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Tumour Review

Breast cancer vaccines: Heeding the lessons of the past to guide a path forward

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ABSTRACT

The ability of cancer immunotherapy to generate lasting responses in a broad spectrum of tumors has generated great enthusiasm in medical oncology. A number of new immune-based compounds have now been approved based on the recent success of immune checkpoint blockade, either administered as monotherapy or in combination with other agents. Because clinical activity is limited only to subsets of patients, two major goals of cancer immunotherapy are (1) to reliably identify responders to these current treatments, and (2) to increase the number of patients who can respond to immunotherapy by developing new strategies. These goals are critically important since the hallmark of immune-based therapies is the induction of durable immunologic and clinical responses that result in overall survival benefit. Innovative combination strategies have great potential for bringing the benefit of immunotherapy to more patients. The use of cancer vaccines to actively induce immune effectors together with other drugs, which may include immune checkpoint blockade, chemotherapy, and/or molecularly targeted agents, is a particularly attractive strategy. Cancer vaccines have been tested both to prevent or intercept the development of cancer, and to decrease established tumor burdens. No vaccine has yet been approved for either breast cancer treatment or prevention. Here, we review the history of breast cancer vaccine development, and highlight near-term opportunities for moving forward.

Introduction

Cancer vaccines represent an active immunotherapy designed to stimulate the patient's immune system to recognize and kill tumor cells. Optimally, cancer vaccines stimulate type 1 $CD4^+$ and $CD8^+$ T cell responses against tumor-associated antigens (Ags) (TAAs) and/or tumor specific Ags (TSAs) [1,2]. The main classes of TAAs and TSAs are summarized in Table 1. Known tumor Ags relevant to breast cancer (BC) are shown in Table 2.

The main advantages of cancer vaccines are minimal toxicity, the generation and amplification of a highly specific adaptive immune response, and the establishment of immunologic memory with the potential to control and eliminate residual disease by swiftly responding to TAA/TSA exposure over time. Cancer vaccines can be used for (1) prevention (e.g., targeting viruses that are involved in the malignant transformation, such as human papilloma virus (HPV) in cervical cancer); (2) interception (e.g., targeting TAAs/TSAs expressed very early in the process of tumor progression, such as human telomerase reverse transcriptase (hTERT) or p53); and (3) therapy (targeting known TAAs expressed by established tumor such as HER2) [3]. Cancer vaccines for disease prevention and interception are used in settings of pre-cancer or in the presence of low disease burden when immune suppression is expected to be less, whereas therapeutic cancer vaccines are used in the setting of established disease, where immune escape is thought to have selected the most aggressive tumors and potentially less immunogenic tumor cells, and the tumor microenvironment (TME) tends to be highly immune suppressed [4].

Preventive cancer vaccines produce stronger and broader $CD4^+$ T helper 1 (Th1) and $CD8^+$ cytotoxic T lymphocyte (CTL) responses in the presence of low disease burden [5–9] or in pre-cancerous lesions [10–15] than therapeutic cancer vaccines. Therapeutic cancer vaccines have been tested in many different tumor types, with a variety of Ags

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Table 1
The main classes of TAAs and TSAs in cancer.

Tumor Associated Antigens	
Type of Antigen	**Examples**
Overexpressed self-proteins	HER-2
Proteins involved in tissue differentiation	Tyrosinase (Melanoma); Mammoglobin (Breast Cancer)
Proteins normally expressed in immune privileged tissues and upregulated in tumor cells	CTAs: - MAGE, - NY-ESO-1
Overexpressed heavily glycosylated transmembrane proteins playing fundamental roles in tumor invasion, metastasis, angiogenesis, proliferation, apoptosis, inflammation and immune regulation	TACA
Tumor Specific Antigens	
Type of Antigen	**Examples**
Proteins of oncogenic viruses that are expressed by transformed cells	HPV, HBV, HERV-K (endogenous retroviruses)
Unique mutated proteins that give rise to neoantigens generated as products of somatic and frame shift mutations	//
Novel proteins generated by the formation of fusion proteins as the result of chromosomal breakpoints/re-joining	BCR/ABL in chronic myelogenous leukemia

Legend:
ABL: Abelson; **BCR:** Breakpoint Cluster Region; **CTAs:** Cancer-testis antigens; **HBV:** Hepatitis B Virus; **HER-2:** Human epidermal growth factor receptor-2; **HERV-K:** Human endogenous retrovirus K**; HPV:** Human Papilloma Virus; **MAGE:** Melanoma Antigen-Encoding Gene; **NY-ESO-1:** New York Esophageal Squamous Cell Carcinoma 1; **TACA:** Tumor-associated carbohydrate antigen; //: not available

(including non-mutated shared TAAs and patient-specific mutated Ags), adjuvant combinations, and delivery methods used [16,17]. Fig. 1 summarizes the main mechanism of action and the different types of therapeutic cancer vaccines used in BC.

Despite the vaccine-mediated induction of Ag-specific immune responses (e.g., the production of Ag-specific T cells and antibodies (Abs)) in both pre-clinical and clinical settings, clinical outcomes in phase III BC vaccine trials have been disappointing [2,18,19]. The two key phase III BC vaccine trials were performed (1) in the metastatic setting, where a mucin 1 (MUC-1) based tumor associated carbohydrate Ag (TACA) vaccine was administered in the Theratope study [18], and (2) in the adjuvant setting, where the HER2-based peptide vaccine nelipepimut-S (NeuVax) was given to high risk BC patients after standard adjuvant therapy with the aim to decrease the risk of recurrence (secondary prevention) [19].

Negative results from BC vaccine trials are thought to be linked to: (1) patient selection factors (disease burden, extent of prior therapy, and tumor immune contexture) [20,21]; (2) the selection of TAAs to target; (3) the low immunogenicity of the vaccine as a result of the Ag chosen, or due to the vaccine delivery platform used (e.g., peptide *vs.* dendritic cell (DC) *vs.* DNA *vs.* RNA *vs.* tumor cell lysate) [22–25]; (4) concurrent therapies used in combination with vaccination (e.g., chemotherapy and targeted therapies) [26,27]; and (5) mechanisms of immune escape, including alterations in Ag processing machinery [28–30], the loss of Human Leukocyte Ag class I (HLA-I) expression [31], and the down-regulation of tumor Ag expression [32,33].

The current era of immune checkpoint blockade (ICB) therapy has revolutionized treatments in a variety of tumors of different histologies, with multiple agents approved by United States (US) Food and Drug Administration (FDA) and the European Medicine Agency (EMA) (reviewed in [34]). Capitalizing on the recent success of ICB strategies, combining ICB with cancer vaccines might improve the activity and effectiveness of ICB by expanding tumor-specific T cells and simultaneously preventing the activation of inhibitory immune checkpoint pathways expressed by the vaccine-induced activated T cells [35].

The principal aims of this review are to summarize the cancer vaccine strategies that have been tested in BC, suggest new research directions, and review the challenges that may be associated with them.

Clinical trials evaluating vaccines for breast cancer

BC represents the third most studied tumor for cancer vaccination, following melanoma and cervical cancer [36]. The most common TAAs targeted in BC are HER2, MUC-1, carcinoembryonic antigen (CEA) and hTERT (Table 2). BC vaccines have been tested with various adjuvants, like granulocyte-macrophage colony-stimulating factor (GM-CSF), toll like receptor (TLR) agonists, virosomes and cytokines, and with other drugs such as chemotherapeutics, the anti-HER2 monoclonal Ab trastuzumab, targeted agents (lapatinib, …) or immune checkpoint agonists or ICB. Published and ongoing trials of BC vaccines are summarized in Tables 3 and 4.

Most BC vaccines have been given in the metastatic setting, where disease burdens are high and immune tolerance and suppression are firmly established. Phase I studies [37–48] in the aggregate demonstrated that vaccines are safe, well-tolerated and immunologically active in that they stimulate Ag-specific immune responses. Tumor regressions were also sometimes observed, although these did not tend to correlate with vaccine-induced immunity [42,47,49,50] (Table 3). Despite early evidence of activity, the phase III randomized controlled trials (RCT) of the Theratope vaccine in metastatic BC and the nelipepimut-S vaccine for early BC in the adjuvant setting (the PRESENT trial) failed to demonstrate clinical benefit from the vaccination [18,19].

Theratope, the sTn keyhole limpet hemocyanin (KLH) vaccine targeting a TACA epitope found on cancer-associated mucins, was administered to 1028 metastatic BC patients across 126 centers. This trial failed to show an improvement in time to progression (TTP) or overall survival (OS), despite producing clinically significant Ab titers specific for sTn in patients treated with the vaccine. In the control arm, patients received a placebo containing the KLH protein without the sTn Ag [18], and patients in each arm also received a low dose of cyclophosphamide (300 mg/m^2) to mitigate the suppressive influence of regulatory T cells (Tregs). The negative results of this study might be in part attributable to the administration of KLH with low dose cyclophosphamide in the control arm, possibly inducing a non-specific stimulation of the immune system in this group of patients. Moreover, the broad patient population (including all BC subtypes, and without any evaluation of the expression of the sTn Ag, which is found only in 30–40% of BC) [51] together with the requirement for advanced disease might have influenced the

Table 2
A Summary Of The Main Breast Cancer Targeted Antigens.

