



## REVIEW ARTICLE

# Current Approaches in Development of Immunotherapeutic Vaccines for Breast Cancer

Adil Allahverdiyev, Gamze Tari, Melahat Bagirova, Emrah Sefik Abamor

Department of Bioengineering, Faculty of Chemical and Metallurgical Engineering, Yildiz Technical University, Istanbul, Turkey

Cancer is the leading cause of death worldwide. In developed as well as developing countries, breast cancer is the most common cancer found among women. Currently, treatment of breast cancer consists mainly of surgery, chemotherapy, hormone therapy, and radiotherapy. In recent years, because of increased understanding of the therapeutic potential of immunotherapy in cancer prevention, cancer vaccines have gained importance. Here, we

review various immunotherapeutic breast cancer vaccines including peptide-based vaccines, whole tumor cell vaccines, gene-based vaccines, and dendritic cell vaccines. We also discuss novel nanotechnology-based approaches to improving breast cancer vaccine efficiency.

**Key Words:** Breast neoplasms, Immunology, Immunotherapy, Vaccines

## INTRODUCTION

Cancer remains the most important health issue of the day. It is estimated that more than 26 million people will be diagnosed with cancer, resulting in 13 millions death in 2030 [1].

Cancer frequency is increasing by 1% to 2% per year in almost every country in the world. Breast cancer (BC) accounts for approximately 30% of female cancers in the United States and approximately 25% of all cancers worldwide. The risk of developing BC during her lifetime is an estimated 10% to 12.8% [1,2].

Biological factors primarily associated with BC are the estrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor receptor 2 (HER2), and proliferation. Triple-negative breast cancer (TNBC) is characterized by ER, PR, and HER2 expression deficiency. Studies aimed at finding a tumor marker specific for BC have been continuing for many years. However, no biochemical or immunochemical substance that

can be quantified has been determined yet [3].

The main methods used in BC therapy are surgery, radiotherapy, chemotherapy, hormone therapy, and targeted therapy [4]. The most important goal of developing any effective cancer treatment method must be the exclusive destruction of tumor cells while preventing cancer recurrence. Immunotherapy is a new generation treatment method developed to overcome side effects of conventional treatments while increasing effectiveness by providing tumor-specific treatment via targeting of tumor cells with antigen differences [5].

Owing to the weak antigenicity of antigenic structures in cancer cells, cancerous cells cannot be detected via immunosurveillance or eliminated by immunocytes, which allows the tumor to develop unhindered [6].

Immunotherapy is an approach that stimulates immune system cells and renders them more effective. In addition to supporting the immune system, the wider goal of immunotherapy is to allow immune system cells to recognize tumor antigens more effectively and specifically attack and destroy cancer cells [7].

## GENERAL APPROACHES ON VACCINE DEVELOPMENT AGAINST BREAST CANCER

### Peptide-based vaccines

In order to develop an effective therapeutic vaccine against BC, self-antigens of breast tumor cells that serve as tumor targets are used. The HER2 protein, specific for both trastuzum-

**Correspondence to:** Adil Allahverdiyev

<https://orcid.org/0000-0002-7031-5986>

Department of Bioengineering, Faculty of Chemical and Metallurgical Engineering, Yildiz Technical University Davutpasa Campus, Davutpasa Street No. 127, 34210 Esenler, Istanbul, Turkey  
Tel: +90-212-383-46-39, Fax: +90-212-383-46-25  
E-mail: adilmoglu@gmail.com

This research was supported by Scientific Researches and Project Coordinatorship of Yildiz Technical University, Istanbul, Turkey with project support numbers FYL-2017-3202 and 2015-07-04-KAP04.

Received: May 31, 2018 Accepted: August 27, 2018

ab and pertuzumab monoclonal antibodies, was the first antigen to be identified as a target for BC immunotherapy. HER2, carbohydrate antigens (such as carcinoembryonic antigen), and Mucin-1 (MUC-1) are the most commonly studied BC antigens for the purpose of vaccine formulation. However, antibody response to HER2 and MUC-1 antigens in BC patients is very low. Peptide vaccines have been used mostly with the granulocyte macrophage colony stimulating factor (GM-CSF) cytokine adjuvant to enhance efficacy [8].

Of the different combinations that may target MUC1 tumor associated antigen (TAA), the BLP25 MUC1 lipopeptide-containing mixture has been used against BC, in addition to chemotherapy and showed an increase in CD8<sup>+</sup> T responses. Currently, the possibility of using the MUC1 antigen in vaccine formulation is being tested in many clinical trials.

Bacille Calmette-Guérin (BCG) conjugated with MUC1 antigen and human interleukin-2 (IL-2) vaccine caused an increase in the cytotoxic T lymphocyte (CTL) response in mouse models [8]. This vaccination approach has been shown to be more effective than the formulation containing the polyinosinicpolycytidylic (poly-IC) stabilized with poly-lysine and carboxymethylcellulose (poly-ICLC) adjuvant [9].

Adjuvants and helper peptides have been used to improve the clinical efficacy of peptide vaccines. An example of this approach is study conducted by Dillon et al. [10], using the major histocompatibility complex class I-restricted BC-associated and Toll-like receptor 3 agonist poly-ICLC adjuvant.

Mittendorf et al. [11] demonstrated the efficacy of the HER2/neu derived peptide GP2 with nine amino acids (654–662: IISAVVGIL) peptide vaccine in preventing tumor recurrence, when GP2 is used together with the GM-CSF adjuvant.

Torres-García et al. [12] evaluated parasite-derived immunomodulatory 18-amino acid (aa) GK-1 peptide adjuvant *in vivo* and reported an enhanced inhibitory effect on invasive BC and its metastasis to lungs.

Currently, NeuVax (Galena Biopharma, San Ramon, USA), and Pan-T-cell epitope+a neolactoseries antigen Lewis Y+the ganglioside GD2 mimicking carbohydrate peptide containing vaccine P10s-PADRE (AmbioPharm Inc., North Augusta, USA), are being evaluated in clinical trials for use against BC. Further examples of clinical trials on peptide vaccines are shown in Table 1 [10,13-19].

### Whole-cell vaccines

Vaccine platforms based on direct application of whole tumor cells or cell extracts to the patient, as an alternative to antigen-specific vaccination with peptide or protein subgroups, are direct polyvalent immunization strategies [20]. Whole-cell tumor vaccines present multiple and unknown cancer anti-

gens in contrast to epitope-specific vaccines. However, according to current research these vaccines are poorly immunogenic [21].

Whole-cell tumor vaccines are prepared by irradiating allogeneic or autologous cancer cells or cell lysates, which inhibits their ability to replicate inside the patient following vaccination [22]. Irradiating cancer cells enhances tumor recognition by CTLs and antigen-presenting cells [23].

Certain modifications have been made in order to increase vaccine immunogenicity. The GVAX (Aduro Biotech, Berkeley, USA) vaccine includes irradiated allogeneic tumor cell lines which are modified to secrete the cytokine GM-CSF [24]. The potency of the GM-CSF secreting whole cell BC vaccine can be enhanced by using chemotherapeutic agents.

