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REVIEW

HER2-Positive Breast Cancer Immunotherapy: A Focus on Vaccine Development

Atefeh Arab[1] · **Rezvan Yazdian-Robati**[2] · **Javad Behravan**[3,4,5]

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Abstract

Clinical progress in the field of HER2-positive breast cancer therapy has been dramatically improved by understanding of the immune regulatory mechanisms of tumor microenvironment. Passive immunotherapy utilizing recombinant monoclonal antibodies (mAbs), particularly trastuzumab and pertuzumab has proved to be an effective strategy in HER2-positive breast cancer treatment. However, resistance to mAb therapy and relapse of disease are still considered important challenges in clinical practice. There are increasing reports on the induction of cellular and humoral immune responses in HER2-positive breast cancer patients. More recently, increasing efforts are focused on using HER2-derived peptide vaccines for active immunotherapy. Here, we discuss the development of various HER2-derived vaccines tested in animal models and human clinical trials. Different formulations and strategies to improve immunogenicity of the antigens in animal studies are also discussed. Furthermore, other immunotherapeutic approaches to HER2 breast cancer including, CTLA-4 inhibitors, immune checkpoint inhibitors, anti PD-1/PD-L1 antibodies are presented.

Keywords Breast cancer · HER2 · Immunotherapy · Vaccine

Introduction

Several therapeutic approaches have been established for treating breast cancer including radiotherapy, surgery, hormone therapy, chemotherapy, and immunotherapy. Cancer immunotherapy includes several modalities including but not limited to cancer vaccines, monoclonal antibodies (mAbs), adaptive cell therapy, chimeric antigen receptor T cell therapy (Miliotou and Papadopoulou 2018) and in oncolytic virus therapy, all aiming to attack and kill tumor cells (McCarthy 2006). It has been documented that the most immunogenic breast cancer subtypes are the human epidermal growth factor receptor 2 (HER2)-positive and triple-negative breast cancer (TNBC). Thus, activating the patient's own immune system to abolish the tumor cells is a promising and a relatively new therapeutic approach. The advantage of active immunotherapy is the development protective effect against tumor tissue, resetting the immune system to an anti-tumor surveillance status (Williams et al. 2017). Active immunotherapy via vaccination is based on the ability of immune system to differentiate between self-antigens that are expressed normally on the surface of cells and those overexpressed abnormally on tumor cells. Tumor-associated antigens (TAA) like HER2 are identified as appropriate sources for peptide vaccination in breast cancer (Marmé 2016). Using immune checkpoint inhibitors is another very successful immunotherapy approach tested in a variety of cancers. mAbs targeting the cytotoxic T lymphocyte-associated antigen (CTLA)-4, programmed cell death (PD)-1 and programmed death-ligand 1 (PD-L1) have impressive outcomes in several malignancies and have been investigated as single agents or as combinations with chemotherapy medications in breast cancer (Schmid et al. 2018; Solinas et al. 2017).

✉ Javad Behravan
behravanj@mums.ac.ir; javad.behravan@uwaterloo.ca

1 Pharmaceutical Sciences Research Center, Ardabil University of Medical Sciences, Ardabil, Iran

2 Molecular and Cell Biology Research Center, Faculty of Medicine, Mazandaran University of Medical Sciences, Sari, Iran

3 Biotechnology Research Center, Mashhad University of Medical Sciences, Mashhad, Iran

4 School of Pharmacy, University of Waterloo, Waterloo, ON, Canada

5 Theraphage Inc., Kitchener, ON, Canada

Tumor Microenvironment

Immune cells as major components of the tumor microenvironment (TME) play crucial role in the recognition/prevention, early eradication and progression of cancer. Immune system elements such as dendritic cells (DCs), macrophages, natural killer (NK) cells and adaptive immune cells penetrate in TME and their presence in TME signals an anti-tumor immune response (Tan et al. 2018).