Antigen	Type of Tumor Antigen	Type of vaccine	Other Compound(s)
MUC-1	TACA	Peptide vaccine	L-BLP25 *plus* standard neoadjuvant chemotherapy or adjuvant hormonal therapy
HER2/neu	TAA	Peptide vaccine	Standard therapy followed by adjuvant trastuzumab *plus* NeuVax (peptide nelipepimut-S (E75))
HER2/neu	TAA	Peptide vaccine	HER-2/neu ICD peptide-based vaccine +/− Polysaccharide Krestin, concurrent with HER2-targeted monoclonal antibody therapy
HER2/neu	TAA	Peptide vaccine	Standard therapy followed by adjuvant dHER2 ASCI *plus* AS15
HER2/neu	TAA	Peptide vaccine	Standard adjuvant therapy followed by GP2/GM-CSF, containing a HER2/Neu-derived epitope (GP2) combined with GM-CSF
HER2/neu	TAA	Peptide vaccine	A combination peptide vaccine of 2 chimeric peptides of the promiscuous T cell epitope derived from MVF (amino acid residues 288–302) co-synthesized with B-cell epitopes derived from the HER-2/neu a.a. 597–626 and HER-2/neu a.a. 266–296
HER2/neu	TAA	Peptide vaccine	HER2-derived peptide vaccine after systemic chemotherapy or radiotherapy
MAGE-A1, MAGE-A3, MAGE-A10, CEA, NY-ESO-1, HER2 proteins	CTA	Peptide vaccine	A peptide vaccine based on 9 MHC class I-restricted BC-associated peptides combined with a MHC class II-restricted helper peptide derived from tetanus toxoid *plus* a TLR3 agonist (Poly ICLC)
Tri Tn Glycotope (MAG-Tn3)	TACA	Peptide vaccine	Standard therapy followed by adjuvant Magtrivacsein *plus* AS15
Patients' own tumor antigens	TSA	Peptide vaccine	Poly ICLC (Personalized synthetic long peptide vaccine)
CMP P10s fused to the pan HLA DR-binding epitope (PADRE)	TACA	Peptide mimotope-based vaccine	P10s-PADRE +/- standard chemotherapy +/- adjuvant MONTANIDE™ ISA 51 VG
Globo-H hexasaccharide-1	TACA	Carbohydrate-based vaccine	Globo H-DT OBI-833
HER2/neu	TAA	Dendritic cell	DC1
HER2/neu	TAA	Dendritic cell	AdHER2/neu
TBVA	TAA	Dendritic cell	αDC1-TBVA vaccine *plus* gemcitabine
p53 / ***Survivin*** / ***hTERT***	TAA/ TAA/ TAA	Dendritic cell	Autologous dendritic cells transfected with p53, survivin and hTERT encoding mRNA *plus* cyclophosphamide
Patients' own tumor antigens	TSA	Dendritic cell	Autologous dendritic cell vaccine *plus* standard neoadjuvant chemotherapy
Cyclin B1 / ***WT-1*** / ***CEF***	TAA/ TAA	Dendritic cell	Cyclin B1/WT-1/CEF (Antigen)-loaded dendritic cell vaccine *plus* standard neoadjuvant chemotherapy
p53	TAA	Dendritic cell	Ad.p53 *plus* 1-MT
Patients' own tumor antigens	TSA	Dendritic cell	Dendritic cell/Tumor cell fusion vaccine *plus* IL-12
HER2/neu	TAA	Viral	Standard therapy/ +/- trastuzumab followed by/concomitant MVA-BN-HER2, a vaccinia Ankara (Bavarian Nordic)-HER2 virus
MUC-1 / ***CEA***	TACA/ TAA	Viral	PANVAC (recombinant vaccinia virus and recombinant fowl poxvirus encoding MUC-1 and CEA) *plus* docetaxel
//		Viral	Metronomic cyclophosphamide *plus* oncolytic virus JX-594 (a modified vaccinia poxvirus engineered with GM-CSF gene addition and thymidine kinase gene deletion which limits viral replication to cells with high levels of thymidine kinase, such as cancer cells with a mutated *RAS* or *p53* gene)
hNIS	TAA	Viral	MV-NIS, an oncolytic measles virus encoding *hNIS*, after failure of standard treatments
//		Viral	HF10 (a replication-competent HSV-1 oncolytic virus) injected on cutaneous and/or superficial lesions
p53	TAA	Viral	p53MVA (a genetically engineered modified vaccinia Ankara virus expressing wild type *p53* transgene) *plus* pembrolizumab
HER2/neu	TAA	Bacterial	ADXS31-164 (a live attenuated Listeria monocytogenes bioengineered with a chimeric human epidermal growth factor receptor 2 fused to a truncated form of the Lm protein listeriolysin O) after failure of standard treatment
A variety of TAAs delivered by tumor vaccine cells	TAA	GM-CSF-secreting BC vaccine	Allogeneic GM-CSF-secreting BC vaccine *plus* cyclophosphamide and doxorubicin
hTERT	TAA	DNA vaccine	Standard therapy followed by adjuvant INO-1400 +/- IL-12

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Table 2 (*continued*)

Antigen	Type of Tumor Antigen	Type of vaccine	Other Compound(s)
CD105	TAA	DNA vaccine	STEMVAC: a plasmid DNA vaccine containing the mammalian expression vector pUMVC3 (pNGVL3) encoding epitopes of CD105 (Endoglin), Yb-1, SOX2, CDH3, and MDM2 proteins
Yb-1	TAA		
SOX2	TAA		
CDH3	TAA		
MDM2	TAA		
Patients' own tumor antigens selected after genome profiling of patient's BC cells	TSA	DNA vaccine	Personalized polyepitope DNA vaccine
Fusion antigen	NV	NV	NV

Legend:
AdHER2/neu: Autologous adenovirus HER2-transduced dendritic cell vaccine; **Ad.p53**: Adenovirus p53-transduced dendritic cell vaccine; **BC**: breast cancer; **CDH3**: cadherin 3; **CEA**: carcino-embryonic antigen; **CMP**: Carbohydrate Mimetic Peptide; **αDC1-TBVA vaccine**: a tumor blood vessel antigen peptide-pulsed alpha-type-1 polarized dendritic cell vaccine; **Globo H-DT OBI-833**: carbohydrate-based vaccine based on Globo H hexasaccharide 1 antigen conjugated to DT-CRM197, a non-toxic, mutated form of diphtheria toxin; **GM-CSF**: granulocyte-macrophage colony-stimulating factor; **hTERT**: human Telomerase Reverse Transcriptase; **ICD**: Intracellular Domain; **IL**: interleukin; **L-BLP25**: liposomal vaccine; **MAG**: multiple antigenic glycopeptide; **MAGE**: Melanoma Antigen-Encoding Gene; **MDM2**: Murine Double Minute 2; **MHC**: Major Histocompatibility Complex; **1-MT**: 1-Methyl-D-Tryptophan; **MUC-1**: mucin 1; **MVF**: measles virus fusion protein; **hNIS**: human thyroidal sodium iodide symporter; **NV**: not validated; **NY-ESO-1**: New York Esophageal Squamous Cell Carcinoma 1; **P10s-PADRE**: Peptide mimotope-based vaccine; **PANVAC**: recombinant vaccinia virus and recombinant fowl poxvirus encoding MUC-1 and CEA; **SOX2**: SRY-Box 2; **STEMVAC**: multi-antigen vaccine comprised of Th1 epitopes derived from five breast cancer stem cell/EMT immunogenic proteins; **TAA(s)**: tumor associated antigen(s); **TACA**: Tumor Associated Carbohydrate Antigen; **TBVA**: Tumor Blood Vessel Antigen; **TLR**: Toll-like Receptor; **TSA**: tumor specific antigen; **WT-1**: Wilms tumor; **Yb-1**: Y-box binding protein 1; //: not available