Huang et al. [25] added IL-2 cytokine and blocked immunosuppressive anionic phospholipid phosphatidylserine in the whole cell cancer vaccine to increase its efficiency. An increase in the tumor-specific cytotoxicity response and interferon (IFN)- $\gamma$  cytokine release has been observed in vaccinated mice. Convit et al. [26] prepared a vaccine formulation including whole-tumor cells, formalin, and BCG adjuvant to improve vaccine immunogenicity and proved the tolerability, safety, and nontoxicity of the formulation based on the results of animal research. The results of this study indicated an overall survival of 60% in humans. The vaccine formulation resulted in a lower tumor growth rate and boosted immune cell activation in the mouse model. Promising results in the term of a decrease in the infiltration of suppressor cells and improved immune memory have been observed [27].

Immunotherapy with vascular endothelial growth factor receptor 2 (VEGFR2) model antigen showed great potential as a tumor vaccine. Researchers irradiated the adenovirus encoding VEGFR2 (AdVEGFR2)-infected whole cancer cell vaccine-based immunotherapy to improve its efficacy. The results of this study indicate that angiogenesis inhibition, CTL activation and increased expression of the high mobility group box 1 (HMGB1) and 70 kilodalton heat shock proteins in AdVEGFR2-infected cells may be used as an effective strategy in cancer immunotherapy [28].

MDA-MB-231 cell line has been used as a whole tumor cell vaccine in a stage IV BC clinical trial. This cell line is an HLA-A21, HER2/neu1 allogeneic BC cell line, which was genetically modified to express the costimulatory molecule CD80 (B7-1). This formulation was used with GM-CSF and BCG adjuvant, resulting in a costimulatory signal and improved antigen presentation capability in patient T cells. It has been proved that the vaccine formulation is safe and feasible [29]. Further examples of clinical trials on whole cell vaccines are shown in Table 1.

**Table 1.** Clinical trials on breast cancer vaccines

ID of clinical trial	Vaccine name	Study phase	Vaccine formulation	Trial enrollment	Trial period	Results
NCT00925548	Stimuvax (tecemotide, L-BLP25) - STRIDE (Stimulating Immune Response In a Variable Breast Cancer)	III	L-BLP25 MUC1 lipopeptide-containing mixture	ER+ and/or PR+, inoperable locally advance, recurrent, or metastatic BC	2009–2010	Permanently terminated due to clinical signs and symptoms of BC after investigational new drug application for tecemotide.
ABCSG-34	Stimuvax (tecemotide, L-BLP25)	II	L-BLP25 MUC1 lipopeptide-containing mixture	Preoperative treatment of women with primary BC	2012–2017	In 2017 San Antonio Breast Cancer Symposium; 93.2% of patients in the neoadjuvant chemotherapy group resulted in high-risk Endopredict score which is related to residual cancer burden. In the neoadjuvant endocrine therapy group, 44% of patients have a low Endopredict score and 39% of patients have a high Endopredict score.
-	-	I	MUC1- KLH conjugation+QS-21 adjuvant	High risk BC without evidence of disease	1995–1996	Increased antibody response, no significant effect on T cell response, some recurrences and side effects [13].
-	-	III	Oxidized mannan-conjugated MUC-1	Postmenopausal women treated surgically with or without local radiation for stage II ER+ breast carcinoma	1997–2006	60% recurrence rate in the control group following 12–15 years in ER+ patients, while it has been reported to be quite low (12%) in the vaccinated group [14].
NCT01479244	NeuVax (nelipepimut-s)	III	E75-GM-CSF conjugation	Disease-free, node+ BC	2011–2016	Increased CTL responses, DFS time is 85.7%, prolonged median time to recurrence [15,16].
NCT01570036	NeuVax (nelipepimut-s)	II	E75-GM-CSF conjugation in combination with herceptin	HER2 IHC1+/2+ and node+ BC	2012–currently ongoing	No result posted yet.
NCT02297698	NeuVax (nelipepimut-s)	II	E75-GM-CSF conjugation in combination with trastuzumab	High-risk, HER2+ or node+ BC	2014–currently ongoing	No result posted yet.
NCT01390064	P10s-PADRE	I	Pan-T-cell epitope and a neolactoseries antigen Lewis Y (LeY) and the ganglioside GD2 mimicking carbohydrate peptide (CMP)+MONTANIDE™ ISA 51 VG adjuvant	Stage IV BC	2011–currently ongoing	Well-tolerated, induces functional proapoptotic antibodies, provide potential clinical benefit [17].
NCT02229084	P10s-PADRE (Chemovax)	I/II	P10s-PADRE with chemotherapy, MONTANIDE™ ISA 51 VG adjuvant or chemotherapy and adjuvant	Stage I, II or III ER+ BC	2014–currently ongoing	No result posted yet.
NCT02938442	P10s-PADRE (Chemovax)	II	P10s-PADRE with MONTANIDE™ ISA 51 VG	TNBC	2016–currently ongoing	No result posted yet.
NCT01532960	Breast 41	I	9 Major histocompatibility complex (MHC) class I-restricted BC-associated peptides (from MAGE-A1, -A3, and -A10, CEA, NY-ESO-1, and HER2 proteins) with TLR3 agonist poly-ICLC adjuvant	Stage I–IV BC	2012–2015	High peptide specific CTL responses, no dose-limiting toxicity. Terminated due to lack of ELISpot responses [10].

(Continued to the next page)

Table 1. Continued

ID of clinical trial	Vaccine name	Study phase	Vaccine formulation	Trial enrollment	Trial period	Results
NCT02826434	PVX-410	Ib	Tetra-peptide vaccine XBP1 (2 splice variants), CD138 and CS1+durvalumab	Stage II/III TNBC	2016–currently ongoing	No result posted yet.
NCT03362060	PVX-410	I	Tetra-peptide vaccine XBP1 (2 splice variants), CD138 and CS1+ pembrolizumab	TNBC	2017–currently ongoing	No result posted yet.
NCT00179309	PANVAC	II	Recombinant vaccinia virus and recombinant fowl poxvirus encode MUC-1 and CEA TAAs, as well as T cell costimulatory molecules, intracellular adhesion molecule-1 and leukocyte function-associated antigen-3 in combination with docetaxel	Metastatic BC	2005–2013	Safe and doubles the PFS compared to chemotherapy.
NCT02960594	INO-1400, INO-9012 and INO-1401	I	A plasmid encoding hTERT	Many types of cancers and BC patients who are at high risk of tumor relapse	2014–currently ongoing	The immune system of the patients has been activated to intensify CTL.
NCT02348320	-	I	Personalized polyepitope DNA vaccine encoding patient's own immunogenic TAAs which are selected after genome profiling of the patient's BC cells	TNBC	2015–currently ongoing	Safety and immunogenicity of vaccine are under examination.
NCT01730118	Ad/HER2/neu DC	I	Autologous adenovirus HER2-transduced DC vaccine	Metastatic BC	2012–currently ongoing	No result posted yet.
NCT00923143	-	I/II	HER2/neu pulsed DC1	DCIS	2009–currently ongoing	Safety and immune response of vaccine are under examination.
NCT01042535	Ad.p53 DC	I/II	Adenovirus p53-transduced DCs in the combination with 1-methyl-D-tryptophan	Metastatic BC	2009–2014	Well tolerated, safe, median PFS approximately 7 weeks.
NCT00978913	-	I	DCs transfected with survivin, hTERT and p53 mRNA with cyclophosphamide	BC and malignant melanoma	2009–2014	Feasible and well-tolerated. 41% of treated melanoma patients remained stable and 3 of 18 patients underwent tumor diminution [18]. No result for BC yet.
NCT00622401	-	I/II	DCs/tumor cell fusion vaccine+IL-12	Stage IV BC	2009–2014	Terminated, patients had adverse events [19].
NCT00093834	-	IV	Allogeneic tumor cells modified to secrete the cytokine GM-CSF in combination with cyclophosphamide and doxorubicin	Stage IV BC	2004–2008	Improved the vaccine activated immunity in patients with advanced cancer than the minimal residual disease [20].