The theory of "immunoediting" which includes elimination, equilibrium and escape phases describes the role of immune system in the progression and development of cancer (Schreiber et al. 2011). In the "elimination phase", immune cells identify and eradicate cancer cells to prevent tumor growth. In the second stage "equilibrium phase'', scarce cancerous cells that evade the elimination phase remain dormant, while immune cells prevent tumor cell outgrowth. Cancer cells that avoid immune recognition and elimination step, enter the "escape stage" and proceed to proliferate aggressively (Ayoub et al. 2019). Activation of $CD8^+$ cytotoxic T lymphocytes (CTLs) is the major part of anticancer immunity that exert anti-tumor activity by secreting tumor necrosis factor (TNF)-α and interferon (IFN)-γ along other cytotoxins (Su et al. 2016; Sugie and Toi 2017). The number of CTLs in TME and their capacity to distinguish TAA have great influence in inhibiting tumor growth and development of malignancy (De La Cruz et al. 2016; Zhou et al. 2016). Tumor cells can evade immune system by lowering the expression or by modification of surface antigens, down-regulation of major histocompatibility complex (MHC) class I proteins, T cell receptor signaling defect and down-regulating of the expression of co-stimulatory molecules (Tan et al. 2018). Other mechanisms to escape immune detection are blocking regulatory pathways activation, development of immunosuppressive TME via regulatory T cells (Tregs), increasing the myeloid-derived suppressor cells, production of tumor growth factor β and interleukin (IL)-10 cells (Ayoub et al. 2019). Tumor infiltrating lymphocytes (TILs) consist of T and B lymphocytes, NK cells, DCs and macrophages surrounding cancer cells (Chin et al. 1992). Identification of TILs in TME can predict the immunogenic nature of cancer and response to therapy and improved prognosis. The quantity of TILs and the phenotype of infiltrated cells determine the clinical outcome of the therapy. Presence of $CD8^+$ CTLs are essential for tumor cell destruction and is related to a lower rate of fatality in estrogen receptor (ER)-negative, ER-positive and HER2-positive tumors. $CD4^+$ T-helper (Th) cells are positively correlated with forkhead box P3 (Foxp3) $CD4^+$ Treg cells and have negative effects on the CTL function. $CD4^+$ T cells have distinct functions during tumor development. Th1 cells are the predominant subset of $CD4^+$ T cells in the early stage of tumor and are important for immunosurveillance. However, in the advanced stage of cancer, $Foxp3^+$ Treg and Th17 cells become the dominant subsets of $CD4^+$ TILs which may contribute in promoting tumor growth (Huang et al. 2015).

It has been found that $CD4^+$ T cells are associated with larger tumor sizes and higher tumor stages, positive lymph node status and expression of HER2 in advanced breast cancer patients (Ayoub et al. 2019; Tan et al. 2018). Strong correlation between the expression of lymphocyte genes and reduction of cancer recurrence rates has been observed in HER2-positive breast cancer (Alexe et al. 2007). As reported in a study of 387 cases of HER2-positive breast cancer patients, there was 3% relative reduction in the risk of recurrence for each one percent increase in TILs (Salgado et al. 2015).

HER2-positive Breast Cancer Immunotherapy

HER2, known as ErbB-2 (Erythroblastosis homolog B2) CD340 or p185, is a 185 kD oncoprotein that is encoded by the ErbB2 gene. It consists of three domains including an intracellular domain with tyrosine kinase property, a transmembrane domain and extra cellular domain (Ladjemi et al. 2010; Slichenmyer and Fry 2001). Higher expression of HER2 in breast cancer is associated with an increase in aggressiveness in clinical behavior, more invasiveness, recurrence and in the absence of immunotherapeutic options, poor chemotherapeutic outcome (Behravan et al. 2018). A growing body of evidence clearly suggest that the interaction between tumor and immune cells in HER2-positive tumors is a critical step in host cellular immunity (Curigliano et al. 2016). The ability of HER2 in breast cancer cells to interact with any existing receptor tyrosine kinase binding partner leads to T cells and antibody responses induction against the HER2 protein. Approximately 20–30% of all invasive breast cancer cases are classified in the subclass of HER2-positive tumors (Cui et al. 2018).Endogenous HER2 specific antibodies and T cell activities against HER2 are observed in breast cancer patients overexpressing HER2, suggesting that stimulation of anti-HER2 immune response could be exploited to eliminate cancerous cells in HER2-positive breast cancer (De La Cruz et al. 2016; Disis et al. 2004). Moreover, overexpression of HER2 on the surface of tumor cells is a major marker for tumor growth and represents a promising target for cancer immunotherapy (Ladjemi et al. 2010).

Targeting of HER2 by Monoclonal Antibodies

Immunotherapy using mAbs has been improved as an immune-based therapeutic strategy to directly target HER2

in breast cancer (De La Cruz et al. 2016). Trastuzumab (Herceptin) as a first humanized mAb directly targets the extracellular domain of HER2 protein. It has been approved by the Food and Drug Administration (FDA) since 1998 for metastatic HER2 overexpressing breast cancer in combination with chemotherapy. Several clinical trials have demonstrated that a combination of trastuzumab with chemotherapy has prolonged the disease-free survival (DFS) in patients with metastatic HER2/neu-overexpressing breast cancer (Knutson et al. 2016; Slamon et al. 2001). The rational for combination of immunotherapy and chemotherapy is due to their synergistic effects. It was indicated that antibodies potentiate the cytotoxicity effects of chemotherapeutic agents in tumor cell lines overexpressing growth factor receptors (Aboud-Pirak et al. 1988; Pietras et al. 1994; Takahashi et al. 1988). The mechanism of the observed synergistic effects could be the depletion of DNA repair activity by binding of antibodies to the epidermal growth factor receptor extracellular epitopes or to HER2 itself (Arteaga et al. 1994; Pietras et al. 1998). Currently, a new drug named Kadcyla (T-DM1 or ado-trastuzumab emtansine) is approved by the FDA for treating the patients with metastatic breast cancer already treated with taxane and trastuzumab either alone or in combination. T-DM1 is an antibody–drug conjugate that comprises of trastuzumab covalently linked to the cytotoxic agent DM1, a microtubule polymerization inhibitor, to deliver emtansine to the antigen expressing cells. It was reported that T-DM1 was able to reduce the risk of recurrence or prevent death in HER2-positive breast cancer patients harboring remaining residual tumor after neoadjuvant therapy and surgery (Arteaga et al. 1994). Pertuzumab (Perjeta) is another humanized mAb that blocks HER2 signaling and improves the survival rates when administrated with trastuzumab and docetaxel in metastatic breast cancer (Leung et al. 2018; Swain et al. 2015). Pertuzumab specifically binds to HER2 extracellular subdomain II and blocks HER2 dimerization (Franklin et al. 2004). The most probable mechanism of synergistic effects of trastuzumab and pertuzumab is their different modes of function in targeting HER2-positive breast cancer (Nami et al. 2018).