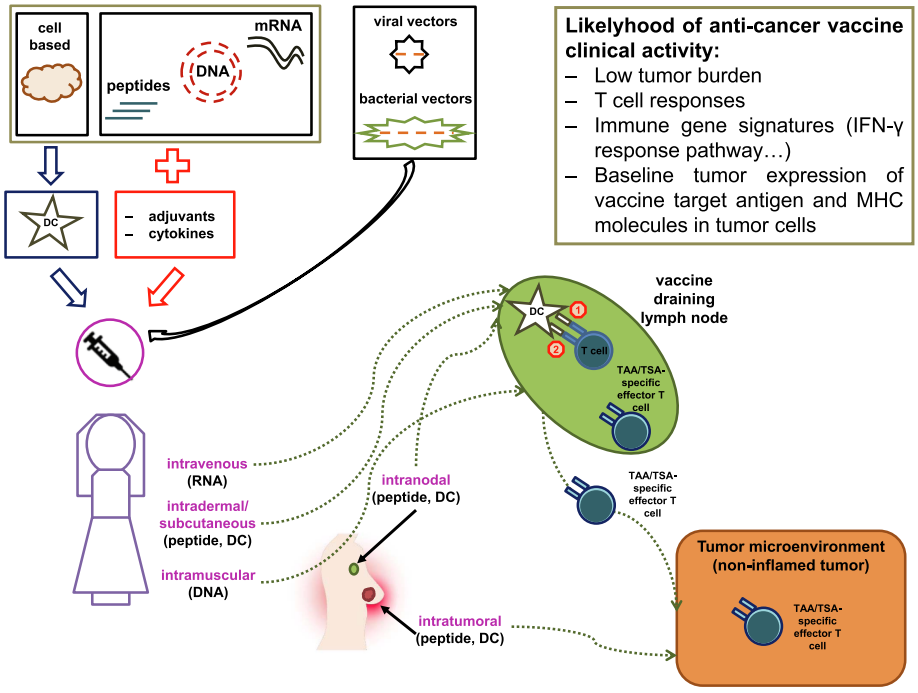

Fig. 1. The main types of therapeutic breast cancer (BC) vaccines and their mechanism of action. The figure summarizes the main types of BC vaccines that have been tested in the clinic so far, as well as the various routes of administration and their principal mechanism of action. The tested BC vaccines are: RNA, DNA, peptide, cell-based, viral, bacterial, and dendritic cell (DC)-based. The main targets of vaccination are the DCs localized in the vaccine-draining lymph node (LN), whose role is to present the processed tumor associated antigen (TAA) or tumor specific antigen (TSA) to T cells (either $CD4^+$ and $CD8^+$) localized in these secondary lymphoid organs. This interaction generates TAA/TSA specific effector T cells that are able to leave the LN and circulate in the blood in order to reach their targets localized in the tumor microenvironment, where the killing of tumor cells takes place. Features that are associated with a higher likelihood of response to vaccines are listed in a box. Bibliographic reference for the Figure: Melief CJM, Zappasodi R, Garassino MC, Di Nicola M. Vaccines (Dendritic Cell Vaccines, Peptide Vaccines, DNA Vaccines, RNA Vaccines, Oncolytic Viruses). ESMO Handbook of Immuno-Oncology, 2018; Pages 23–42.

results of the study. Consistent with this, a subgroup analysis of vaccinated patients who were also on endocrine therapy (ET) had longer TTP and OS than the control group of patients on ET [52]. Moreover, vaccinated patients on ET who developed higher Ab responses (> the median) had greater clinical benefit than similar patients who developed lower Ab responses (< the median). These hypothesis-generating results are consistent with data supporting the immune-modulating effect of ET [53–55].

In the adjuvant setting, the phase III PRESENT trial testing nelipepimut-S, an anti-HER2 peptide vaccine given with subcutaneous GM-CSF in lymph node (LN)+ BC patients with low expression of HER2 (1+ or 2+ by immunohistochemistry, IHC), failed to demonstrate any improvement in disease-free survival (DFS) and was stopped in July 2016 [19].

NeuVax, the HLA-A2/A3-restricted extracellular HER2-domain-derived E75 peptide vaccine (nelipepimut-S), is a class I Major Histocompatibility Complex (MHC-I) epitope. This vaccine was able to induce E75 HER2-specific $CD8^+$ T cell immune responses in BC patients in very early clinical trials [56]. In two phase II adjuvant trials, patients who were disease-free after standard therapy but at high risk for recurrence, HLA-$A2^+$ or HLA-$A3^+$ patients expressing any degree of HER2, were vaccinated with 4 to 6 monthly injections, while HLA-$A2^-$/$A3^-$ patients served as concurrent unvaccinated controls [57]. These studies demonstrated a good safety profile and revealed the induction of vaccine dose-dependent anti-HER2 immunity. Interestingly, vaccinated patients with low expression of HER2 demonstrated more robust immune responses than vaccinated patients with high levels of HER2 expression, with larger HER2-specific delayed-type hypersensitivity

Table 3
Published Clinical Trials on Breast Cancer Vaccines.

Adjuvant setting

Clinical trial reference	Trial phase	Trial status	Compound(s)	TAAs	Breast Cancer Subtypes	Primary Objective(s)	Outcome(s)
NCT01570036 (57)	II	Completed	Standard therapy followed by adjuvant trastuzumab *plus* NeuVax *vs.* Standard therapy followed by adjuvant trastuzumab	HER2 peptide nelipepimut-S (E75)	HLA A2/A3 + HER2 + and node positive BC	RR/ DFS	RR at 20 months: 5.6% (vaccinated group); 14.2% (unvaccinated group) (P = 0.04). RR at 26 months: 8.3% (vaccinated group); 14.8% (unvaccinated group) (P = 0.15). DFS rate at 5 years (subgroup of patients that received an optimal vaccination dose): 94.6% (vaccinated group); 80.2% (unvaccinated group) (P = 0.05)
NCT01479244 (19)	III	Completed	Standard therapy followed by adjuvant NeuVax *vs.* Standard therapy followed by placebo	HER2 peptide nelipepimut-S (E75)	Early stage HLA A2/A3 + HER2 IHC 1+/2 + and node positive BC	DFS	RR at 18 months (Interim analysis): 9.8% (vaccinated group); 6.3% (unvaccinated group) (P = 0.07). Based on these data, the IDMC recommended the study to be stopped for futility
// (63)	I	Completed	Ii-Key/HER-2/neu MHC class II peptide AE37 vaccine *plus* GM-CSF	HER2/neu	HLA-A2 + HER2 IHC 1 + to 3+. Disease-free, node negative BC	Safety/ Immune response	Toxicities: Grade 1 adverse events = 40%; Grade 2 adverse events = 60%. The vaccine induced immunologic responses *in vitro* and *in vivo* to AE37
NCT00524277 (66)	II	Completed	Ii-Key/HER-2/neu MHC class II peptide AE37 vaccine *plus* GM-CSF *vs.* Placebo	HER2/neu	HLA-A2 + HER2+, node-positive or high-risk node-negative BC	RR	RR at 25 months: 12.4% (vaccinated group); 13.8% (unvaccinated group) (P = 0.70)
NCT00058526 (70)	I	Completed	Standard therapy followed by adjuvant dHER2 ASCI *plus* AS15	HER2/neu	Stage II – III HER2+ BC	Safety Immune response	Toxicities: Grade 2 adverse events = 92%; Grade 3 adverse events = 8%. The vaccine induced a dose-dependent immunologic response *in vitro* and *in vivo*

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Table 3 (*continued*)

Adjuvant setting							
Clinical trial reference	Trial phase	Trial status	Compound(s)	TAAs	Breast Cancer Subtypes	Primary Objective(s)	Outcome(s)
// (67)	I	Completed	GP2 vaccine *plus* GM-CSF	HER2/neu	HLA-A2+ HER2 IHC 1 + to 3+ disease-free, node negative BC	Safety Immune response	Toxicities: Maximum local toxicity; Grade 1 adverse events = 38.9%; Grade 2 adverse events = 61.1%; Maximum systemic toxicity: Grade 0 adverse events = 5.6%; Grade 1 adverse events = 61.1%; Grade 2 adverse events = 33.3%. The vaccine induced immunologic responses *in vitro* and *in vivo* to GP2
NCT00524277 (68)	II	Completed	Standard adjuvant therapy followed by GP2/GM-CSF *vs.* Placebo	HER2/neu	HLA-A2+ HER2+ node-positive or high-risk node-negative BC	DFS	5-year DFS rate: 88% (vaccinated group); 81% (unvaccinated group) (P = 0.43)
ISRCTN71711835 (75)	II	Completed	Standard adjuvant therapy followed by oxidized mannan–MUC1 vaccine *vs.* Placebo	MUC-1	Stage II HER-2-, ER + BC	RR/ Time of Recurrence	RR at 1.5 years: 12.5% (vaccinated group); 60% (unvaccinated group). Median Time of Recurrence: 118 months (vaccinated group); 65.8 months (unvaccinated group) (P = 0.009)
// (76)	I	Completed	MUC1-KLH conjugate vaccine *plus* the immunostimulant QS-21	MUC-1	BC with one of the following characteristics: Stage IV after eradication of all detectable disease - Stage I, II, or III with rising tumor markers - Stage III with an initially unresectable primary tumor after adjuvant therapy	Safety/ Immune response	Toxicities: Grade 1–2 adverse events = 89%; Grade 3 adverse events = 11%. The vaccine induced immunologic responses to MUC1-KLH
NCT00005956 (102)	I	Completed	HER2 ICD protein-containing DC vaccine	HER2/neu	Stage II (≥6 positive lymph nodes), III, or stage IV BC with > 50% HER2 overexpressing tumor cells who were disease-free after surgery and adjuvant therapy	Immune response/ Time to Recurrence	Delayed-type hypersensitivity reactions occurred in 6/7 patients. At more than 5 year follow-up, 6/7 had detectable anti-ICD antibodies. 1 patient experienced a pulmonary recurrence at 4 years from their study immunizations. All patients are alive and disease-free at 4.6–6.7 year follow-up

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Table 3 (*continued*)