MUC1 = Mucin-1; ER = estrogen receptor; PR = progesterone receptor; BC = breast cancer; KLH = keyhole limpet hemocyanin; GM-CSF = granulocyte macrophage colony stimulating factor; CTL = cytotoxic T lymphocyte; DFS = disease-free survival; HER2 = human epidermal growth factor receptor 2; IHC = immunohistochemistry; TNBC = triple-negative breast cancer; CEA = carcinoembryonic antigen; TLR3 = Toll-like receptor 3; poly-ICLC = carboxymethylcellulose, polyinosinic-polycytidylic acid, and poly-L-lysine double-stranded RNA; TAA = tumor associated antigen; DC = dendritic cell; hTERT = human telomerase reverse transcriptase; DCIS = ductal carcinoma *in situ*; PFS = progression-free survival; IL-12 = interleukin 12.

Although using tumor cell antigens has improved the efficacy of these applications, the inability of recognition of immunocompetent agents, which are suppressed by tumor cells,

to recognize the immunogens may be one of the greatest reasons underlying the inability of these vaccine formulations to achieve the desired activity.

### Gene-based vaccines

In recent years, innovative gene-based vaccines have also been developed for use in treating BC. Recombinant viral vector vaccines based on New York Vaccinia, modified virus of Ankara (MVA) and canarypox virus have been designed to express TAAs such as HER2, p53, and MUC1, and have been generally tested in clinical trials involving metastatic BC patients [30].

Kwilas et al. [31] designed a poxviral-based cancer vaccine using MVA including the Twist transgene and a triad of costimulatory molecules (B7-1, intercellular adhesion molecule 1, lymphocyte function-associated antigen 3) and evaluated its efficiency in metastatic BC and prostate cancer models. They targeted the Twist transcription factor which plays an important role in metastasis, poor prognosis, and drug resistance, and observed both CD4<sup>+</sup> and CD8<sup>+</sup> Twist-specific T-cell responses *in vivo*.

Although these viral vaccines produce highly potent CTL responses to encoded TAAs, they pose a risk in clinical practice because of the potential for inducing strong immunity to the viral construct and, in some cases, neutralizing antibodies. In addition, a major issue preventing intensive use of the viral vector-based vaccines is the high level of immune response to viral antigens as compared to coded antigens. A similar issue exists in adenovirus-based vaccines. Elimination of certain high immunogenic motifs such as the *E1a* and *E2b* genes may attenuate antibody neutralization after vaccination [32].

However, development of DNA vaccines has afforded a new perspective to cancer immunotherapy. The approval of Oncept™ (Merial, Duluth, USA), a plasmid coding for human tyrosinase, by Food and Drug Administration for canine melanoma proved the importance of DNA vaccines in 2010 [33].

Nazarkina et al. [34] designed a polyepitope DNA vaccine encoding immunogenic peptides of HER2 and mammaglobin-1 tumor antigens and optimized its delivery to dendritic cell (DC). It was demonstrated that secretion of IL-6 from DNA vaccine transfected DC, indicating DC maturation, and has great capacity to induce an immune response. Further examples of clinical trials on whole cell vaccines are shown in Table 1.

Although promising results have been obtained using recombinant vaccines in BC immunotherapy, a major risk associated with the use of these vaccine formulations is the possibility of oncogene activation and stimulation of cancer, as well as the possibility that genetic material in the vaccine may fuse with the genome of healthy cells in the patient.

### Dendritic cell-based vaccines

One of the most important approaches developed for treat-

ing BC is the DC-based immunotherapeutic vaccine. DC-mediated vaccine development approaches against BC are based on preparing synthetic peptides in accordance with the structure of antigens on BC cells, and obtaining immunogenic antigens by killing autologous or allogeneic BC cells using various means such as gamma irradiation, chemical sensitization, treatment with chemotherapeutic drugs or freeze-thaw-based techniques and loading the isolated antigens *in vitro* to DCs. In addition, tumor cells may be fused with DCs using polyethylene glycol (PEG) or electrofusion technology, thereby providing tumor antigen presence on DCs [35].

Her2/neu and MUC1 are peptide antigens that are widely used in DC vaccines. Lapuleucel-T (APC8024), overexpressing HER2 peptide, which has been developed for use in cancer patients and tested in phase I clinical trials, is obtained via the recombinant fusion protein BA7072 in DCs of peripheral blood mononuclear cells (PBMC) origin *in vitro* [36]. This fusion protein is a product of the conjugation of intracellular and extracellular domains of HER2 with the GM-CSF adjuvant. This study proved the safety and anti-HER2 effect of Lapuleucel-T. It also indicated that this formulation may cause less side effects compared to Sipuleucel-T vaccine [36].

However, since such vaccination approaches can only be used in BC patients overexpressing HER2 or MUC-1 peptides, their efficacy for use in other types of BC that do not express these peptides or do not have a specific antigen, such as TNBC, is considered to be insufficient.

Additionally, certain studies using genetically modified DCs have been reported. Sakai et al. [37] used recombinant adenovirus-transduced bone marrow-derived DCs (BMDCs) encoding neuroproteins in the vaccination of the BALB-neuT BC mouse models. The vaccine has shown positive results such as specific anti-neu antibody production, improved interferon gamma release, and delayed carcinoma development in mice. However, this study is valid only for HER2/neu-expressing BCs and is considered to be inadequate for other BC cell.

Other studies involve the use of all tumor antigens in order to remove limitations caused by utilizing single or multiple antigens in DC-based immunotherapeutic approaches. These studies are based on the fusion of tumor cells and DCs (PEG and electrofusion), or loading of tumor cells into DCs using various techniques for obtaining antigens (gamma irradiation, treatment with chemotherapeutic drugs, and so forth).

In one such study, Gong et al. [38] demonstrated that lysis of cancer cells may be achieved by evaluating *in vitro* CTL response of hybrid cells formed via performing PBMC-based DC-tumor cell fusion PEG. In 2007, Koido et al. [39] performed PEG-mediated fusion with DCs of MCF-7 and BT-20

tumor cells and estimated the efficacy of this vaccine both *in vitro* and in a mouse model *in vivo*. Although *in vitro* studies have shown high CTL responses, the main disadvantage of this study is that the vaccine prepared using human BC cell lines, was tested only in mouse cancer models. This prevents achievement of reliable results. Bird et al. tested PEG fusion-based DC vaccines in dog models in two different studies in 2008 and 2010. The former involved the fusion of DCs with allogeneic cancer cells [40] whereas the latter involved fusion with autologous cancer cells [41]. Effective CTL responses were not observed in these studies. It is proposed that DC vaccines based on PEG fusion are different from DC function and antigen presentation, and that vaccine based on such formula may suppress immune responses by activating the Treg cells.

