9% in low-grade control patients, relative risk reduction = 84%, $p = 0.01$) [45].

NeuVax™ is currently being evaluated in a phase III registration trial, the PRESENT trial (NCT01479244). This study enrolled patients with HLA-A2+/A3+, node-positive, HER2 IHC 1+, 2 + breast cancer who had no evidence of disease after standard treatment. Patients were randomized to receive either E75 + GM-CSF or GM-CSF alone. A 6-inoculation primary series was followed by booster inoculations every 6 months through 3 years. The trial's primary endpoint is 3-year DFS.

4.1. Combination immunotherapy: trastuzumab and vaccine

Both cellular and humoral anti-neu immune responses are needed to eliminate HER2/neu-expressing tumors [46–48]. Anti-HER2 antibody-induced tumor regression is T cell dependent [49]. Moreover, antibody-dependent cellular cytotoxicity mediated by natural killer cells is a known mechanism of action of trastuzumab, which is an IgG antibody with a conserved Fc portion. Antibody-dependent cellular cytotoxicity induces tumor cell lysis with consequent release of antibody-coated tumor antigens. Dendritic cells take these antibody-coated tumor antigens up and present them on MHC class I molecules through the cross-presentation process. Hence, trastuzumab effectively turns the tumor into a vaccine [50].

In brief, trastuzumab augments tumor cells susceptibility to lysis by vaccine-generated $CD8^+$ T cells, suggesting possible benefit of an immunotherapy combination strategy [51–54].

A small study of patients who received trastuzumab for metastatic HER2-positive breast cancer demonstrated the generation of HER2-specific $CD4^+$ T cell responses as well as anti-HER2 antibody responses that augmented significantly on therapy, and were associated with improved clinical response [54].

In early-stage E75 trials, 12 patients with HER2 IHC 3 + breast cancer received standard-of-care trastuzumab, which was then followed by vaccination. None of these patients recurred after 5 years of follow-up [55], thus supporting the potential synergy between trastuzumab and a $CD8^+$ T cell-eliciting vaccine. A second MHC class I peptide derived from the HER2 protein that stimulates a $CD8^+$ T cell response, GP2, has been evaluated in phase I and II clinical trials [56]. At a median follow-up of 34 months, in HER2-overexpressing patients, DFS was 100% in patients who received the vaccine after trastuzumab ($n = 48$) and 89% in control patients ($n = 50$) ($p = 0.08$). The combination of trastuzumab and a vaccine designed to elicit a HER2-specific helper T cell response is safe without cardiac toxicity in phase I trials [57].

Since the combination of trastuzumab and an MHC class I, $CD8^+$ T cell-eliciting vaccine was proven safe, this strategy has been further evaluated in two phase II adjuvant therapy trials. One of these studies (NCT02297698) randomizes patients with high-risk HER2+ breast cancer to receive trastuzumab ± the E75 + GM-CSF vaccine. This study includes patients with residual disease after optimal neoadjuvant therapy, or, for those undergoing surgery as first intervention, those with nodal involvement (any positive lymph nodes for hormone receptor-negative tumors, ≥ 4 positive lymph nodes for patients with hormone receptor-positive tumors). The second study (NCT01570036) randomized 275 patients with HER2 IHC 1 + or 2 + tumors, a group that does not receive HER2-targeted therapy as standard treatment, to receive trastuzumab + the E75 + GM-CSF vaccine (NeuVax) or trastuzumab + GM-CSF. All pts received one year of trastuzumab. NeuVax or GM-CSF was given every 3 weeks for six times starting with the third dose of trastuzumab, then boosted every six months for 4 times. Findings of a prespecified interim analysis of this randomized phase IIb study have been presented at the 2018 ESMO annual meeting. The activity of the NeuVax vaccine/trastuzumab combination in reducing recurrence of HER2 low-expressing, node positive and/or TNBC was compared with trastuzumab plus GM-CSF in 275 patients. The primary endpoint was DFS at 24 months. In patients with HER2 low-expressing TNBC, estimated 24-month DFS was 91.1% in the vaccine arm and 69.9% in the control arm (hazard ratio 0.26; $p = 0.02$). This level of significance was not replicated in the overall study population or in node-positive patients. Both regimens appeared to be well tolerated; there were no grade 4–5 adverse events and no between-treatment differences in cardiac, local or systemic toxicities.