Metastatic setting							
Clinical trial reference	Trial phase	Trial status	Compound(s)	TAAs	Breast Cancer Subtypes	Primary Objective(s)	Outcome(s)
NCT01042535 (96)	I/II	Completed	Ad.p53 *plus* 1-MT	p53	p53 positive Stage IV BC	MTD/ ORR	Toxicities: Grade 1–2 adverse events = 58%; Grade 3 adverse events = 0%.- The MTD of 1-MT was 1.600 mg BID + Ad.p53DC vaccine. ORR: Best response to immunotherapy in phase 2 = 1 SD, 8 PD. Phase 2 median TTP = 6.85 weeks (3.8–18.1). Phase 2 median OS = 18.1 weeks (3.8–52)
NCT00179309 (90)	II	Completed	PANVAC *plus* Docetaxel *vs.* Docetaxel alone	MUC-1 CEA	Stage IV BC	PFS	PFS = 7.9 months (vaccinated group); 3.9 months (unvaccinated group) (P = 0.09)
// (92)	I	Completed	Peptide vaccine contains 3 peptides from the extracellular domain of HER-2/neu: P4, P6 and P7 associated to a reconstituted influenza virosomes	HER2/neu	Stage IV ER/PgR+ HER2 IHC 1+/2+ BC	Safety/ Immune Response	Toxicities. Grade 1–2 adverse events = 40%; Grade 3 adverse events = 0%; Immune response rate = 80%
NCT00093834 (46)	I	Completed	Allogeneic GM-CSF-secreting BC vaccine *plus* cyclophosphamide and doxorubicin	A variety of TAAs delivered by the tumor vaccine cells	Stage IV BC	Safety/ Immune Response	Toxicities: Grade 1–2 adverse events = 100%; Grade 3 adverse events = 0%. No dose-limiting toxicities were observed. The vaccine induced HER2-specific immunity
NCT00399529 (88)	II	Completed	Allogeneic HER2+ GM-CSF-secreting BC vaccine *plus* cyclophosphamide and trastuzumab	HER2/neu	Stage IV HER2+	Safety/ CBR	Toxicities: Grade 1–2 adverse events = 100%; Grade 3 adverse events = 25%; CBR at 6 months = 55% P = 0.013
NCT02432963 (97)	I	Active, not recruiting	p53MVA *plus* pembrolizumab	p53	p53 positive Stage IV TNBC	Safety/ Clinical Response	Toxicities: Grade 3 adverse events = 64%; Grade 5 adverse events = 9%; Clinical Response ORR = 27% (3/11 patients)
NCT00807781 (98)	I	Completed	Plasmid Mammaglobin-A DNA-based vaccine	Mammaglobin-A	HLA A2/A3+ Stage IV BC with stable disease	Safety PFS	Toxicities: Grade 1–2 adverse events = 57%; Grade 3 adverse events = 0%;

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Table 3 (*continued*)

Metastatic setting							
Clinical trial reference	Trial phase	Trial status	Compound(s)	TAAs	Breast Cancer Subtypes	Primary Objective(s)	Outcome(s)
NCT00003638 (18)	III	Completed	Theratope STn-KLH vaccine *plus* cyclophosphamide *vs.* Placebo-KLH *plus* cyclophosphamide	Synthetic antigen that mimics the STn-antigen, conjugated to the high-molecular-weight protein carrier KLH	Stage IV BC	TTP/ OS	PFS at 6 months: 53% (vaccinated group); 33% (screen failure group) P = 0.011 TTP: 3.4 months (vaccinated group); 3.0 months (unvaccinated group), Cox P = 0.353; Log-rank test P = 0.305. OS: 23.1 months (vaccinated group); 22.3 months (unvaccinated group), Cox P = 0.916; Log-rank test P = 0.972
NCT00194714 (45)	I/II	Completed	HER2 cytotoxic T-cell peptide-based vaccine *plus* maintenance trastuzumab	HER2/neu	HLA-A2+ HER2+, Stage IV BC	Safety/ Immune response	Toxicities: Grade 1–2 adverse events = 99%; Grade 3 adverse events = 1%; Immunogenicity: Immune response rate = 84%
NCT00140738 (71)	I/II	Completed	dHER2 ASCI *plus* AS15 as first- or second-line therapy following response to trastuzumab-based maintenance therapy	HER2/neu	Stage IV HER2+ BC	Safety/ CBR	Toxicities: Grade 1–2 adverse events = 92%; Grade 3 adverse events = 8%; CBR = 30%
// (77)	I	Completed	Globo H-KLH conjugate vaccine *plus* the immunostimulant QS-21	Globo H	Stage IV BC without evidence of disease after therapy or those with active disease who were on hormonal therapy	Safety/ Immune response	Toxicities: Grade 1–2 adverse events = 89%; Grade 3 adverse events = 11%. The vaccine induced immunologic responses and complement-dependent cytotoxicity
// (80)	I	Completed	hTERT peptide I540 vaccine *plus* MONTANIDE™ ISA 51 VG adjuvant and GM-CSF	hTERT	HLA-A2 + Stage IV BC refractory to at least one conventional therapy for the metastatic disease	Immune response/ OS	Immune Response: High immune responders = 9/16; Non/low immune responders = 7/16. OS: 32.2 months (High responders); 17.5 months (Non/low responders) (P = 0.03)
UMIN000001844 (1 0 4)	II	Completed	Personalized peptide vaccination *plus* standard therapy	Antigens individually selected on the basis of pre-existing host immunity	Stage IV recurrent BC	Safety/ Efficacy	Toxicities: Grade 1–2 adverse events = 89%;

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Table 3 (*continued*)

Metastatic setting							
Clinical trial reference	Trial phase	Trial status	Compound(s)	TAAs	Breast Cancer Subtypes	Primary Objective(s)	Outcome(s)
							Grade 3 adverse events = 11%; Efficacy: ORR = 14%; PFS (TNBC) = 7.5 months; OS (TNBC) = 11.1 months; PFS (luminal/HER2-) = 12.2 months; OS (luminal/HER2-) = 26.5 months; PFS (HER2 +) = 4.5 months; OS (HER2 +) = 14.9 months
Other settings							
Clinical trial reference	Trial phase	Trial status	Compound(s)	TAAs	Breast Cancer Subtypes	Primary Objective(s)	
NCT01532960 (105)	I	Terminated (futility considering the immune responses generated by the vaccine)	A peptide vaccine based on 9 MHC class I-restricted BC-associated peptides combined with a MHC class II-restricted helper peptide derived from tetanus toxoid *plus* a TLR3 agonist (Poly ICLC)	MAGE-A1, MAGE-A3, MAGE-A10, CEA, NY-ESO-1, HER2 proteins	HLA-A1, -A2, -A3, or -A31 + Stage I–IV BC	Safety/ Immune Response Rate	Toxicities: Grade 1–2 adverse events = 100%; Grade 3 adverse events = 0%; Immune Response Rate = 0%
NCT02061332 (101)	I/II	Completed	HER-2 pulsed Dendritic cell vaccine	HER2/neu	HER2+, DCIS Early Invasive BC	Safety/ Immune and clinical responses to vaccine via intralesional, intranodal or both intra- lesional and intranodal injection	Vaccination by all injection routes was well tolerated. There was no significant difference in immune and clinical response rates by vaccination route of administration

Legend: Ad.p53: Adenovirus p53-transduced dendritic cell vaccine; **ASCI**: Antigen-Specific Cancer Immunotherapeutic; **BC**: breast cancer; **CEA**: carcino-embryonic antigen; **CEF**: Cytomegalo-, Epstein-Barr and Flu-virus; **CMP**: Carbohydrate Mimetic Peptide; **DCIS**: Ductal carcinoma in situ, **DFS**: Disease-Free Survival; **DLT**: Dose Limiting Toxicity; **ER**: Estrogenic Receptor; **GM-CSF**: granulocyte–macrophage colony-stimulating factor; **HER**: Human epidermal growth factor receptor; **HLA**: Human Leukocyte Antigen; **IHC**: Immunohistochemistry; **KLH**: keyhole-limpet Hemocyanin; **L-BLP25**: liposomal vaccine; **MAGE**: Melanoma Antigen-Encoding Gene; **MHC**: Major Histocompatibility Complex; **1-MT**: 1-Methyl-D-Tryptophan; **MTD**: Maximum Tolerated Dose; **MUC1**: mucin 1; **NY-ESO-1**: New York Esophageal Squamous Cell Carcinoma 1; **ORR**: Objective Response Rate; **OS**: Overall Survival; **P10s-PADRE**: Peptide mimotope-based vaccine; **p53MVA**: a genetically engineered modified vaccinia Ankara virus expressing wild type p53 transgene; **PANVAC**: recombinant vaccinia virus and recombinant fowl poxvirus encoding MUC-1 and CEA; **PFS**: Progression-free Survival; **PgR**: Progesterone Receptor; **RR**: Recurrence Rate; **STn:** Sialy1-Tn; **TAAs**: tumor associated antigens; **TLR:** Toll-like Receptor; **TNBC**: Triple Negative Breast Cancer; **TTP**: Time To Progression; **WT-1**: Wilms tumor

Table 4
Ongoing Clinical Trials on Breast Cancer Vaccines.