Interest in electrofusion technology in the production of DC-based vaccines has increased recently owing to its usefulness in avoiding toxic effects of PEG fusion as well as increasing the efficiency of fusion. Zhang et al. [42] performed the fusion of DCs with MCF-7 BC cell line using an electrofusion device. Analysis of the immune system-stimulating effect of *in vitro* generated hybrid cells, determined that, in addition to high T lymphocyte proliferation and cytokine release responses, MCF-7-specific CTL responses could also be obtained. However, research has indicated that DC-based vaccines generated by allogeneic tumor cells in this manner, may only be useful for vaccination protocols on an experimental basis.

In another study, autologous BC cells were electrofused to autologous DCs, where it was reported that formulations in which other products of the fusion mixture were used, demonstrated a more effective immunostimulatory effect than the ones using purified hybrid cells [43]. Zhang et al. [44] performed electrofusion of PBMC-derived DCs with MDA-MB-231 cell lines, evaluated *in vitro* T cell proliferation, IL-12 and IFN- $\gamma$  release, and tumor-specific CTL responses, and reported more effective responses compared to control groups. The results of this study showed that fusion with of DCs with whole BC cells produced an effective cancer specific anti-tumor T cell response *in vitro*, which made such a procedure a promising candidate for use in adoptive immunotherapy. Studies indicate that immunotherapeutic dendritic/cancer cell fusion hybrids may inhibit the induction of CTL responses due to soluble factors derived from tumor cells and that the formation of immunosuppressive regulatory T cells is supported [45].

Isolation of antigens from cancer cells, used as an antigen source, to be loaded to DCs can be achieved in a similar manner as Neidhardt-Berard et al. [46], who performed MCF-7

cell line culturing in serum-free medium and gamma irradiation and HCC1806 cell line anti-Fas monoclonal antibody, CH11 and cycloheximide desensitization in the form of killing. The researchers tested *in vitro* CTL responses and IFN- $\gamma$  cytokine release by loading the antigens to DCs and enabling those with T lymphocytes. While this approach demonstrated the efficacy of stimulating immunosystem cells, it also emphasized that different methods of antigen isolation may be necessary since the killing methods used, did not demonstrate similar levels of efficacy in all BC cells. In a study conducted by Saito et al. [47], T47D and Hs578T cell lines were been killed using betulinic acid. The researchers induced CTL formation by culturing the tumor lysates with DCs and T lymphocytes. Cyclin B1-MUC1- and survivin-specific CTL responses were determined *in vitro*. However, betulinic acid killing is not a commonly reported technique in the literature.

Delirez et al. [48] determined the immunostimulatory efficacy of DCs loaded with gamma  $\gamma$  irradiation killed human autologous BC cells *in vitro*. Antigen-loaded DCs were cultured with autologous T lymphocytes from patients, resulting in high T cell proliferation and IFN- $\gamma$  cytokine production response. Individual-specific CTL response emphasizes the importance of autologous cell use in the vaccine protocol. However, the gamma irradiation based killing method is not effective in killing every cancer cell.

The freeze-thaw method, which causes instantaneous temperature changes in the cell leading to isolate of whole antigens, is a method that has been proved reliable for many. This method is often used in anticancer studies because of the low number of antigens identified on cancer cells and the ability to release certain idiotypic epitopes or antigens, which are products of mutational events occurring throughout the tumorigenic process in the cancerous cells [49].

The heat shock soluble proteins obtained by the freeze-thaw method increased antigen presentation and expression of helper stimulating molecules in DCs [50]. As an example of this method, the *in vitro* and *in vivo* immunotherapeutic efficacy of a vaccine formulation prepared by loading freeze-thawed antigens of BC cells onto DCs was evaluated by Delirez et al. [51]. The study concluded that both CD4<sup>+</sup> and CD8<sup>+</sup> T lymphocyte-mediated immunity was acquired *in vitro*.

Another immunotherapeutic approach in the treatment of BC is a vaccination involving loading of DCs with antigens of cancer stem cells, which are the main factors that cause inadequacies in traditional treatments. Although only a few studies using this approach have been reported, it may be beneficial both in preventing recurrence as well as metastasis of cancer stem cells, and in providing immunosuppression by permanent memory cells in case the resting cancer stem cells be-

come active.

Nguyen et al. [52] assessed the specificity of DCs developed to overcome cancer stem cells both *in vitro* and *in vivo*. 4T1 tumor cells from BALB/c females were developed using the verapamil-resistance selection method and their antigens collected via freeze-thaw method were loaded onto mouse BMDCs. This improved vaccine formulation stimulated breast cancer stem cell (BCSC)-specific CTL responses in *in vitro* studies. Improvements were also observed in tumor size, as indicated by a decrease in the tumor size of the treated group *in vivo* in tumor-induced Balb/c mouse models of BCSC tumors. These results demonstrated that DCs may be used in cancer stem cells as well as in the treatment of drug-resistant cancer cells.

Following demonstration of the importance of DC vaccines in BC immunotherapy in both *in vitro* and *in vivo* studies, clinical trials in this field as well as studies on DC vaccines have increased dramatically. Detailed explanations of these studies are presented in Table 1.

### Nanotechnology on breast cancer vaccines

Recent developments in the field of nanotechnology show a great promise in providing solutions to the insufficiencies currently affecting the development of effective vaccines against BC. Nanoparticles (NPs) confer many advantages to vaccine technology such as increased circulation time, immunogenicity, biocompatibility and solubility of hydrophobic antigens, controlled as well as targeted release and protection of peptide antigens from enzymatic degradation. Nanosized particles in the 10–50 nm are able to easily reach DCs in the lymphatic system. Also, larger NPs are mostly captured by macrophages. Thus nano-designed vaccine formulations can boost immune system components much more effectively [53].

Polymer-peptide conjugates are one of the most common nano delivery systems used in vaccine development. Razazan et al. [54] conjugated GP2, a HER2/neu-derived peptide, to distearoylphosphoethanolamine-N-(maleimide[polyethylene glycol]-2000) micelles. Next, the peptide, including micelle, was inserted into liposomes composed of (dimyristoylphosphatidylcholine [DMPC], dimyristoylphosphoglycerol [DMPG]) phospholipids, and fusogenic lipid dioleoyl phosphatidylethanolamine (DOPE) containing monophosphoryl lipid A (MPL) adjuvant (DMPC-DMPG-DOPE-MPL-GP2). The researchers have evaluated the immune response of BALB/C mice immunized with the vaccine formulation. Liposome-DOPE-MPL-GP2 vaccine formulation produced the high level of IFN- $\gamma$  release and increased CTL responses. The prophylactic effect of liposome-DOPE-MPL-GP2 was determined as a mean tumor growth delay of 92.22%.