Results of a CLEOPATRA (Clinical Evaluation of Pertuzumab and Trastuzumab) study after 51 months follow up indicated that adding of pertuzumab to docetaxel and trastuzumab therapy led to the improvement of overall survival. Therefore, this therapeutic regimen was established as the first line treatment for HER2-positive metastatic breast cancer (Swain et al. 2015). Despite the high efficiency of these anti-HER2 antibodies, the problem of drug resistance and eradication of the minimal residual disease should be considered (Mosaffa et al. 2012; Elahian et al. 2009; Sabahi et al. 2010). Therefore, new treatment strategies to improve disease therapy outcome are needed (Ladjemi et al. 2010).

Vaccines for HER2-positive Breast Cancer

Active cancer immunotherapy has received an extensive attention in recent years. A cancer vaccine elicits the patient's immune system to produce an anti-tumor response that would produce or activate a wide range of immune regulators such as CTLs, antibodies and Th cells. The vaccination strategy has some advantages over mAbs mediated immunotherapy including fewer administrations, cost effectiveness, and generation of immunological memory. The immune memory advantage helps the immune system to recognize and respond to the antigens in future exposures and protect against the relapsing tumor (Ayoub et al. 2019; Williams et al. 2017). Active immunotherapy with vaccines is based on the induction of a tumor–destructive environment using type I immunity (Th1) toward cancerous cells distinguished with tumor antigens. Immune responses against HER2-positive breast cancer are induced by both CTLs and $CD4^+$ T cells (Costa et al. 2017). CTLs as key elements in anticancer immunity are activated by the anticancer vaccines and secrete IFN-γ, TNF-α and several other cytokines (Jinushi 2015). $CD8^+$ cells are capable to recognize HER2 peptides presented by MCH class I molecules, induce cell cycle arrest and apoptosis, and kill the tumor cells by producing IFN-γ (Mukai et al. 2002). $CD4^+$ T cells also play an important role in breast cancer therapy and are associated with stronger anti-HER2 immunity response (Datta et al. 2016). Different strategies including specific tumor antigen-derived peptides, proteins, DNA, RNA, whole tumor cells and DCs can be used for breast cancer vaccine development (Doyle et al. 2018; Kim et al. 2014; Norell et al. 2010; Tomasicchio et al. 2019).

HER2-derived Peptide Vaccines

Immunogenic HER2-derived peptides include peptides from different parts of HER2 molecule consisting E75 (from the extracellular domain), AE37 (intracellular domain), and GP2 (transmembrane domain). E75 (HER2/neu 369–377: KIFGSLAFL) represents an immunodominant CTL epitope with high affinity for human leukocyte antigen HLA-A2 and HLA-A3 molecules (Datta et al. 2015; Kawashima et al. 1998; Patil et al. 2010). It is expressed in about 60–75% of the population and is capable to stimulate T cells against HER2 expressing breast cancer cells. The effects of E75 in induction of immune system of patients with overexpressing HER2 cancers has been established in preclinical studies (Fisk et al. 1995; Kuerer et al. 2002; Patil et al. 2010; Pietras et al. 1994; Sotiropoulou et al. 2003). Moreover, the efficacy of E75 has been studied at varying dose schedules in clinical trials (Table 1). The highest and most advanced clinical trials on the HER2-derived peptides are those of E75 peptide (Nelipepimut-S). In a phase I/II trial, 187 women

Table 1 List of clinical trials of HER2 directed peptide vaccines (Clinical trials.gov)