Neoadjuvant setting Clinical trial reference	Trial phase	Trial status	Compound(s)	TAAs	Breast Cancer Subtypes	Primary Objective(s)
2011–004822-85 EudraCT Number	II	Completed	L-BLP25 (Peptide vaccine) *plus* standard neoadjuvant chemotherapy	MUC1 lipopeptide-containing mixture	TNBC or ER+/HER2- BC	RCB at the time of surgery
NCT01431196	II	Completed	Autologous dendritic cell vaccine *plus* standard neoadjuvant chemotherapy	Patients' own tumor antigens	Stage II – III HER2- BC	pCR
NCT02229084	I/II	Enrolling by invitation	P10s-PADRE *plus* standard neoadjuvant chemotherapy	CMP P10s fused to the pan HLA DR-binding epitope (PADRE)	Stage I – II – III ER+ BC	Anti-P10s immunoglobulin-G response rate
NCT02938442	II	Recruiting	P10s-PADRE *plus* standard neoadjuvant chemotherapy	CMP P10s fused to the pan HLA DR-binding epitope (PADRE)	Stage I – II – III TNBC	pCR
NCT02018458	I/II	Active, not recruiting	Cyclin B1/WT-1/CEF (antigen) -loaded dendritic cell vaccine *plus* standard neoadjuvant chemotherapy	Cyclin B1/WT-1/CEF	TNBC or ER+/HER2- BC	Safety and Toxicities

Adjuvant setting Clinical trial reference	Trial phase	Trial status	Compound(s)	TAAs	Breast Cancer Subtypes	Primary Objective(s)
NCT02826434	Ib	Recruiting	Standard therapy followed by adjuvant PVX-410 *plus* durvalumab	XBP1 (2 splice variants) CD138/CS1	HLA-A2 + Stage II – III TNBC	Safety, Toxicities and DLT
NCT02960594	I	Completed	Standard therapy followed by adjuvant INO-1400 (DNA vaccine based on a plasmid encoding hTERT) +/- IL 12	hTERT	All BC subtypes at high risk of tumor relapse	Safety and Toxicities
NCT00923143	I/II	Active, not recruiting	DC1	HER2/neu	HER2+ DCIS	Safety and Toxicities
NCT02297698	II	Active, not recruiting	Standard therapy followed by adjuvant trastuzumab *plus* NeuVax	HER2 peptide nelipepimut-S (E75)	HER2 IHC 3+ and node positive BC	Invasive DFS
NCT01479244	III	Completed	Standard therapy followed by adjuvant NeuVax	HER2 peptide nelipepimut-S (E75)	Early stage HER2 IHC 1+/2+ and node positive BC	DFS
NCT02364492	I	Recruiting	Standard therapy followed by adjuvant Magtrivacsein *plus* AS15	MAG-Tn3	HER2-negative localized BC at high risk of relapse	DLT
NCT01152398	I	Completed	Standard adjuvant therapy followed by MVA-BN-HER2 vaccine	HER2/neu	Localized or locally advanced HER2+ ER/PgR + or - BC	Safety and Toxicities

Metastatic setting Clinical trial reference	Trial phase	Trial status	Compound(s)	TAAs	Breast Cancer Subtypes	Primary Objective(s)
NCT00978913	I	Completed	Autologous dendritic cells transfected with p53, Survivin and hTERT encoding mRNA *plus* cyclophosphamide	p53/Survivin/hTERT	Stage IV BC and malignant melanoma	Safety and Toxicities
NCT01730118	I	Recruiting	AdHER2/neu	HER2/neu	1 + to 3 + IHC HER2/neu Stage IV BC	Cardio-toxicity, Anti-HER2/neu antibody concentration, Antibody dilution titers
NCT03362060	I	Recruiting	Standard therapy followed by adjuvant PVX-410 *plus* durvalumab	XBP1 (2 splice variants)/CD138/CS1	HLA-A2+ Stage IV TNBC	Immune Response
NCT00622401	I/II	Completed	Dendritic cell/ Tumor cell fusion vaccine *plus* IL-12	Patients' own tumor antigens	Stage IV BC	Safety and Toxicities
NCT02310464	I	Recruiting	Globo H-DT OBI-833	Globo-H hexasaccharide-1	Stage IV BC	Safety and Toxicities

(*continued on next page*)

Table 4 (*continued*)

Metastatic setting Clinical trial reference	Trial phase	Trial status	Compound(s)	TAAs	Breast Cancer Subtypes	Primary Objective(s)
NCT02276300	I	Recruiting	HER2-derived peptide vaccine after at least 1st line systemic chemotherapy/radiotherapy for advanced disease with documented stable disease or objective response	HER2/neu	HLA-A2+ HER2/neu 2 + IHC Stage IV BC	Safety and Toxicities
NCT02479230	I	Recruiting	αDC1-TBVA vaccine *plus* gemcitabine	Tumor Blood Vessel Antigen	HLA-A2+ Stage IV BC	Safety and Toxicities
NCT00485277	I	Completed	MVA-BN-HER2 vaccine, with and without trastuzumab, following 1st or 2nd line chemotherapy	HER2/neu	HER2+ Stage IV BC	Safety and Toxicities
NCT01376505	I	Recruiting	A combination peptide vaccine of 2 chimeric peptides of the promiscuous T cell epitope derived from MVF (amino acid residues 288–302) co-synthesized with B-cell epitopes derived from the HER-2/neu a.a. 597–626 and HER-2/neu a.a. 266–296, after failure of standard treatment	HER2/neu	Stage IV BC	Immune Response and CBR
NCT02386501	Ib	Completed	ADXS31-164 after failure of standard treatment	HER2/neu	HER2+ Stage IV Solid Tumors (including BC)	Safety, Toxicities and DLT
NCT01922921	I/II	Active, not recruiting	HER-2/neu ICD peptide-based vaccine +/-Polysaccharide Krestin, concurrently with HER2-targeted monoclonal antibody therapy	HER2/neu	HER2+ Stage IV BC	Safety and Toxicities
NCT02157051	I	Recruiting	STEMVAC (a plasmid DNA vaccine containing the mammalian expression vector pUMVC3 (pNGVL3) encoding epitopes of CD105 (Endoglin), Yb-1, SOX2, CDH3 and MDM2 proteins)	CD105/Yb-1/SOX2/CDH3/MDM2	HER2- Stage III/IV BC	Immune Response, Safety and Toxicities
NCT01017185	I	Completed	HF10 injected on cutaneous and/or superficial lesions	//	Stage IV BC with cutaneous and/or superficial lesions	Local Tumor Response
NCT01846091	I	Suspended (Per study design)	MV-NIS, an oncolytic measles virus encoding the hNIS, after failure of standard treatment	hNIS	Stage IV BC	MTD, Safety and Toxicities
NCT02630368	I/II	Recruiting	Metronomic cyclophosphamide *plus* oncolytic virus JX-594 (a modified vaccinia poxvirus engineered with GM-CSF gene addition and thymidine kinase gene deletion which limits viral replication to cells with high levels of thymidine kinase, such as cancer cells with a mutated RAS or p53 gene)	//	HER2- Stage IV BC	MTD and ORR
Other settings Clinical trial reference	Trial phase	Trial status	Compound(s)	TAAs	Breast Cancer Subtypes	Primary Objective(s)
NCT02348320	I	Recruiting	Personalized polyepitope DNA vaccine	Patients' own tumor antigens selected after genome profiling of the patient's BC cells	Persistent TNBC after neoadjuvant chemotherapy	Safety and Toxicities
NCT02427581	I	Suspended	Poly ICLC (Personalized synthetic long peptide vaccine)	Patients' own tumor antigens	Persistent TNBC after neoadjuvant chemotherapy	Safety and Toxicities

Legend: AdHER2/neu: Autologous adenovirus HER2-transduced dendritic cell vaccine; **ADXS31-164**: live attenuated Listeria monocytogenes bioengineered with a chimeric human epidermal growth factor receptor 2 fused to a truncated form of the Lm protein listeriolysin O; **BC**: breast cancer; **CDH3**: cadherin 3; **CBR**: Clinical Benefit Rate; **CMP**: Carbohydrate Mimetic Peptide; **DC1**: Dendritic cell vaccine; **αDC1-TBVA vaccine**: a tumor blood vessel antigen peptide-pulsed alpha-type-1 polarized dendritic cell vaccine; **DCIS**: ductal carcinoma in situ; **DFS**: Disease-Free Survival; **DLT**: Dose Limiting Toxicity; **ER**: Estrogenic Receptor; **Globo H-DT OBI-833**: carbohydrate-based vaccine based on Globo H hexasaccharide 1 antigen conjugated to DT-CRM197, a non-toxic, mutated form of diphtheria toxin; **GM-CSF**: granulocyte–macrophage colony-stimulating factor; **HER**: Human epidermal growth factor receptor; **HLA**: Human Leukocyte Antigen; **HF10**: a replication-competent oncolytic Herpes Simplex virus-1; **hTERT**: human Telomerase Reverse Transcriptase; **ICD**: Intracellular Domain; **IHC**: Immunohistochemistry; **IL**: interleukin; **L-BLP25**: liposomal vaccine; **MAG-Tn3**: multiple antigenic glycopeptide composed of tri Tn glycotope; **MDM2**: Murine Double Minute 2; **MTD**: Maximum Tolerated Dose; **MUC1**: mucin 1; **MVA-BN-HER2**: Modified Vaccinia Ankara-Bavarian Nordic-HER2; **MVF**: measles virus fusion protein; **hNIS**: human thyroidal sodium iodide symporter; **ORR**: Objective Response Rate; **P10s-PADRE**: Peptide mimotope-based vaccine; **pCR**: pathologic Complete Response; **PgR**: Progesterone Receptor; **PVX-410**: Tetra-peptide vaccine; **RCB**: Residual Cancer Burden; **SOX2**: SRY-box 2; **STEMVAC**: multi-antigen vaccine comprised of Th1 epitopes derived from five breast cancer stem cell/EMT immunogenic proteins; **TAAs**: tumor associated antigens; **TNBC**: Triple Negative Breast Cancer; **XBP1**: X-box binding protein 1; **Yb-1**: Y-box binding protein 1; //: not available

(DTH) responses, significantly higher maximum *ex vivo* E75 (HER2)-specific $CD8^+$ T cell proliferation and increases in E75-specific $CD8^+$ T cells from pre-vaccination to post-vaccination, and more durable E75-specific CTL responses. Not surprisingly, not only patients over-expressing HER2 but also Ag-*naïve* patients (HER-2 IHC 0) were able to respond immunologically to the vaccine. The recurrence rate in vaccinated patients was lower with respect to unvaccinated controls at 20 months follow-up, though this effect lost significance at 26 months follow-up [57]. A 24-months landmark analysis of DFS revealed that patients benefitting the most from NeuVax had tumors with HER2 IHC 1+/2+, or grade 1 or 2 as opposed to unvaccinated controls. Trends were observed for the subgroup of patients with LN-positive disease [56]. These findings could reflect the presence of immunologic tolerance in HER2 over-expressing patients (HER2 3+ or FISH ≥ 2) [58]. The subgroup of patients who received optimal doses (1000 μg E75 + 250 μg GM-CSF) had a significantly improved 5-year DFS with respect to controls [59,60].