Similarly, Arab et al. [55] prepared E75 antigens attached liposomes consisting of distearoylphosphocholine/distearoylphosphoglycerol/cholesterol (Chol)/DOPE nanovaccine to improve immunogenicity of the peptide and evaluated immune responses in vaccinated mice. The results of this study indicated that the vaccine formulation had dramatically increased the CTL response. The significantly prolonged survival time was indicated as a mean tumor growth delay of 72.4%.

Another nano carrying system for peptide vaccines was designed as the AE36 HER2/neu-derived peptide with or without the CpG adjuvant. The AE36 peptide was encapsulated in liposomes and its prophylactic and therapeutic effects were evaluated using mouse models. The liposomal formulations significantly increased the antitumor immune function of AE36 in both treatment and prophylaxis [56].

Alipour Talesh et al. [57] designed a cationic nanoliposome carrier system composed of 1,2-dioleoyl-3-trimethylammonium propane-cholesterol for vaccine formulation including natural multi-epitope HER2/neu-derived P5 peptide and poly-IC immunoadjuvant. The release of IFN- $\gamma$  was higher in the vaccinated mouse group than in the control group. It was also observed that the nanovaccine formulation produced 2.7 times higher tumor growth delay in vaccinated mice.

A liposomal formulation composed of DMPC:DMPG:Chol:DOPE, encapsulated P5 and loaded MPL was used as a vaccine against BC on BALB/c mouse models. Enzyme-linked immunosorbent assay, cytotoxicity and intracellular cytokine assays, which followed immunization demonstrated that this formulation was unable to induce either CTL or CD4 T cell responses. Researchers concluded that using anionic conventional liposomes had resulted in a low uptake by DCs because of their negative surface charge [58].

Jalali et al. [59] evaluated the efficacy of four different multi-epitope HER2/neu peptide antigens (p5, p453, p373, and p1209) encapsulated in liposome-polycation-DNA (LPD) NPs for the induction of immune response in TUBO tumor mouse models. Two of the four tested peptides (p5 and p435)-LPD NPs caused higher IFN- $\gamma$  release and cytotoxic responses in comparison with the control groups. Fifty percent of the mice that had been vaccinated with the p5-LPD and p435-LPD formulations remained tumor free for 80 days after eradication of existing tumors. It was shown that 45% of NPs remained at the injection site after 24 hours, leading to a higher circulation time for the vaccine. The results of this study indicated that p5 and p435 peptides together with the nano delivery design had enhanced antigen-specific immunity.

Glaffig et al. [60] combined the tumor-associated MUC1 glycopeptide with the immunostimulating T-cell epitope P2 from tetanus toxoid, coupled to a dendrimer-like hyper-

branched polyglycerol (hbPG) composed of azido-terminated triethyleneglycol acyl spacer and alkyne-functionalized hbPG-polymers. This structure led to improved water solubility of the vaccine formulation. A significant induction of immune response was observed in mouse models especially for the recognizing the human tumor cell (MCF-7) by IgG antibodies.

Nanodelivery systems are not only used in peptide antigen vaccines, but also in delivering gene-based vaccines. An example of such formulation is the lipid/calcium/phosphate NP-based mRNA vaccine encoding tumor antigen MUC1 combined with immune checkpoint blocker anti-CTLA-4 (CTLA-associated protein 4) monoclonal antibody. Here, the researchers demonstrated that the nanovaccine formulation dramatically improved the anticancer immune response compared to the vaccine alone or monoclonal antibody alone, in TNBC mouse model [61].

Alginate acid-coated chitosan nanoparticles (A.C.NPs) have been used as oral delivery carriers for the legumain DNA vaccine to overcome its lack of efficiency in clinical application. Degradation of the vaccine in an acidic environment was evaluated *in vitro* and better protection of DNA vaccine via A.C.NPs has been proved by agarose gel electrophoresis. The antitumor effect of A.C.NPs-legumain DNA vaccine in BC mouse models was demonstrated by the lower tumor growth ratio and improved T lymphocyte responses [62].

Jadidi-Niaragh et al. [63], aiming to overcome the inefficacy of conventional DC-based BC vaccines caused by the immunosuppressive microenvironment of the tumor, used the vaccine in combination with CD73-specific small interfering RNA (siRNA)-loaded chitosan-lactate NPs. CD73-specific siRNA to decrease CD73 expression in tumor cells caused by its association with immunosuppression by adenosine, tumor-associated macrophages, regulatory T (Treg) and myeloid-derived suppressor cells. It augmented CTL responses, increased T cell proliferation and release of IFN- $\gamma$  and IL-17, and enhanced the antitumor response. The results of this study indicated that 57% (4 of 7) of vaccinated mice showed significantly prolonged survival time with complete tumor remission.

Iranpour et al. [64] tested the ability of DCs, loaded with BC cell lysate in combination with three different poly-lactico-glycolic acid (PLGA) NP formulations, to induce antigen release, DC maturation, and T lymphocyte responses through these cells. It was shown that PLGA-NPs increased antigen presentation on DCs resulting in a higher amount of IFN- $\gamma$  and IL-12 immunocytokine release from T lymphocytes. Kokate et al. [65] designed CpG-PLGA-NP-Tag (CpG-oligodeoxynucleotide [ODN]-coated tumor antigen [Tag] encapsulating NP) and evaluated the efficacy of this formulation *ex vivo* and *in vivo*. It was demonstrated that CpG-NP-Tag NPs were ac-

tively captured and presented by DCs. *In vivo* results showed a decrease not only in tumor size but also in angiogenesis, in addition to which an improved cytotoxic response was observed.

Unlike conventional approaches which use immunogen molecules in nanocarrier system, Campbell et al. [66] only encapsulated an endogenous protein derived from HMGB1 as an adjuvant in the NP. Hp91 peptide was packaged inside PLGA-NPs and its effectiveness in combination with HER2 antigen was evaluated. It was shown that Hp91-PLGA-NPs stimulated DCs and improved CTL and cytokine responses, compared to free HER2 peptide *in vitro*. *In vivo* results demonstrated that mice vaccinated with Hp91-PLGA-NPs and HER2 stayed tumor-free for a longer time, than mice vaccinated only with HER2.

Hartmann et al. [67] used cationic nanohydrogel particles to carry MUC1 antigen and CpG-ODN adjuvant in order to improve antitumor responses. They demonstrated that antigen loaded cationic nanohydrogel particles were immunologically neutral when used alone. By contrast, when the design NPs were loaded with MUC1 and CpG-ODN adjuvant, expression of co-stimulatory molecules and T cell proliferation were significantly boosted *in vitro*. Augmented antibody responses against MUC1 were also observed following *in vivo* vaccination of Balb/c mice.

Virus-like particles (VLPs) are some of the most intelligent nanotools with a great ability to encapsulate materials and chemically modify the external surface. VLP-based nanosystems are used for targeted delivery and controlled release of vaccine formulations in many types of infections and cancers [68]. Optimal nano-size, particulate nature and adjuvant activity of VLPs impart a great power to enhance strong immune responses to these particles [69].

Palladini et al. [70] developed a high-density display of HER2 antigen on the surface of VLPs and evaluated its prophylactic and therapeutic effects on mice. It was shown that the vaccine formulation overcomes B-cell tolerance and stimulates anti-HER2 IgG production. Fifty percent of the mice which were vaccinated with the HER2-VLP formulation survived without tumor growth for 1 year. The HER2-VLP vaccine has shown a promise in the prevention of tumor growth as also for the treatment of HER2+ BC.