Vaccine	Adjuvant	Additional intervention	Condition	Study population	Phase	Status	Clinicaltrials.gov Identifier
E75 peptide	GM-CSF	Trastuzumab	HER2 positive breast cancer	High risk, HLA-A2 + or HLA-A3 + or HLA-A24 + or HLA-A26 + patients	II	Active, not recruiting	NCT02297698
E75 peptide	GM-CSF	–	Breast cancer	Patients with Low to intermediate HER2 expression and HLA-A2 or HLA-A3 haplotype	III	Completed	NCT01479244
E75 peptide	GM-CSF	–	Ductal breast carcinoma in Situ	Pre or postmenopausal, HLA-A2-positive patients	II	Active, not recruiting	NCT02636582
E75 peptide	GM-CSF	Trastuzumab	Breast cancer	Node-positive or node-negative, HER2 1 + and 2 + and HLA-A2 and HLA-A3 patients	II	Completed	NCT01570036
E75 peptide	GM-SCF	–	Breast cancer	HER2-positive, HLA-A2 and HLA-A2 patients	I/II	Completed	NCT00841399
E75 peptide	GM-SCF	–	Breast cancer	HLA-A2 and HLA-A3	I/II	Completed	NCT00854789
GP2 peptide	GM-CSF	Trastuzumab	Breast cancer	HER2-positive (IHC3 + or FISH ≥ 2 and HLA-A2 and HLA-A3 patients	I	Completed	NCT03014076
GP2 peptide	GM-CSF	AE37 peptide	Breast cancer	HER2/neu-positive, node-positive, or high-risk node-negative, HLA-A2 patients	II	Unknown[a]	NCT00524277
9 peptides from HER2/neu, CEA, CTA	poly-ICLC			HLA-A1, -A2, -A3, or -A31-positive patients with high risk, HER2 expressing cancer	I	Terminated	NCT01532960
HER2/neu peptide vaccine			Breast cancer	HER2 (IHC 3 +), stage II/III patients	I	Completed	NCT01632332
HER2/neu peptide vaccine	GM-SCF	Rintatolimod	Breast cancer	HER2 (IHC 1 + 2 + 3 +) stage II, III (A,B,C) and IV breast cancer	I/II	Active, not recruiting	NCT01355393
HER2/neu peptide vaccine			Breast cancer	HER2 (IHC 3 +) HLA-A2-positive, stage IV	II	Terminated	NCT01729884
HER2/neu peptide vaccine	GM-CSF	Cyclophosphamide	Breast cancer	HER2 (IHC 2 + or 3 +), stage IV breast cancer	II	Completed	NCT00791037
HER2/neu peptide vaccine MUC-1 peptide vaccine	GM-CSF	CpG oligodeoxynucleotide incomplete Freund's adjuvant	Breast cancer	HER2-positive, Triple-negative, Muc-1-positive and HLA-A2 patients	Early phase I	Completed	NCT00640861

Table 1 (continued)

Vaccine	Adjuvant	Additional intervention	Condition	Study population	Phase	Status	Clinicaltrials.gov Identifier
HER2/neu peptide vaccine			Breast and ovarian cancers	HER2 (IHC 2+, 3+), HLA-A2-positive breast or ovarian cancer with stage IV	I/II	Active, not recruiting	NCT00194714

[a]Study has passed its completion date and status has not been verified in more than 2 years

with node-positive and node-negative cancer harboring HER2 expressing tumors (IHC 1+ to 3+) were participated. 108 patients with HLA-A2/3 were administered the E75 peptide plus granulocyte–macrophage colony stimulating factor (GM-CSF) and 79 HLA-A2/3 negative patients were selected in the control group. The 5 year DFS was improved in vaccinated patients (89.7%) compared to control group) 80.2%; $P=0.08$) (Mittendorf et al. 2014).

Based on the observed promising outcome, the clinical efficacy and safety of E75 + GM-CSF (NeuVaxTM, Galena Biopharma, Inc., Portland, OR, USA) vaccine in prevention of breast cancer recurrence has been investigated in a controlled phase III study (PRESENT trial, NCT01479244). In this study, 758 HLA-A2/A3 breast cancer patients with the following criteria: (1) early stage node positive and (2) low-to-intermediate level of HER2 expression after completed standard of therapy, were enrolled. Preclinical data indicated that combining of passive immunotherapy with active immunotherapy might have a synergistic effect. Antibody-dependent cell-mediated cytotoxicity (ADCC) is considered as one of the main mechanisms of action of trastuzumab. Through the ADCC mechanism, trastuzumab-coated tumor antigens are released from tumor cells and taken up by DCs and presented on MHC class I molecules. Therefore, trastuzumab in combination with active immunotherapy, led to the greater expansion of peptide specific CTLs and higher cytotoxicity via enhancement of uptake and cross presentation of HER2 specific epitopes by DCs (Clifton et al. 2016).

Observations from early stages of E75 trial indicated that there was a potential synergistic effect between trastuzumab and E75 vaccine. In a clinical trial study, 12 HER2 (IHC 3+) patients received trastuzumab followed by an E75 vaccine. The patients were observed for 5 years. No cancer recurrences were seen in any of patients after 5 years of follow up (Mittendorf et al. 2012). This was followed by two other studies on the concurrent administration of E75 vaccine and trastuzumab. In the first study, high-risk HER2 breast cancer patients randomized to either traustuzumab plus nelipepimut-S (E75) + GM-CSF vaccine treatment, or only trastuzumab. There were no disease recurrences after 36 months of follow up in patients treated with trastuzumab followed by vaccination with nelipepimut-S(NCT02297698) (Mittendorf et al. 2015). In the second study, patients with low-expression of HER2 (IHC 1+ or 2+) breast cancer (after completion of standard therapy), were vaccinated with nelipepimut-S + GM-CSF and trastuzumab or trastuzumab + GM-CSF. No significant differences in toxicity especially cardiotoxicity among immunized patients were observed. This trial is ongoing, and the clinical efficacy will be available after completion of the study (NCT01570036) (Clifton et al. 2019). GP2 is another nanopeptide (654-662: IISAVVGIL) derived from the transmembrane domain of the HER2/neu protein. GP2 has been known as a subdominant