Another dose-escalation trial enrolling patients with no evidence of disease, HLA-A2+ or A3+, HER2-positive breast cancer has been conducted [58].

Patients received every month for six months inoculations of the GP2 (HER2: 654–662) peptide vaccine, which is comprised of the HLA-A2- and A3-restricted, HER2-derived peptide GP2 combined with GM-CSF, administered concurrently with trastuzumab. Local and systemic toxicity, including cardiac toxicity, were regularly monitored. Immunologic responses were evaluated *in vitro* by using an IFN-γ ELISPOT assay and *in vivo* by measuring the local reaction. Seventeen patients received the vaccine. No dose limiting nor greater than grade 2 toxicities were observed. The median left ventricular ejection fraction (LVEF) did not change from baseline after vaccination. Mean local reaction at first injection was 28 ± 10 mm and increased to 68 ± 8 mm at the final injection ($p < 0.01$). Mean ELISPOT response to GP2 increased from 47 ± 19 at baseline to 144 ± 60 ($p = 13$) after vaccination. According to safety and immunologic data, the appropriate dose was found to be 1000 μg of GP2 + 250 μg of GM-CSF. Based on these results, the GP2+GM-CSF vaccine demonstrated to be safe and to stimulate an immunologic response when given concurrently with trastuzumab, which was strongest in patients without a pre-existing immunity to HER2.

A combination of trastuzumab and $CD8^+$ T cell-eliciting HER peptide vaccine may induce synergistic immunotherapeutic benefit in patients with HER2+ breast cancer.

These positive results prompted the initiation of a randomized phase II trial (NCT00524277), evaluating the efficacy of combining a CD8 T cell-eliciting vaccine with trastuzumab in HER2-positive breast cancer patients. Patients were treated with either GP2 peptide + GM-CSF vaccine, GM-CSF (sargramostim), or AE37 (another cancer vaccine) + GM-CSF vaccine. This trial is ongoing but no longer recruiting participants. At ASCO 2014 Breast Cancer Symposium the initial results from 190 women with breast cancer were presented. Among the 182 patients out of 190 who completed the trial, disease-free survival (DFS) was 94% in patients treated with the vaccine vs 85% in patients treated with trastuzumab only, suggesting that treatment with GP2 plus GM-CSF along with trastuzumab may reduce the risk of recurrence.

5. Challenges

Despite huge improvements, the development of neoantigen vaccines is still challenging. Time to manufacture neoantigen vaccines is still quite long, from tissue acquisition to vaccine delivery it may take from 3 to 5 months [59,60]. Identification and validation of candidate neoantigens as well as manufacture of neoantigen vaccines under good manufacturing practice conditions are still very expensive [30]. Furthermore, neoantigen prediction algorithms need further optimization, as $CD4^+$ T-cell responses to neoantigen vaccines are more frequent than $CD8^+$ T-cell responses, even if the neoantigens included in the vaccines were prioritized based on predicted MHC class I binding [59,60].

Immunotherapies are evaluated using immune-related response criteria (irRC) [61,62]. Besides respecting these immune-related response criteria, neoantigen vaccines have to rely on effective immune monitoring to evaluate vaccine-induced immune responses before clinical end points are obtained.

Conclusions

Neoantigen vaccines have shown very promising results in inducing neoantigen-specific T-cell responses. First, they have been meticulously tested in phase I clinical trials. Then, they have demonstrated encouraging results in various phase II clinical trials,

and currently cancer vaccines are being tested in phase III trials, in order to prevent breast cancer recurrences in patients at high risk of recurrence after optimal standard of care therapy. Given the promise of a very specific long-term antitumor immune response, the development of cancer vaccines continues to be of great interest. Combinations of neoantigen vaccines and other immunotherapies is key to evade the cancer immune escape.