These data served as the foundation for the phase III PRESENT adjuvant trial, which enrolled over 750 HLA-$A2^+$ BC patients after standard treatment [19]. Patients were randomized to receive NeuVax with GM-CSF or GM-CSF alone. The PRESENT trial was terminated due to futility when an interim analysis was triggered after 70 qualifying DFS (=primary endpoint) events occurred among the enrolled 750 patients failing to show a clinical benefit with vaccination.

Cancer vaccines are not currently available for use as a standard of care for BC, but there is increasing interest in clinical research that evaluates them using other vaccine platforms and combination immunotherapy strategies, including incorporation of ICB. In the following paragraphs, we summarize other BC vaccines that have been investigated.

Peptide vaccines

Peptide vaccines targeting HER2

The most commonly targeted TAA in BC is the HER2 cell surface receptor. As we have described above, a HER2-derived peptide vaccine reached advanced clinical testing, but the phase 3 trial failed to demonstrate a clinical benefit. One of the drawbacks of the NeuVax is that it is an MHC-I peptide epitope vaccine that does not induce Th immunity. Early HER2 vaccine trials clearly demonstrated that a HER2-specific MHC-I peptide epitope vaccine alone elicits only short-lived $CD8^+$ T cell responses [38]. Therefore, in order to stimulate Th and B cell in addition to $CD8^+$ T cell responses, vaccines that include both MHC-I and MHC-II epitopes were developed [61].

Multi-epitope vaccines incorporating MHC-II epitopes were investigated in an early-phase clinical trial where patients with stage IV BC, ovarian, or non-small-cell lung cancer (NSCLC) were enrolled [40]. One vaccine consisted of three MHC-II epitopes derived from the HER2 protein's extracellular domain (ECD). These epitopes encompassed HLA-A2-restricted MHC-I epitopes within the MHC-II epitopes. The majority of patients that received this vaccine developed $CD8^+$ T cell-mediated immunity [39]. A second cohort of patients received a vaccine comprised of MHC-II epitopes, one of which is AE36, derived from the intracellular domain (ICD) of the HER2 protein. All of the vaccinated patients developed T cell and Ab responses, consistent with vaccine-stimulated Th and B cell responses [61]. This vaccine was also tested in combination with trastuzumab in 22 patients with HER2+ metastatic BC [45]. This trial demonstrated the safety of this vaccine combination, with boosting of HER2-specific immunity, the induction of epitope spreading within HER2 and to other tumor Ags, and a decline in serum levels of the immunosuppressive transforming growth factor-β.

One of the drawbacks of MHC-II peptide epitopes is a lower binding affinity than many MHC-I epitopes. A modified form of AE36, called AE37, is covalently linked to the Ii-Key peptide LRMK, which has been added to improve Ag presentation by enhancing epitope charging [58,62,63]. AE37 is an HLA-II binding, unrestricted $CD4^+$ T cell-eliciting peptide vaccine derived from the transmembrane domain of the HER2 receptor. Its administration with the adjuvant GM-CSF in a phase I trial demonstrated that the vaccine is safe and capable of inducing HER2-specific $CD4^+$ T cell peptide-specific immune responses and HER2-specific DTH responses [63]. Lower levels of circulating immune suppressive Tregs post-vaccination were also observed [64]. After a median follow up of 17 months, the recurrence rate in vaccinated patients overall was reduced by 42%. Further analyses demonstrated a 49% reduction in disease recurrence in patients with HER2- tumors, with no observed reduction in patients with HER2+ tumors. The vaccine effect occurs specifically in patients with tumors expressing low levels of HER2 [65]. In a randomized phase II trial of clinically disease-free LN+ and high-risk LN- BC patients with tumors expressing any degree of HER2, adjuvant AE37 *plus* GM-CSF *vs,* GM-CSF alone were administered to 153 and 145 patients, respectively. No differences in recurrence rate and DFS were observed. A trend toward improved benefit emerged in patients with HER2 low tumors, underlining the importance of patient selection for immunotherapy [66].

GP2, a HER2-derived HLA-A2- and HLA-A3-restricted epitope, when administered in association with GM-CSF, generated $CD8^+$ GP2- and E75- specific T cell responses and GP2-specific pre- to post-vaccination DTH responses in all patients in a phase I study of high risk disease-free, LN- BC [67]. GP2 *plus* GM-CSF did not generate any significant benefit in DFS as shown by a primary analysis from the phase II trial randomizing HLA-$A2^+$ disease free, LN+ and high-risk LN- BC patients with tumors expressing HER2 (IHC: 1+ to 3+) to receive GP2 *plus* GM-CSF or GM-CSF alone [68]. In a vaccine strategy designed to stimulate both $CD8^+$ and $CD4^+$ immune responses, GP2 (a HLA-A2 and HLA-A3 restricted, $CD8^+$ eliciting epitope) has also been given with AE37 (a HLA unrestricted, MHC-II, $CD4^+$ eliciting epitope) and GM-CSF, in disease-free LN+ or high-risk, LN- BC or ovarian cancer patients after the completion of standard adjuvant treatment [69]. The rationale underlying this combination was to stimulate a more robust immune response (as shown by the significant T cell proliferation that was induced after exposure to AE37/GP2 *plus* GM-CSF dual peptide vaccine in *in vitro* studies), considering the efficacy of these vaccines employed singularly. Finally, an ongoing phase II trial randomizes HLA-$A2^+$ and HLA-$A2^-$ patients with LN+ or high-risk LN- early stage BC to receive GP2 peptide with GM-CSF or GM-CSF alone in HLA-$A2^+$ patients; or AE37 in association with GM-CSF or GM-CSF alone in HLA-$A2^-$ patients, after the completion of standard adjuvant therapy (NCT00524277). Results are still awaited from all of these trials.

A recombinant HER2 protein (dHER2) that includes the ECD and part of the ICD of HER2 receptor was administered with the immunostimulant AS15 to patients with HER2+ metastatic BC or to patients with early stage HER2+ BC in two different trials. These studies showed that the vaccine was safe, and had clinical activity [70,71]. Notably, the vaccine induced HER2-specific Ab responses in trastuzumab *naïve* patients with HER2-overexpressing stage II and III BC after standard adjuvant treatments [70]. This vaccine was also tested in combination with trastuzumab as consolidation after 1^{st} or 2^{nd} line trastuzumab-based therapy in a phase I/II study that enrolled HER2+ metastatic BC patients, leading to 2 objective responses (OR) and prolonged stable disease (SD) in 10/40 patients [45,71]. Another clinical trial tested increasing doses of a HER2 ICD vaccine given with GM-CSF in advanced BC and ovarian cancer patients, demonstrating safety and the induction of HER2-specific T cell and Ab immunity in most patients [72]. In follow up to these initial findings, a randomized phase I/II trial (NCT01922921) is evaluating the combination of the HER2-ICD protein vaccine with anti-HER2 monoclonal Abs (trastuzumab +/- pertuzumab) +/− a TLR-2 agonist, and the immune-modulating polysaccharide krestin (PSK) in HER2+ metastatic BC patients. Preliminary results showed that the combination is safe and well-tolerated [73].

Peptide vaccines targeting carbohydrate antigens

The glycoprotein MUC-1 is one of the first BC TAAs studied and used in immunotherapy (Table 2) to generate tumor-specific B and T-cell responses. In the early trials that supported the phase 3 Theratope RCT, higher Ab titers and longer OS were observed if low dose cyclophosphamide was given three days prior to vaccination [74]. However, as discussed earlier in this review, the Theratope RCT failed to meet its primary endpoint despite this early evidence of vaccine activity. Vaccines other than Theratope that target TACA in BC include the oxidized-mannan-MUC-1 [75], the MUC1-KLH conjugate *plus* the adjuvant QS-21 [76] and the globo H (GH)-KLH conjugates *plus* QS-21 [77] (Table 3).

A phase II trial of oxidized-mannan-MUC-1 in patients with stage II BC reduced the risk of relapse by about 48% and showed a statistically significant improvement in OS [75].

A vaccine comprised of MUC-1-KLH *plus* QS-21 was also safe and well tolerated in disease-free BC patients, and resulted in vaccine-induced immunity [76]. High immunoglobulin (Ig)M and IgG Ab titers against MUC-1 were observed. No evidence of T-cell activation was detected.