epitope with poor binding affinity to HLA-A2 molecule (Ayoub et al. 2019; Clive et al. 2012; Cui et al. 2018). Initially in various studies, GP2 peptide immunogenicity was investigated, and the peptide GP2 was introduced as a promising antigen for breast cancer vaccine application (Brossart et al. 1998; Mittendorf et al. 2006; Peiper et al. 1997; Yoshino et al. 1994). In a phase I clinical trial, the safety, immunogenicity and the best doses of the GP2 peptide plus GM-CSF were assessed. Patients were lymph node-negative and expressed HER2 based on immune histochemical analysis (IHC 1–3 +). All patients received standard therapy and were disease free. HLA-A2 + patients were vaccinated with increasing doses of the GP2 peptide and GM-CSF. The safety of vaccine was demonstrated with minimal local and systematic toxicity including erythema, induration, pruritis, inflammation and fatigue. The trial indicated that the vaccine was able to elicitaGP2 specific immune response in patients (Carmichael et al. 2010). A phase II trial was then conducted to investigate the GP2 vaccine efficacy in preventing recurrence in node-positive and high-risk node-negative HER2 breast cancer patients (IHC 1–3 +) (NCT00524277). Disease-free patients with HLA-A2-positive after receiving standard therapy, were divided in two groups including a control group (only received GM-CSF) and a treatment group (received GP2 vaccine + GM-CSF). The 5-year DFS rates in (IHC 3 + and FISH 1 +) patients vaccinated with GP2 was increased compared to the control group. However, the benefits were not statistically significant for the patients ($P = 0.08$). Using intention-to-treat analysis, after 34 months of follow up, the 5 year DFS rate was estimated to be 88% in the treatment group and 81% in GM-CSF group ($P = 0.43$) (Mittendorf et al. 2016). In addition, this research reported that a combination therapy of GP2 + GM-SCF vaccine with trastuzumab in HER2 overexpressing breast cancer patients was safe with limited local or systemic toxicity and without any increase in cardiotoxicity. A list of clinical trials of GP2 vaccines are listed in Table 1.

AE37 is another HER2-derived peptide (Ii-key hybrid of MHC II peptide AE36 (HER2/neu 776–790) is identified to be capable in inducing both $CD8^+$ and $CD4^+$ cells. The presence of Ii-key, a four-amino-acid LRMK, enhances vaccine effectiveness by increasing epitope charging resulting in improved antigen presentation (Gillogly et al. 2004). Fifteen disease-free, node-negative patients received hybrid AE37 and GM-CSF for 6 months on a dose escalation method in a phase I study. AE37 was safe and found to induce HER2 specific immune response both in vivo and in vitro (Benavides et al. 2008). Furthermore, the AE37 peptide vaccine showed potency in the absence of an immunoadjuvant (Holmes et al. 2008). Due to the positive results from phase I trial, a phase II trial (NCT00524277) was conducted to analyze the benefits of AE37 + GM-SCF vaccine in preventing the recurrence rate in node-positive and high-risk node-negative breast cancer patients. The patients were inoculated with AE37 and GM-CSF did not show a significant difference in recurrence rate and 5 year DFS, but data from this study indicates of the benefit of AE37 + GM-SCF vaccine in reduction the recurrence rate in TNBC patients which need further clinical investigation (Mittendorf et al. 2014).