Another approach using VLPs on BC immunotherapy was aimed at neutralizing relapse, metastatic progression and therapeutic resistance caused by BCSC. The cystine-glutamate antiporter protein xCT (SLC7A11) plays an important role for BCSC function. Bolli et al. [69] developed a vaccine formulation to target the xCT protein using a virus-like particle nanosystem (AX09-0M6), and proved its effectiveness in produc-

ing anti-xCT IgG2 antibodies and inhibiting tumor growth and pulmonary metastases (~42%) in mouse models.

Patel et al. [71] designed influenza VLPs modified to express BC HER2 converted to a glycosylphosphatidylinositol (GPI)-anchored form. A high level of T helper cell 1 and T helper cell 2 type anti-HER2 antibody response and protection against HER2-expressing tumor challenge in mouse models were because of GPIHER-2-VLP vaccination.

## CONCLUSION

Studies on immunotherapy have increased dramatically following the realization of the importance of cancer vaccines in overcoming the disadvantages of traditional therapies and provoking long-lasting protective antitumor immunity. Peptide-based vaccines, whole tumor cell vaccines, genetic-based vaccines and DC vaccines, are being evaluated for the use against BC, in both preclinical and clinical studies. Although the results of these vaccine studies show enhanced antitumor responses, it appears that some improvements may be needed owing to low immunogenicity of tumor antigens, genomic fusion risk of recombinant vaccines, absence of usable active adjuvants and the risk of uncontrolled vaccine release. It is known that even the weakest antigen, when used in combination with a suitable adjuvant, may produce stronger immune responses than the most immunogenic molecule acting alone. Therefore, development of new generation vaccine adjuvants may be essential. Additionally, each patient may have different BC phenotype because of the heterogeneity of this cancer. Accordingly, development of patient-specific vaccines may be very important. Immune tolerance and suppression pathways in the tumor microenvironment are also issues that need to be considered. Combining vaccines with other therapeutic agents as well as other antitumor immunity enhancing approaches may lead to better in-clinic responses. Furthermore, recent developments in nanotechnology show great promise as tools that may be used in developing effective immunotherapeutic vaccines for BC. Nanotechnology enables controlled release and targeted therapy, which are properties required to improve the efficacy of many types of BC vaccines. Efforts to develop an effective vaccine against BC may lead to improved antitumor clinical outcomes and therapeutic support for women fighting BC worldwide.

## CONFLICT OF INTEREST

The authors declare that they have no competing interests.

## REFERENCES

1. American Cancer Society. Cancer Facts & Figures 2018. Atlanta: American Cancer Society; 2018.
2. Ferlay J, Soerjomataram I, Ervik M, Dikshit R, Eser S, Mathers C, et al. GLOBACAN 2012 v1.0. Cancer incidence and mortality worldwide: IARC CancerBase No. 11. International Agency for Research on Cancer. <http://globocan.iarc.fr>. Accessed May 30th, 2018.
3. Goldhirsch A, Winer EP, Coates AS, Gelber RD, Piccart-Gebhart M, Thürlimann B, et al. Personalizing the treatment of women with early breast cancer: highlights of the St Gallen International Expert Consensus on the Primary Therapy of Early Breast Cancer 2013. *Ann Oncol* 2013;24:2206-23.
4. Pham PV. Breast Cancer Stem Cells & Therapy Resistance. Cham: Springer International Publishing; 2015.
5. Yang Y. Cancer immunotherapy: harnessing the immune system to battle cancer. *J Clin Invest* 2015;125:3335-7.
6. Abbas AK, Lichtman AH, Pillai S. Basic Immunology: Functions and Disorders of the Immune System. 5th ed. St. Louis: Elsevier; 2015.
7. Alatrash G, Jakher H, Stafford PD, Mittendorf EA. Cancer immunotherapies, their safety and toxicity. *Expert Opin Drug Saf* 2013;12:631-45.
8. Chung MA, Luo Y, O'Donnell M, Rodriguez C, Heber W, Sharma S, et al. Development and preclinical evaluation of a Bacillus Calmette-Guérin-MUC1-based novel breast cancer vaccine. *Cancer Res* 2003;63:1280-7.
9. Reichenbach DK, Finn OJ. Early in vivo signaling profiles in MUC1-specific CD4(+) T cells responding to two different MUC1-targeting vaccines in two different microenvironments. *Oncoimmunology* 2013; 2:e23429.
10. Dillon PM, Petroni GR, Smolkin ME, Brenin DR, Chianese-Bullock KA, Smith KT, 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.
11. Mittendorf EA, Ardavanis A, Litton JK, Shumway NM, Hale DE, Murray JL, et al. Primary analysis of a prospective, randomized, single-blinded phase II trial evaluating the HER2 peptide GP2 vaccine in breast cancer patients to prevent recurrence. *Oncotarget* 2016;7:66192-201.
12. Torres-García D, Pérez-Torres A, Manoutcharian K, Orbe U, Servín-Blanco R, Fragosó G, et al. GK-1 peptide reduces tumor growth, decreases metastatic burden, and increases survival in a murine breast cancer model. *Vaccine* 2017;35:5653-61.
13. Gilewski T, Adluri S, Ragupathi G, Zhang S, Yao TJ, Panageas K, et al. Vaccination of high-risk breast cancer patients with mucin-1 (MUC1) keyhole limpet hemocyanin conjugate plus QS-21. *Clin Cancer Res* 2000;6:1693-701.
14. Vassilaros S, Tsibanis A, Tsikkinis A, Pietersz GA, McKenzie IF, Apostolopoulos V. Up to 15-year clinical follow-up of a pilot Phase III immunotherapy study in stage II breast cancer patients using oxidized mannan-MUC1. *Immunotherapy* 2013;5:1177-82.
15. Peoples GE, Gurney JM, Hueman MT, Woll MM, Ryan GB, Storrer CE, 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.
16. Clifton GT, Peoples GE, Mittendorf EA. The development and use of