References

[1] Schreiber RD, Old LJ, Smyth MJ. Cancer immunoediting: integrating immunity's roles in cancer suppression and promotion. Science 2011;331:1565–70.
[2] Criscitiello C, Esposito A, Trapani D, et al. Prognostic and predictive value of tumor infiltrating lymphocytes in early breast cancer. Cancer Treat Rev 2016;50:205–7.
[3] Kroemer G, Senovilla L, Galluzzi L, et al. Natural and therapy-induced immunosurveillance in breast cancer. Nat Med 2015;21:1128–38.
[4] Schmid P, Adams S, Rugo HS, et al. Atezolizumab and nab-paclitaxel in advanced triple-negative breast cancer. N Engl J Med 2018.
[5] Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell 2011;144:646–74.
[6] Luen S, Virassamy B, Savas P, et al. The genomic landscape of breast cancer and its interaction with host immunity. Breast 2016;29:241–50.
[7] Nalbandian G, Paharkova-Vatchkova V, Mao A, et al. The selective estrogen receptor modulators, tamoxifen and raloxifene, impair dendritic cell differentiation and activation. J Immunol 2005;175:2666–75.
[8] Svensson S, Abrahamsson A, Rodriguez GV, et al. CCL2 and CCL5 are novel therapeutic targets for estrogen-dependent breast cancer. Clin Cancer Res 2015;21:3794–805.
[9] Solinas C, Carbognin L, De Silva P, et al. Tumor-infiltrating lymphocytes in breast cancer according to tumor subtype: current state of the art. Breast 2017;35:142–50.
[10] Dieci MV, Griguolo G, Miglietta F, et al. The immune system and hormone-receptor positive breast cancer: is it really a dead end? Cancer Treat Rev 2016;46:9–19.
[11] Lim E, Wu D, Pal B, et al. Transcriptome analyses of mouse and human mammary cell subpopulations reveal multiple conserved genes and pathways. Breast Cancer Res 2010;12:R21.
[12] Joffroy CM, Buck MB, Stope MB, et al. Antiestrogens induce transforming growth factor beta-mediated immunosuppression in breast cancer. Cancer Res 2010;70:1314–22.
[13] Vogelstein B, Papadopoulos N, Velculescu VE, et al. Cancer genome landscapes. Science 2013;339:1546–58.
[14] Haricharan S, Bainbridge MN, Scheet P, et al. Somatic mutation load of estrogen receptor-positive breast tumors predicts overall survival: an analysis of genome sequence data. Breast Canc Res Treat 2014;146:211–20.
[15] Stephens PJ, Tarpey PS, Davies H, et al. The landscape of cancer genes and mutational processes in breast cancer. Nature 2012;486:400–4.
[16] Nik-Zainal S, Alexandrov LB, Wedge DC, et al. Mutational processes molding the genomes of 21 breast cancers. Cell 2012;149:979–93.
[17] Chan MS, Wang L, Felizola SJ, et al. Changes of tumor infiltrating lymphocyte subtypes before and after neoadjuvant endocrine therapy in estrogen receptor-positive breast cancer patients–an immunohistochemical study of Cd8+ and Foxp3+ using double immunostaining with correlation to the pathobiological response of the patients. Int J Biol Mark 2012;27:e295–304.
[18] Castle JC, Kreiter S, Diekmann J, et al. Exploiting the mutanome for tumor vaccination. Cancer Res 2012;72:1081–91.
[19] Robbins PF, Lu YC, El-Gamil M, et al. Mining exomic sequencing data to identify mutated antigens recognized by adoptively transferred tumor-reactive T cells. Nat Med 2013;19:747–52.
[20] Wick DA, Webb JR, Nielsen JS, et al. Surveillance of the tumor mutanome by T cells during progression from primary to recurrent ovarian cancer. Clin Cancer Res 2014;20:1125–34.
[21] Alexandrov LB, Nik-Zainal S, Wedge DC, et al. Signatures of mutational processes in human cancer. Nature 2013;500:415–21.