Strategies to Improve Immunogenicity of Peptide Vaccines

Due to poor immunogenicity of single peptide vaccines in inducing suitable immune response and particularly inducing cell-mediated immunity, considerable attention has been devoted to enriching the anti-tumor activity of peptide vaccines by inclusion of different adjuvants and delivery vectors. For this purpose, nano-delivery strategies including liposomal, virus-like particle, polymeric and non-degradable nanoparticle delivery approaches have been considered in recent years. There are potential advantages in using these vaccine delivery vectors over the application of immunologic peptide alone. Liposomes have the potential to help peptide antigen delivery to the lymph nodes and to increase the cellular uptake by DCs resulting in enhancement of peptide immunogenicity (Alving 1991; Krishnamachari et al. 2011; Watson et al. 2012). In addition, liposomes act as immune adjuvants to enhance and prolong the immune response (Nordly et al. 2009). In a study reported by our group, liposomal formulations composed of dioleoylphosphatidylethanolamine (DOPE), distearoylphosphocholine, distearoylphosphoglycerol and cholesterol carrying E75 peptide were developed. Liposomal formulations were used both as delivery vehicles and as immune adjuvants for enhancement of the stimulation of immune response against established rHER2/neu protein overexpressing tumors in BALB/c mice. The liposomal formulation of E75 peptide was able to evoke specific $CD8^+$ CTLs response that protected mice against HER2 tumor challenge in both therapeutic and prophylactic experiments (Arab et al. 2018a). Moreover, and in a similar line of research, we formulated the GP2 peptide in liposomal carriers consisting of dimyristoylphosphatidylcoline, dimyristoylphosphoglycerol, DOPE, cholesterol and monophosphoryl lipid A (as an adjuvant). The GP2 containing nano-liposome improved the immunogenicity of the GP2 peptide in inducing T cell immunity in BALB/c mice model of TUBO xenograft cancer and potentially reduced the tumor development and, prolonged survival time in mice (Razazan et al. 2017). Furthermore, we have reported that encapsulation of the AE37 peptide in nano-liposomes composed of DOTAP (*N*-[1-(2, 3-Dioleoyloxy) propyl]-*N*, *N*, *N*-trimethylammoniummethyl-sulfate), DOPE and cholesterol with CpG (as an adjuvant) elicited both cellular and humoral immunity leading to potential therapeutic and protective effects in BALB/c mice bearing

HER2 breast cancer (Barati et al. 2017). Phage display is another valuable technique for vaccine discovery which has been exploited by many research groups including our group (Arab et al. 2018b, c, 2019; Frenzel et al. 2016). It has been shown that antigen-presenting cells (APCs) readily take the immunogenic molecules from the displaying phage, process and present them on MHC I and MHC II molecules to induce higher immune responses compared with soluble antigens with no carriers (Prisco and De Berardinis 2012). In addition, there are many other advantages for phage vector application in vaccine delivery that include the phage intrinsic adjuvant activity, higher stability, easy and cost effectiveness of construction and manufacturing, safety profile and the high multivalent phage-display potential. These advantages make phage-derived nanoparticles potential candidates for phage-based vaccine delivery systems (Arab et al. 2019).

Our group evaluated the immune response induction by the E75 peptide displayed on phage λF7 (gpD::E75) in TUBO tumor of BALB/c mice. Before and after vaccination the animals were challenged in vivo with the TUBO xenograft tumor cells (for therapeutic and prophylactic evaluation of the vaccine). Results of this study indicated that stimulation of E75 specific $CD8^+$ T cells resulted in higher titers of IL-4 and IFN-γ in vaccinated mice compared to control (λF7) and buffer groups (Arab et al. 2018b). In addition, we have shown the anti-tumor activity of GP2 and AE37 peptides displayed on phage λF7 (gpD::GP2 and gpD::AE37) in BALB/c mice with the TUBO tumor. The prophylactic and therapeutic activities against TUBO tumor model in mice indicated effective immunogenicity of the designed phage display systems (Barati et al. 2018; Razazan et al. 2019).

The B-subunit of Shiga toxin (STxB) has been also used as a delivery vehicle to optimize the E75 vaccine efficacy by targeting DCs. The STxB-E75 vaccine in combination with GM-CSF and CpG was shown to be more potent than the free E75 peptide or STxB-E75 without adjutants in induction of E75 specific anti $CD8^+$ T cells and inhibited the tumor growth in murine HLA-A-2 expressing low levels of HER2/neu (Tran et al. 2016). Polyactin A (PAA) is an antibiotic which was obtained from a streptococcus strain and used in China for treatment of various disease caused by immune system dysfunction. PAA as an unprecedented immunological adjuvant was also exploited to improve the immune system in peptide-based cancer vaccines (Bi et al. 2008). Culturing of peripheral blood mononuclear cells (PBMCs) in the presence of PAA, IL-4 and TNF-α could sensibly induce differentiation and maturation of DCs from PBMCs and resulted in higher expression of CD80, CD83, CD86 and HLA-DR compared to negative control. These DCs were able to stimulate efficacious T cell responses against E75 peptide in vitro. In an in vivo evaluation of the potential E75 vaccine formulation, C57BL/6-Tg (HLA-A2.1) 1Enge/J transgenic mice were vaccinated with E75 and PAA. This resulted in positive rates of $CD4^+$ and $CD8^+$ T lymphocytes and IFN-γ in splenocytes (Wang et al. 2018).

Anti-idiotype Based Vaccines

One of the most troubling obstacles in cancer vaccination is the immunological tolerance, probably due to antigens of self-origin (Nanda and Sercarz 1995). Anti-idiotype (anti-Id) antibodies in cancer immunotherapy, which mimic TAA are used to circumvent the tolerance phenomenon (Lollini et al. 2005). scFv40 and scFv69 are human anti-Id scFv antibodies fragments that were chosen through a phage-display library screening using the anti-HER2 antibody trastuzumab in sera of immunized BALB/c mice (Teulon et al. 2006). Ladjemi et al. (2011) reported the protection effects of anti-trastuzumab anti-Id scFv69 in vaccinated transgenic BALB/c mice from development of HER2-positive breast tumors. This protection effects could be due to anti-HER2 antibody induction, immune response associated to Th2 cells and reverse HER2 immunological tolerance (Ladjemi et al. 2011).