- the E75 (HER2 369-377) peptide vaccine. *Future Oncol* 2016;12:1321-9.
17. Hutchins LF, Makhoul I, Emanuel PD, Pennisi A, Siegel ER, Jousheghany F, et al. Targeting tumor-associated carbohydrate antigens: a phase I study of a carbohydrate mimetic-peptide vaccine in stage IV breast cancer subjects. *Oncotarget* 2017;8:99161-78.
  18. Borch TH, Engell-Noerregaard L, Zeeberg Iversen T, Ellebaek E, Met Ö, Hansen M, et al. mRNA-transfected dendritic cell vaccine in combination with metronomic cyclophosphamide as treatment for patients with advanced malignant melanoma. *Oncoimmunology* 2016;5:e1207842.
  19. Qi CJ, Ning YL, Han YS, Min HY, Ye H, Zhu YL, et al. Autologous dendritic cell vaccine for estrogen receptor (ER)/progesterone receptor (PR) double-negative breast cancer. *Cancer Immunol Immunother* 2012;61:1415-24.
  20. Emens LA, Armstrong D, Biedrzycki B, Davidson N, Davis-Sproul J, Fetting J, et al. A phase I vaccine safety and chemotherapy dose-finding trial of an allogeneic GM-CSF-secreting breast cancer vaccine given in a specifically timed sequence with immunomodulatory doses of cyclophosphamide and doxorubicin. *Hum Gene Ther* 2004;15:313-37.
  21. Srivatsan S, Patel JM, Bozeman EN, Imasuen IE, He S, Daniels D, et al. Allogeneic tumor cell vaccines: the promise and limitations in clinical trials. *Hum Vaccin Immunother* 2014;10:52-63.
  22. Deacon DH, Hogan KT, Swanson EM, Chianese-Bullock KA, Denlinger CE, Czarkowski AR, et al. The use of gamma-irradiation and ultraviolet-irradiation in the preparation of human melanoma cells for use in autologous whole-cell vaccines. *BMC Cancer* 2008;8:360.
  23. Sharma A, Bode B, Wenger RH, Lehmann K, Sartori AA, Moch H, et al. Gamma-radiation promotes immunological recognition of cancer cells through increased expression of cancer-testis antigens in vitro and in vivo. *PLoS One* 2011;6:e28217.
  24. Simons JW, Sacks N. Granulocyte-macrophage colony-stimulating factor-transduced allogeneic cancer cellular immunotherapy: the GVAX vaccine for prostate cancer. *Urol Oncol* 2006;24:419-24.
  25. Huang X, Ye D, Thorpe PE. Enhancing the potency of a whole-cell breast cancer vaccine in mice with an antibody-IL-2 immunocytokine that targets exposed phosphatidylserine. *Vaccine* 2011;29:4785-93.
  26. Convit J, Montesinos H, Oviedo H, Romero G, Maccarone B, Essensfeld E, et al. Autologous tumor lysate/Bacillus Calmette-Guérin immunotherapy as an adjuvant to conventional breast cancer therapy. *Clin Transl Oncol* 2015;17:884-7.
  27. Godoy-Calderón MJ, Salazar V, González-Marcano E, Convit AF. Autologous tumor cells/bacillus Calmette-Guérin/formalin-based novel breast cancer vaccine induces an immune antitumor response. *Oncotarget* 2018;9:20222-38.
  28. Yan HX, Cheng P, Wei HY, Shen GB, Fu LX, Ni J, et al. Active immunotherapy for mouse breast cancer with irradiated whole-cell vaccine expressing VEGFR2. *Oncol Rep* 2013;29:1510-6.
  29. Dols A, Smith JW 2nd, Meijer SL, Fox BA, Hu HM, Walker E, et al. Vaccination of women with metastatic breast cancer, using a costimulatory gene (CD80)-modified, HLA-A2-matched, allogeneic, breast cancer cell line: clinical and immunological results. *Hum Gene Ther* 2003;14:1117-23.
  30. Guo C, Manjili MH, Subjeck JR, Sarkar D, Fisher PB, Wang XY. Therapeutic cancer vaccines: past, present, and future. *Adv Cancer Res* 2013;119:421-75.
  31. Kwilas AR, Ardiani A, Dirmeier U, Wottawah C, Schlom J, Hodge JW. A poxviral-based cancer vaccine the transcription factor twist inhibits primary tumor growth and metastases in a model of metastatic breast cancer and improves survival in a spontaneous prostate cancer model. *Oncotarget* 2015;6:28194-210.
  32. Larocca C, Schlom J. Viral vector-based therapeutic cancer vaccines. *Cancer J* 2011;17:359-71.
  33. Bergman PJ. Cancer immunotherapy. *Top Companion Anim Med* 2009;24:130-6.
  34. Nazarkina ZhK, Khar'kova MV, Antonets DV, Morozkin ES, Bazhan SI, Karpenko LI, et al. Design of polypeptide DNA vaccine against breast carcinoma cells and analysis of its expression in dendritic cells. *Bull Exp Biol Med* 2016;160:486-90.
  35. Gelao L, Criscitiello C, Esposito A, De Laurentiis M, Fumagalli L, Locatelli MA, et al. Dendritic cell-based vaccines: clinical applications in breast cancer. *Immunotherapy* 2014;6:349-60.
  36. Peethambaram PP, Melisko ME, Rinn KJ, Alberts SR, Provost NM, Jones LA, et al. A phase I trial of immunotherapy with lapuleucel-T (APC8024) in patients with refractory metastatic tumors that express HER-2/neu. *Clin Cancer Res* 2009;15:5937-44.
  37. Sakai Y, Morrison BJ, Burke JD, Park JM, Terabe M, Janik JE, et al. Vaccination by genetically modified dendritic cells expressing a truncated neu oncogene prevents development of breast cancer in transgenic mice. *Cancer Res* 2004;64:8022-8.
  38. Gong J, Avigan D, Chen D, Wu Z, Koido S, Kashiwaba M, et al. Activation of antitumor cytotoxic T lymphocytes by fusions of human dendritic cells and breast carcinoma cells. *Proc Natl Acad Sci U S A* 2000;97:2715-8.
  39. Koido S, Tanaka Y, Tajiri H, Gong J. Generation and functional assessment of antigen-specific T cells stimulated by fusions of dendritic cells and allogeneic breast cancer cells. *Vaccine* 2007;25:2610-9.
  40. Bird RC, DeInnocentes P, Lenz S, Thacker EE, Curiel DT, Smith BF. An allogeneic hybrid-cell fusion vaccine against canine mammary cancer. *Vet Immunol Immunopathol* 2008;123:289-304.
  41. Bird RC, DeInnocentes P, Church Bird AE, van Ginkel FW, Lindquist J, Smith BF. An autologous dendritic cell canine mammary tumor hybrid-cell fusion vaccine. *Cancer Immunol Immunother* 2011;60:87-97.
  42. Zhang Y, Ma B, Zhou Y, Zhang M, Qiu X, Sui Y, et al. Dendritic cells fused with allogeneic breast cancer cell line induce tumor antigen-specific CTL responses against autologous breast cancer cells. *Breast Cancer Res Treat* 2007;105:277-86.
  43. Zhang Y, Luo W, Wang Y, Liu Y, Zheng L. Purified dendritic cell-tumor fusion hybrids supplemented with non-adherent dendritic cells fraction are superior activators of antitumor immunity. *PLoS One* 2014;9:e86772.
  44. Zhang P, Yi S, Li X, Liu R, Jiang H, Huang Z, et al. Preparation of triple-negative breast cancer vaccine through electrofusion with day-3 dendritic cells. *PLoS One* 2014;9:e102197.
  45. Koido S, Homma S, Hara E, Mitsunaga M, Namiki Y, Takahara A, et al. In vitro generation of cytotoxic and regulatory T cells by fusions of human dendritic cells and hepatocellular carcinoma cells. *J Transl Med* 2008;6:51.
  46. Neidhardt-Berard EM, Berard F, Banchereau J, Palucka AK. Dendritic cells loaded with killed breast cancer cells induce differentiation of