Another TACA, GH-KLH conjugate *plus* QS-21, administered in metastatic BC patients resulted in the generation of IgM Abs specific for GH, with little IgG induced. Several patients developed evidence of complement-dependent cytotoxicity [77,78].

Peptide vaccines targeting hTERT

hTERT, the catalytic subunit of human telomerase, is a protein with potential immune-stimulating and anti-neoplastic activities. A phase I trial evaluated the HLA-A2-restricted hTERT I540 peptide in combination with KLH loaded onto *ex vivo* generated autologous DCs. hTERT-specific T lymphocytes were induced in 4/7 patients with advanced BC or prostate carcinoma. Partial tumor regression was seen in 1 patient in association with the induction of $CD8^+$ tumor-infiltrating lymphocytes (TIL) [79]. $CD8^+$ TIL were also detected after peptide vaccination with hTERT administered in an open-label prospective study of HLA-$A2^+$ patients with BC refractory to at least one conventional therapy for metastatic BC [80]. hTERT-specific $CD8^+$ T cells exhibited Ag-specific proliferation, interferon-gamma (IFN-γ) production, and tumor lysis *in vitro*. Patients with an immune response to hTERT had longer OS than patients who lacked an immune response. Ongoing trials are evaluating hTERT vaccines in metastatic BC (NCT00573495 and NCT01660529) and in the adjuvant setting (NCT02960594 [81] and NCT00753415).

Vaccines targeting WT1

Wilms tumor 1 (WT-1), is a protein with transcription factor activity involved in the maintenance of tissue homeostasis; it may be an oncogene in several tumors, including BC [82], where it is expressed in around 30–50% of cases [83]. A WT-1 analogue peptide vaccine is able to induce a $CD8^+$ response, resulting in tumor cell lysis and inhibition of tumor cell proliferation. This WT-1 peptide-based vaccine was administered in 2 patients with BC in a phase I trial enrolling patients with over-expression of the *WT1* gene and HLA-A*2402-positivity, showing a regression of the metastatic lesions, with a good safety profile [84]. The phase II INDUCT trial (NCT01220128) evaluated the safety and immunogenicity of the anti-WT-1 vaccine in association with standard neoadjuvant treatment in stage II/III BC, and results are awaited.

Cell-Based breast cancer vaccines

Tumor cell vaccines are derived from autologous or allogeneic tumor cells. Autologous [85–87] or allogeneic (from cell lines) tumor cell vaccines [36–38], combined with strong adjuvants or cytokines, have been used in several clinical trials in BC.

A GM-CSF secreting tumor cell vaccine that expresses HER2 (among other TAAs) has been tested in two early clinical trials. The first study was designed to identify the optimal doses of chemotherapy to give with the vaccine. The vaccine was safe, and could induce HER2-specific immunity as measured by HER2-specific DTH and Ab responses. The optimal doses of chemotherapy were cyclophosphamide at 200 mg/m^2 and doxorubicin at 350 mg/m^2 [46]. In the second trial the vaccine was given with a low dose cyclophosphamide (300 mg/m^2 based on historical data) and weekly trastuzumab (2 mg/kg, with a 4 mg/kg loading dose as necessary) in 20 HER2 + metastatic BC patients. This vaccination regimen was safe, and demonstrated evidence of clinical activity. Augmented HER2-specific immunity was detected by enhanced DTH and augmented $CD8^+$ T cell responses by ELISPOT [88].

Breast cancer vaccines delivered by bacterial or viral vectors

ADXS31-164, a cancer vaccine containing a live, highly attenuated strain of the Gram + *bacterium* Listeria monocytogenes (LmddA) encoding a fusion protein containing a chimeric peptide comprised of three highly immunogenic epitopes of the human TAA HER2 (chHER2) fused to a non-hemolytic fragment of the listeriolysin O (LLO) protein has been tested in preclinical models, and is now being evaluated in the clinic. This vaccine induces robust HER2-specific T cell responses, decreases regulatory T cells (Tregs), and increases the $CD8^+$ T cell/Treg *ratio* in tumors, delaying tumor growth in HER2 transgenic mice. A multicenter phase Ib first-in-human dose-escalation trial is testing ADXS31-164 in patients with HER2-expressing solid tumors [89]. This vaccine resulted in a decreased proportion of Tregs and myeloid derived suppressor cells, particularly in the TME, but not in secondary lymphoid organs (e.g., LNs) or in the peripheral blood.

CEA is over-expressed in several tumors, including BC, gastrointestinal cancers, and NSCLC. In a clinical trial the recombinant PANVAC poxviral vaccine (containing CEA and MUC-1 in addition to three T-cell costimulatory molecules (B7.1, ICAM-1, and LFA-3 (TRICOM)) *plus* GM-CSF was tested in 12 heavily pre-treated metastatic BC patients, with 1 patient demonstrating a complete response (CR) lasting > 37 months, and 5 patients enjoying SD lasting ≥4 months [47]. The randomized phase II trial of docetaxel +/- PANVAC-V (recombinant vaccinia virus vector vaccine) and PANVAC-F (recombinant fowl pox virus vector vaccine) showed that sequential vaccination with this combination might provide a clinical benefit in patients with metastatic BC relative to chemotherapy alone [90]. This hypothesis-generating study supports more formal testing of this vaccine strategy in larger numbers of BC patients.

Viral vaccines, such as the measles virus fusion epitope sequence (MVF) (a combination peptide vaccine including a T cell epitope that was incorporated into the HER2 B cell epitope), reconstituted influenza virosomes (containing HER2 peptides, representing B cell epitopes) and MVA-BN-HER2 (a poxvirus encoding a modified form of HER2-ECD *plus* 2 tetanus toxoid peptide epitopes) were safe, well tolerated and able to generate HER2-specific immune responses in both the metastatic and adjuvant BC settings [91,92]. Some vaccines, such as MVA-BN-HER2 were potent activators of B cell immunity, and were able to generate broad immune responses that extended to non-HER2-TAAs (epitope spreading) [93]. Preclinical data from mouse models in BC show that the combination of anti-cytotoxic T lymphocyte Ag (CTLA)-4 ICB with the MVA-BN-HER2 vaccine strongly synergized to improve OS in treated mice, generating highly activated polyfunctional T cells (the latter amplified by the anti-CTLA-4 treatment) [94].

Oncolytic viruses such as AD5/3-D24-GM-CSF in combination with either single dose or low-dose metronomic cyclophosphamide gave rise to 1 minor response and 4 SD in a cohort of 14 evaluable metastatic BC patients refractory to other treatments [95].

Adenoviruses used to generate autologous DC vaccines against p53 epitopes (Ad.p53) have been administered in metastatic BC patients (with < 3 lines of chemotherapy received in the metastatic setting, p53 expression by IHC > 5%) in association with indoximod, an indoleamine-pyrrole 2,3-dioxygenase (IDO) inhibitor. The combination was well tolerated, and the best response to this treatment combination was SD in 4/21 of the patients [96].

The phase I trial evaluating the combination of the anti-programmed cell death-1 (PD-1) pembrolizumab *plus* the p53-expressing modified vaccinia Ankara virus (p53MVA) vaccine showed that administration of this combination in patients with advanced solid tumors is safe and may generate clinical benefit, with 3/11 patients achieving SD [97]. Remarkably increased frequencies and persistence of p53-reactive $CD8^+$ T cells and elevation of expression of multiple immune response genes were detected in 2/3 patients.

DNA-based vaccines

In plasmid DNA-based vaccines, Ag presenting cells take up the plasmid DNA, transcribe the gene into an immunogenic protein, and process and present it to the immune system. A phase I clinical trial of a Mammaglobin-A (MAM-A) DNA vaccine in metastatic BC patients revealed that this compound is capable of eliciting MAM-A specific $CD8^+$ T cell responses. Significantly improved PFS was observed in the vaccinated patients, even though the sample size was low (n = 14) [98,99].

Dendritic cell-based vaccines

In vitro activated DCs, cultured with IFN-γ and bacterial lipopolysaccharide (LPS) become polarized and consequently able to secrete IL-12. These DCs were pulsed with HER2-specific HLA-I and -II peptides and were intranodally administered to patients with ductal carcinoma *in situ* of the breast before surgery. Vaccinated subjects had a higher *ratio* of T cell co-stimulatory CD28/inhibitory CTLA-4 expressing T cells, high rates of peptide-specific sensitization of both IFN-γ-secreting $CD4^+$ and $CD8^+$ T cells [49], with accumulation of T and B cells in the breast, and induction of complement-dependent, tumor-lytic Abs. Notably, for the first time an active process of "immunoediting" for HER2+ tumor cells following vaccination was observed, since a decreased HER2 expression in surgical tumor specimens was detected in some patients. High levels of durable HER2 specific $CD4^+$ and $CD8^+$ T cell immunity lasting 52 months after immunization were also observed [100]. A follow up trial in 54 patients with early HER2+ BC evaluated the route of administration (19 patients = receiving intralesional, 19 patients = intranodal, and 16 patients = both intralesional and intranodal injection) [101]. No differences in immune response or clinical activity were observed across the different routes of administration.