Vaccines Based on Large Fragments of HER2 Protein

Although peptide-based vaccines have many advantages in eliciting immune system, but they suffer from many limitations. As discussed above, the majority of peptide vaccines are HLA restricted and epitopes specific for HLA class I that may need Th adjuvant to promote persistence of CTLs response (Knutson et al. 2016). Whole protein vaccines include both HLA class I and II epitopes and also are not HLA restricted (Disis et al. 1999). Animal experiments have shown an induction of immune response after immunization with protein-based vaccines. Vaccination of mice with a complex of extracellular domain (ECD) of human HER2 and anti-human HER2/neu IL-2, IL-12 or GM-CSF fusion protein, inhibited the growth of SK-BR-3 human breast tumor cells significantly and protected mice against the TUBO tumor expressing HER2/neu due to eliciting both antibodies and cellular responses against HER2/neu (Cruz et al. 2003). Twenty-five patients in different stages of HER2-positive breast cancer (II, III, or IV) were vaccinated with HER2 intracellular domain (ICD) protein in combination with GM-CSF once a month for 6 months. Most of the vaccinated patients tolerated and promoted both HER2, ICD specific T cell and humoral responses. After the completion of immunization, more than half of the patients maintained the CTLs immunity for 9–12 months. However, the vaccine dosage did not influence the extent of T cell and antibody responses. The authors nevertheless observed that using a higher dose of the vaccine was correlated with fasteranti-HER2 immunity in patients (Disis et al. 2004). In another study, Kitano et al. (2006) utilized CHP-HER2 (a

protein-based vaccine consisting of a truncated HER2 protein, (amino acids 1–146 of HER2) and cholesterylpullulan (CHP). Patients vaccinated subcutaneously with CHP-HER2 received three doses of the vaccine, each dose separated by a 2 week period, followed by a booster injection. The vaccine was well-tolerated with transient skin reactions (grade 1) at the site of injection and specific $CD8^+$ and/or $CD4^+$ T cell responses were developed in patients against the truncated HER2 protein (Kitano et al. 2006). In the second part of this clinical trial, nine patients were immunized with the vaccine alone followed by administration of the adjuvant GM-CSF or OK-432. Other six patients, from the beginning of the vaccination plan treated with CHP-HER2 plus GM-SCF. The authors demonstrated that 146 different HER2 specific antibodies were induced in 14 patients; however, the immune response was detectable earlier in those immunized by CHP-HER2 with GM-CSF at the first cycle of vaccination. This indicated that GM-CSF has a role in acceleration of immune response in the patients immunized with CHP-HER2 plus GM-CSF at the onset of the immunization (Kageyama et al. 2008). Another anti-HER2 protein-based vaccine, dHER2, a fusion protein consisting of ECD of HER2 and a portion of HER2 ICD plus the immune- stimulant AS15, was evaluated by Curigliano et al. (2016). Forty subjects with HER2-positive metastatic breast cancer were vaccinated by this group with dHER2. Vaccine was safe and only adverse effects grade 1/2 such as myalgia, back pain, chest pain, and diarrhea were reported. Cellular and antibody immunity to the dHER2 were observed. Twenty-five percent of patients producedanti-HER2 long term immunity (Kroemer et al. 2015). Table 2 represents a list of clinical trials for immunogenicity of HER2 protein-based vaccines.

DNA Based Anti-HER2 Vaccines

In DNA (genetic) vaccines, the DNA coding the tumor antigen is presented by a plasmid which is injected into the host. The DNA-based vaccine can be used to stimulate antigen specific adoptive and nonspecific innate immunity (Li et al. 2012). This strategy is considered as one of the most practical ways for cancer immunotherapy because of its simplicity, safety, stability and cost effectiveness (Yang et al. 2014).