- tumor-specific cytotoxic T lymphocytes. *Breast Cancer Res* 2004;6:R322-8.
47. Saito H, Dubsy P, Dantin C, Finn OJ, Banchereau J, Palucka AK. Cross-priming of cyclin B1, MUC-1 and survivin-specific CD8+ T cells by dendritic cells loaded with killed allogeneic breast cancer cells. *Breast Cancer Res* 2006;8:R65.
  48. Delirez N, Moazzeni SM, Shokri F, Shokrgozar MA, Atri M, Kokhaei P. Autologous dendritic cells loaded with apoptotic tumor cells induce T cell-mediated immune responses against breast cancer in vitro. *Cell Immunol* 2009;257:23-31.
  49. Herr W, Ranieri E, Olson W, Zarour H, Gesualdo L, Storkus WJ. Mature dendritic cells pulsed with freeze-thaw cell lysates define an effective in vitro vaccine designed to elicit EBV-specific CD4(+) and CD8(+) T lymphocyte responses. *Blood* 2000;96:1857-64.
  50. Gao Y, Chen X, Gao W, Yang Y, Ma H, Ren X. A new purification method for enhancing the immunogenicity of heat shock protein 70-peptide complexes. *Oncol Rep* 2012;28:1977-83.
  51. Delirez N, Moazzeni SM, Shokri F, Shokrgozar MA, Atri M, Karbassian H. In vitro analysis of T cell responses induced by breast tumor cell lysate pulsed with autologous dendritic cells. *Adv Biosci Biotechnol* 2012;3:126-36.
  52. Nguyen ST, Nguyen HL, Pham VQ, Nguyen GT, Tran CD, Phan NK, et al. Targeting specificity of dendritic cells on breast cancer stem cells: in vitro and in vivo evaluations. *Onco Targets Ther* 2015;8:323-34.
  53. Kakwere H, Ingham ES, Allen R, Mahakian LM, Tam SM, Zhang H, et al. Toward personalized peptide-based cancer nanovaccines: a facile and versatile synthetic approach. *Bioconjug Chem* 2017;28:2756-71.
  54. Razazan A, Behravan J, Arab A, Barati N, Arabi L, Gholizadeh Z, et al. Conjugated nanoliposome with the HER2/neu-derived peptide GP2 as an effective vaccine against breast cancer in mice xenograft model. *PLoS One* 2017;12:e0185099.
  55. Arab A, Behravan J, Razazan A, Gholizadeh Z, Nikpoor AR, Barati N, et al. A nano-liposome vaccine carrying E75, a HER-2/neu-derived peptide, exhibits significant antitumor activity in mice. *J Drug Target* 2018;26:365-72.
  56. Barati N, Nikpoor AR, Razazan A, Mosaffa F, Badiie A, Arab A, et al. Nanoliposomes carrying HER2/neu-derived peptide AE36 with CpG-ODN exhibit therapeutic and prophylactic activities in a mice TUBO model of breast cancer. *Immunol Lett* 2017;190:108-17.
  57. Alipour Talesh G, Ebrahimi Z, Badiie A, Mansourian M, Attar H, Arabi L, et al. Poly (I:C)-DOTAP cationic nanoliposome containing multi-epitope HER2-derived peptide promotes vaccine-elicited anti-tumor immunity in a murine model. *Immunol Lett* 2016;176:57-64.
  58. Shariat S, Badiie A, Amir Jalali S, Mansourian M, Alireza Mortazavi S, Reza Jaafari M. Preparation and characterization of different liposomal formulations containing P5 HER2/neu-derived peptide and evaluation of their immunological responses and antitumor effects. *Iran J Basic Med Sci* 2015;18:506-13.
  59. Jalali SA, Sankian M, Tavakkol-Afshari J, Jaafari MR. Induction of tumor-specific immunity by multi-epitope rat HER2/neu-derived peptides encapsulated in LPD nanoparticles. *Nanomedicine* 2012;8:692-701.
  60. Glaffig M, Palitzsch B, Hartmann S, Schüll C, Nuhn L, Gerlitzki B, et al. A fully synthetic glycopeptide antitumor vaccine based on multiple antigen presentation on a hyperbranched polymer. *Chemistry* 2014;20:4232-6.
  61. Liu L, Wang Y, Miao L, Liu Q, Musetti S, Li J, et al. Combination immunotherapy of MUC1 mRNA nano-vaccine and CTLA-4 blockade effectively inhibits growth of triple negative breast cancer. *Mol Ther* 2018;26:45-55.
  62. Liu Z, Lv D, Liu S, Gong J, Wang D, Xiong M, et al. Alginate acid-coated chitosan nanoparticles loaded with legumain DNA vaccine: effect against breast cancer in mice. *PLoS One* 2013;8:e60190.
  63. Jjadi-Niaragh F, Atyabi F, Rastegari A, Kheshtchin N, Arab S, Hassannia H, et al. CD73 specific siRNA loaded chitosan lactate nanoparticles potentiate the antitumor effect of a dendritic cell vaccine in 4T1 breast cancer bearing mice. *J Control Release* 2017;246:46-59.
  64. Iranpour S, Nejati V, Delirez N, Biparva P, Shirian S. Enhanced stimulation of anti-breast cancer T cells responses by dendritic cells loaded with poly lactic-co-glycolic acid (PLGA) nanoparticle encapsulated tumor antigens. *J Exp Clin Cancer Res* 2016;35:168.
  65. Kokate RA, Chaudhary P, Sun X, Thamaake SI, Maji S, Chib R, et al. Rationalizing the use of functionalized poly-lactic-co-glycolic acid nanoparticles for dendritic cell-based targeted anticancer therapy. *Nanomedicine (Lond)* 2016;11:479-94.
  66. Campbell DF, Saenz R, Bharati IS, Seible D, Zhang L, Esener S, et al. Enhanced anti-tumor immune responses and delay of tumor development in human epidermal growth factor receptor 2 mice immunized with an immunostimulatory peptide in poly(D,L-lactic-co-glycolic) acid nanoparticles. *Breast Cancer Res* 2015;17:48.
  67. Hartmann S, Nuhn L, Palitzsch B, Glaffig M, Stergiou N, Gerlitzki B, et al. CpG-loaded multifunctional cationic nanohydrogel particles as self-adjuncting glycopeptide antitumor vaccines. *Adv Healthc Mater* 2015;4:522-7.
  68. Roldão A, Mellado MC, Castilho LR, Carrondo MJ, Alves PM. Virus-like particles in vaccine development. *Expert Rev Vaccines* 2010;9:1149-76.
  69. Bolli E, O'Rourke JP, Conti L, Lanzardo S, Rolih V, Christen JM, et al. A virus-like-particle immunotherapy targeting epitope-specific anti-xCT expressed on cancer stem cell inhibits the progression of metastatic cancer in vivo. *Oncoimmunology* 2017;7:e1408746.
  70. Palladini A, Thrane S, Janitzek CM, Pihl J, Clemmensen SB, de Jongh WA, et al. Virus-like particle display of HER2 induces potent anti-cancer responses. *Oncoimmunology* 2018;7:e1408749.
  71. Patel JM, Vartabedian VF, Kim MC, He S, Kang SM, Selvaraj P. Influenza virus-like particles engineered by protein transfer with tumor-associated antigens induces protective antitumor immunity. *Biotechnol Bioeng* 2015;112:1102-10.