Morse *et al.* tested a HER2 ICD protein-containing DC vaccine in stage II, III and IV HER2+ (in > 50% tumor cells) disease-free BC patients after surgery and systemic therapy [102]. Immature and mature cultured DCs, or mature Flt3-ligand mobilized peripheral blood DCs were loaded with HER2 ICD, or *tetanus* toxoid, KLH and cytomegalovirus (CMV) peptide as controls, and were administered intradermally/subcutaneously. DTH reactions at the site of injection were observed in most of the patients (6/7). Remarkably at > 5 year follow up 6/7 patients had circulating anti-HER-2 ICD Abs and all patients were alive and disease free at 4.6–6.7 years of follow-up.

Another trial tested an autologous DC vaccine, where DCs were generated with IL-4 and GM-CSF stimulation *in vitro* and pulsed with six HLA-A*0201 binding wild-type p53-derived peptides, then given with IL-2 to HLA-$A2^+$ patients with metastatic BC [103]. One third of the patients with p53 tumor over-expression had SD [50]. Interestingly, the frequency of Tregs was almost doubled after vaccination, and patients with PD had a decrease in the percentage of *naïve* T cells [102,103].

In stage II-III HER2-negative BC, a phase II trial (NCT01431196) tested a vaccine consisting of DCs pulsed with each subject's own tumor, administered after anthracycline- and taxane based neoadjuvant chemotherapy. The study has been completed and results are awaited. Table 5 summarizes the main trials of DC-based BC vaccines.

Table 5
Results from Clinical Trials testing Recombinant or Dendritic Cell-based Breast Cancer Vaccines.

Clinical trial reference	Trial phase	Number of vaccinated patients	Compound(s)	Antigen	Breast Cancer Subtypes	Outcome(s)
Avigan D. *et al.* [42]	I	16/32	Fusion vaccine: KLH-pulsed DCs, derived from co-cultured patient PBMC's derived DCs with tumor cells	BC cells from pleural effusions, malignant ascites, superficial LNs, chest wall/breast tissue, bone marrow	Luminal and HER2+	1 CR: > 50% decline in serum CA 15–3 (MUC-1); rise over time in the tumor-induced percentage of $CD4^+$ and $CD8^+$ T cells expressing intracellular IFN-γ; 1 PR; 1 SD
Svane I.M. *et al.* [50]	I	6 ($HLA\text{-}A2^+$)	p53-peptide-pulsed autologous DCs	3 wild-type and 3 modified p53 peptides	NA	2 SD; 1 transient regression; 1 mixed response
Czerniecki B.J. *et al.* [49]	I	13 (DCIS)	Autologous DC-1s pulsed with HER-2/neu HLA class I and II peptides	6 MHC class II HER-2/*neu*–derived peptides	HER2/neu overexpression (> 2 + intensity) in at least 10% of cells	Induction of HER2/*neu*–reactive $CD4^+$ T cells; Induction of $CD8^+$ T cells that directly recognize HER2-overexpressing BC cell lines; Accumulation of lymphocytes in the breast and changes in residual DCIS; Induction of complement dependent antibody-mediated cytotoxicity
Mohebtash M. *et al.* [47]	I	12	Recombinant fowlpox-CEA(6D)/MUC-1(L93)/TRICOM vaccine	CEA(6D)/MUC-1(L93)	Luminal, HER2-positive, TNBC	1 CR; 4 SD; 7 PD; drop in the cytokines and chemokines produced mainly by tumor cells, such as IL-8, IL-6, and TNF-α, corresponded with clinical response

Legend: BC: breast cancer; **CR**: complete response; **DC**: Dendritic cell; **DCIS**: ductal carcinoma *in situ*; **HER2**: Human epidermal growth factor receptor-2; HLA: Human Leukocyte Antigen; **IFN**: interferon; **IL**: interleukin; **KLH**: Keyhole Limpet Haemocyanin; **LN**: lymph node; **MUC-1**: mucin 1; **NA**: not available; **PBMC**: peripheral blood mononuclear cells; **PD**: progressive disease; **SD**: stable disease; **TNBC**: Triple Negative Breast Cancer; **TNF**: tumor necrosis factor.

Multi-peptide vaccines and personalized vaccines targeting neoantigens

Recently, multi-peptide vaccines and personalized vaccines targeting neoAgs specific to patient tumors have been and are currently tested in early phase clinical trials in BC.

Takahashi *et al* employed a personalized peptide vaccine administered subcutaneously in a phase II clinical trial of 79 patients with metastatic BC, demonstrating the feasibility of a personalized vaccine strategy for BC patients [104]. Here Ags were selected based on the induction of higher peptide-specific IgG responses in pre-vaccination plasma of 4 HLA-matched peptides which were selected from pooled peptide candidates applicable to the four HLA Class IA phenotypes. T cell and Ab responses were assessed through measurement of peptide-specific CTL and IgG responses. CTL boosting was a prognostic factor for OS but not for PFS in triple-negative (TNBC) patients whereas IgG boosting was a prognostic factor for PFS and OS in HER2+ patients. There was no association of immune boosting with PFS or OS in luminal HER-2 negative patients.

A 9-peptide BC vaccine including MHC-I-restricted BC-associated peptides (from MAGE-A1, −A3, and -A10, CEA, NY-ESO-1, and HER2 proteins) *plus* the TLR3 agonist poly-ICLC with a helper peptide derived from *tetanus* toxoid was given in early stage BC [105], demonstrating its safety and feasibility. However, the study was terminated early due to the lack of observed $CD8^+$ T cell responses to the 9 peptides in the vaccine by direct ELISPOT assay in the first 11 participants.

Discussion and conclusions

BC vaccines, like vaccines for other cancer types, have been tested for years, with limited evidence of clinically meaningful activity despite the induction of Ag-specific immunity. So far, none of these agents has shown significant clinical benefit in phase III trials in either the metastatic or adjuvant setting. The lack of success to date is likely primarily related to suboptimal vaccine formulations (tumor Ags and adjuvants) that have limited potency for inducing tumor-specific T cells, lack of informative biomarkers of response, and dominant pathways of immune suppression at the tumor site. Remarkably, BC vaccines have historically often been tested as single agents in patients with metastatic disease, where both disease burden and the suppressive TME play a major role in limiting the activity of T cells. To overcome these limitations vaccines have also been tested in the adjuvant setting, where disease burden is minimal. In this setting vaccines have been incorporated into combination regimens including chemotherapy or targeted therapy (e.g., trastuzumab) with the aim of increasing their therapeutic activity. So far, a combination approach of cancer vaccines with chemotherapy and ICB might represent one of the most promising strategies aiming to increase vaccine activity and efficacy. In addition, multi-epitope vaccines and/or personalized vaccines delivering multiple Ags that may be expressed by patients' tumors (neoAgs) are being tested. However neoAg vaccines represent nowadays a big challenge, for a variety of factors, e.g., long time and good practice conditions for manufacture, costs for identification and validation of neoAgs, and the need for further optimization of neoAg prediction algorithms. Another crucial point are the criteria for the evaluation of the efficacy of neoAg vaccines, that should be mainly based on immune monitoring evaluating vaccine-induced immune responses, instead of clinical end points that are defined by immune-related response criteria, reviewed in [106,107].

Patient selection, vaccine delivery route (e.g., subcutaneous *vs.* intratumoral, to maximize bioavailability in the TME), timing (choice of the setting: adjuvant, neoadjuvant or metastatic), their combination and/or their optimal sequence with other therapies will need to be carefully investigated. Multi-modality strategies, adapted to individual patients' baseline immune response against the tumor (e.g. through TIL assessment or the evaluation of the general immune status, like the evaluation of C-reactive protein, neutrophil to lymphocyte ratio, lactate dehydrogenase, eosinophils) might represent some of the strategies to select patients for personalized treatments in order to increase the efficacy of immunotherapy in BC [108]. Appropriate clinical trial designs and efficient immune monitoring approaches will also be critical for the effective clinical activity of BC vaccines.

Although advances in our understanding of immunoregulation and the interactions between tumor cells and the immune system have led to the development of strategies that manipulate both positive and negative immune checkpoints, many challenges still remain in BC [109]. Blockade of the PD-1/PD-L1 pathway can unleash the activity of pre-existing T cells [110], and applying antagonists of PD-1/PD-L1-mediated immune suppression is one promising way to overcome barriers to vaccine-induced T cell activity within the TME.

Despite the clinical activity of targeting the PD-1/PD-L1 pathway in advanced BC, the efficacy of ICB itself remains suboptimal with most patients failing to respond, and most of the clinical activity observed in patients to date is in TNBC treated in the 1st line metastatic setting, and who express PD-L1 on tumor-infiltrating immune cells ($PD\text{-}L1^+IC^+$) [109,111–114]. Recently, the phase III Impassion130 trial demonstrated the superiority of the combination of the anti-PD-L1 Ab atezolizumab *plus* nab-paclitaxel *vs.* nab-paclitaxel as a 1st line treatment for metastatic $PD\text{-}L1^+IC^+$ TNBC [111]. Concurrent or sequential PD-1/PD-L1 ICB administered with BC vaccines might represent a future strategy aimed at enhancing immunotherapy response rates by inducing greater T cell infiltration in the breast TME.

In the end, BC vaccines will likely have the greatest impact in adjuvant and neoadjuvant treatment settings, for the secondary prevention of relapse in patients with a personal history of BC, and ultimately for the primary prevention in individuals at high risk for the development of BC.

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Declaration of Competing Interest

Authors declare that no conflict of interest exist.

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