[22] Rizvi NA, Hellmann MD, Snyder A, et al. Cancer immunology. Mutational landscape determines sensitivity to PD-1 blockade in non-small cell lung cancer. Science 2015;348:124–8.
[23] Snyder A, Makarov V, Merghoub T, et al. Genetic basis for clinical response to CTLA-4 blockade in melanoma. N Engl J Med 2014;371:2189–99.
[24] van Rooij N, van Buuren MM, Philips D, et al. Tumor exome analysis reveals neoantigen-specific T-cell reactivity in an ipilimumab-responsive melanoma. J Clin Oncol 2013;31:e439–42.
[25] Brown SD, Warren RL, Gibb EA, et al. Neo-antigens predicted by tumor genome meta-analysis correlate with increased patient survival. Genome Res 2014;24:743–50.
[26] Rajasagi M, Shukla SA, Fritsch EF, et al. Systematic identification of personal tumor-specific neoantigens in chronic lymphocytic leukemia. Blood 2014;124:453–62.
[27] McGranahan N, Furness AJ, Rosenthal R, et al. Clonal neoantigens elicit T cell immunoreactivity and sensitivity to immune checkpoint blockade. Science 2016;351:1463–9.
[28] Segal NH, Parsons DW, Peggs KS, et al. Epitope landscape in breast and colorectal cancer. Cancer Res 2008;68:889–92.
[29] Heemskerk B, Kvistborg P, Schumacher TN. The cancer antigenome. EMBO J 2013;32:194–203.
[30] Li L, Goedegebuure P, Mardis ER, et al. Cancer genome sequencing and its implications for personalized cancer vaccines. Cancers 2011;3:4191–211.
[31] Blum JS, Wearsch PA, Cresswell P. Pathways of antigen processing. Annu Rev Immunol 2013;31:443–73.
[32] Schumacher TN, Schreiber RD. Neoantigens in cancer immunotherapy. Science 2015;348:69–74.
[33] Cherryholmes GA, Stanton SE, Disis ML. Current methods of epitope identification for cancer vaccine design. Vaccine 2015;33:7408–14.
[34] Desai DV, Kulkarni-Kale U. T-cell epitope prediction methods: an overview. Methods Mol Biol 2014;1184:333–64.
[35] Patronov A, Doytchinova I. T-cell epitope vaccine design by immunoinformatics. Open Biol 2013;3:120139.
[36] Singh SP, Mishra BN. Major histocompatibility complex linked databases and prediction tools for designing vaccines. Hum Immunol 2016;77:295–306.
[37] Backert L, Kohlbacher O. Immunoinformatics and epitope prediction in the age of genomic medicine. Genome Med 2015;7:119.
[38] Soria-Guerra RE, Nieto-Gomez R, Govea-Alonso DO, et al. An overview of bioinformatics tools for epitope prediction: implications on vaccine development. J Biomed Inf 2015;53:405–14.
[39] van der Burg SH, Arens R, Ossendorp F, et al. Vaccines for established cancer: overcoming the challenges posed by immune evasion. Nat Rev Canc 2016;16:219–33.
[40] Dillon PM, Petroni GR, Smolkin ME, et al. A pilot study of the immunogenicity of a 9-peptide breast cancer vaccine plus poly-ICLC in early stage breast cancer. J Immunother Cancer 2017;5:92.
[41] Clifton GT, Gall V, Peoples GE, et al. Clinical development of the E75 vaccine in breast cancer. Breast Care 2016;11:116–21.
[42] Peoples GE, Gurney JM, Hueman MT, et al. Clinical trial results of a HER2/neu (E75) vaccine to prevent recurrence in high-risk breast cancer patients. J Clin Oncol 2005;23:7536–45.
[43] Mittendorf EA, Clifton GT, Holmes JP, et al. Final report of the phase I/II clinical trial of the E75 (nelipepimut-S) vaccine with booster inoculations to prevent disease recurrence in high-risk breast cancer patients. Ann Oncol 2014;25:1735–42.
[44] Benavides LC, Gates JD, Carmichael MG, et al. The impact of HER2/neu expression level on response to the E75 vaccine: from U.S. Military Cancer Institute Clinical Trials Group Study I-01 and I-02. Clin Cancer Res 2009;15:2895–904.
[45] Clifton GT, Mittendorf EA, Peoples GE. Adjuvant HER2/neu peptide cancer vaccines in breast cancer. Immunotherapy 2015;7:1159–68.
[46] Reilly RT, Gottlieb MB, Ercolini AM, et al. HER-2/neu is a tumor rejection target in tolerized HER-2/neu transgenic mice. Cancer Res 2000;60:3569–76.
[47] Reilly RT, Machiels JP, Emens LA, et al. The collaboration of both humoral and cellular HER-2/neu-targeted immune responses is required for the complete eradication of HER-2/neu-expressing tumors. Cancer Res 2001;61:880–3.
[48] Wolpoe ME, Lutz ER, Ercolini AM, et al. HER-2/neu-specific monoclonal antibodies collaborate with HER-2/neu-targeted granulocyte macrophage colony-stimulating factor secreting whole cell vaccination to augment CD8+ T cell effector function and tumor-free survival in Her-2/neu-transgenic mice. J Immunol 2003;171:2161–9.
[49] Park S, Jiang Z, Mortenson ED, et al. The therapeutic effect of anti-HER2/neu antibody depends on both innate and adaptive immunity. Cancer Cell 2010;18:160–70.
[50] Ferris RL, Jaffee EM, Ferrone S. Tumor antigen-targeted, monoclonal antibody-based immunotherapy: clinical response, cellular immunity, and immunoescape. J Clin Oncol 2010;28:4390–9.
[51] Dhodapkar KM, Krasovsky J, Williamson B, et al. Antitumor monoclonal antibodies enhance cross-presentation ofcCellular antigens and the generation of myeloma-specific killer T cells by dendritic cells. J Exp Med 2002;195:125–33.
[52] Rafiq K, Bergtold A, Clynes R. Immune complex-mediated antigen presentation induces tumor immunity. J Clin Invest 2002;110:71–9.
[53] Knutson KL, Clynes R, Shreeder B, et al. Improved survival of HER2+ breast cancer patients treated with trastuzumab and chemotherapy is associated with host antibody immunity against the HER2 intracellular domain. Cancer Res 2016;76:3702–10.
[54] Taylor C, Hershman D, Shah N, et al. Augmented HER-2 specific immunity during treatment with trastuzumab and chemotherapy. Clin Cancer Res 2007;13:5133–43.
[55] Mittendorf EA, Clifton GT, Holmes JP, et al. Clinical trial results of the HER-2/neu (E75) vaccine to prevent breast cancer recurrence in high-risk patients: from US Military Cancer Institute Clinical Trials Group Study I-01 and I-02. Cancer 2012;118:2594–602.
[56] Carmichael MG, Benavides LC, Holmes JP, et al. Results of the first phase 1 clinical trial of the HER-2/neu peptide (GP2) vaccine in disease-free breast cancer patients: United States Military Cancer Institute Clinical Trials Group Study I-04. Cancer 2010;116:292–301.
[57] Disis ML, Wallace DR, Gooley TA, et al. Concurrent trastuzumab and HER2/neu-specific vaccination in patients with metastatic breast cancer. J Clin Oncol 2009;27:4685–92.
[58] Clifton GT, Litton JK, Arrington K, et al. Results of a phase ib trial of

combination immunotherapy with a CD8+ T cell eliciting vaccine and trastuzumab in breast cancer patients. Ann Surg Oncol 2017;24:2161–7.
[59] Ott PA, Hu Z, Keskin DB, et al. Corrigendum: an immunogenic personal neoantigen vaccine for patients with melanoma. Nature 2018;555:402.
[60] Sahin U, Derhovanessian E, Miller M, et al. Personalized RNA mutanome vaccines mobilize poly-specific therapeutic immunity against cancer. Nature 2017;547:222–6.
[61] Hoos A. Development of immuno-oncology drugs - from CTLA4 to PD1 to the next generations. Nat Rev Drug Discov 2016;15:235–47.
[62] Wolchok JD, Hoos A, O'Day S, et al. Guidelines for the evaluation of immune therapy activity in solid tumors: immune-related response criteria. Clin Cancer Res 2009;15:7412–20.