Many vaccine studies have provided evidence for the efficacy of HER2 DNA vaccine in the prevention of tumor development in HER2 transgenic mice and transplantable tumor models (Chen et al. 1998; Jacob et al. 2006; Marchini et al. 2013; Rovero et al. 2000; Yang et al. 2014). A DNA sequence coding for full extracellular domain or four extracellular subdomains of human HER2/neu was cloned into pCMV6-Neo vector. An anti-HER2 antibody was raised in the serum of BALB/c mice vaccinated with full extracellular domain of HER2, whereas none of extracellular subdomains induced detectable levels of anti-HER2 antibody (Sadri-Ardalani et al. 2016). In a similar work, it was reported that a DNA vaccine, p185 with plasmid coding both the transmembrane domain and the extracellular domain of rat-p185 could protect transgenic BALB/c mice against TUBO cancer cells (Rovero et al. 2000). DNA sequences corresponding to the ligands and immune- adjuvants have been fused to improve the immunogenicity of tumor antigens. For instance, a research group fused the human heat shock protein, hsp70 to the extracellular domain of rat HER2/neu (NeuEDhsp70). Hsp70 could promote DC activation resulting in immune-enhancing cytokine production and priming antigen specific T cells (Todryk et al. 2003). The NeuEDhsp70 DNA vaccine promoted Neu-specific antibody and cellular immune responses in vivo and significantly reduced the metastasis with enhancing the survival rate in a spontaneous metastatic breast tumor model (Kim et al. 2005). Similarly, Pakravan et al. (2010) have reported on a DNA vaccine made of GP96 + HER2/neu. A reduction in $CD4^+CD25^+Foxp3^+$ at the tumor site and enhancement of IFN-γ/IL-4 levels were observed in BALB/c mice harboring TUBO tumor after vaccination with GP96 + HER2/neu DNA vaccine (Pakravan et al. 2010). Chemokines such as CCR7 ligands Epstein–Barr-virus-induced-molecule-1-ligand-chemokine (ELC/CCL19) and secondary lymphoid-tissue chemokine (SLC/CCL21) have great roles in regulating the innate and adoptive immune responses and have been investigated in two different studies (Förster et al. 2008; Stewart and Smyth 2009; Viola et al. 2006). In one study, Nguyen-Hoai et al. (2012a) used CCL21 as an attractive adjuvant to boost the immunogenicity of HER2/neu DNA-based vaccine in a BALB/c mice model. Results of this study demonstrated that co-expression of CCL21 and HER2/neu induced immune responses via TH1 cells and improved the protective effects of the HER2/neu DNA vaccine. CCL21 also showed a synergistic activity with GM-CSF in increasing the tumor protective effects in mice vaccinated with HER2/neu DNA vaccine (Nguyen-Hoai et al. 2012a). In another study, the adjuvant activity of CCL19 in HER2/neu DNA vaccine was evaluated in BALB/c mice harboring HER2 cancer. Co-administration of pDNA (CCL19) with HER2/neu DNA vaccine improved the protective efficacy of the vaccine (Nguyen-Hoai et al. 2012b). In a preclinical study, a polypeptide DNA vaccine was designed which consisted of the full length of HER2 protein and mammaglobin-1. Poly Th epitopes were added to the construct to obtain an optimal induction of immune system by $CD8^+$ and $CD4^+$ T cells. The expression and effectiveness of the vaccine was tested in human DCs. The level of $CD86^+$ and $CD83^+$ cells increased significantly on transfected DCs which could establish interactions with T cells on their surface. Furthermore, the production of IL-6 was increased with DC maturation. This demonstrated the ability of the test vaccine to induce immune response (Nazarkina et al. 2016). The first

Table 2 List of clinical trials using whole vaccines prepared from HER2 protein, whole cell, dendritic cells and DNA (clinicaltrials.gov)

Vaccine	Adjuvant	Additional intervention	Condition	Study population	Phase	Status	Clinicaltrials.gov identifier
HER2/neu intracellular domain protein	GM-CSF	Trastuzumab	Breast cancer	Patients with locally advanced HER2-positive (IHC 2+or 3+) stage IIIB–IV	II	Active, not recruiting	NCT00343109
HER2/neu intracellular domain protein	Polysaccharide- K	Trastuzumab or pertuzumab	Breast cancer	Stage IV, HER2-positive, (IHC 2+or 3+)	I/II	Active, not recruiting	NCT01922921
HER2 protein AUTOVAC (PX104.1.6)	–	–	Breast cancer	HER2-positive (IHC: 1+, 2+or 3+)	I	Completed	NCT00068614
Cholesterol-bearing hydrophobized pullulan HER2 protein 146 (CHP-HER2) and NY-ESO-1 protein (CHP-NY-ESO-1)	OK-432 (Picibanil)		Esophageal cancer Lung cancer Stomach cancer Breast cancer Ovarian cancer	HER2 (IHC 1+or more) and/or NY-ESO-1-expressing cancers	I	Completed	NCT00291473
dHER2 (truncated version of the HER2 protein)	AS15 ASCI	Lapatinib	Metastatic breast cancer	HER2 (IHC 3+) Metastatic breast cancer Refractory to Trastuzumab	I/II	Completed	NCT00952692
HER2/neu intracellular domain protein	–	–	Breast cancer	Stage III or stage IV breast cancer that overexpresses HER2	–	Completed	NCT00363012
dHER2 Recombinant Protein	AS15		Neoplasm, breast cancer	HER2 (IHC 3+) Metastatic breast cancer	I/II	Completed	NCT00140738
Recombinant dHER2 protein	AS15		Breast cancer	HER2-overexpressing Patients with high-risk breast cancer	I	Completed	NCT00058526
MVA-BN-HER2 highly attenuated non-replicating vaccinia virus, MVA-BN®, engineered to encode a modified form of the HER2 protein			Breast cancer	HER2 (IHC 3+) after adjuvant therapy	I	Completed	NCT01152398
MVA-BN-HER2 highly attenuated non-replicating vaccinia virus, MVA-BN®, engineered to encode a modified form of the HER2 protein			Breast cancer	Patients with metastatic HER2-positive breast cancer	I	Completed	NCT00485277

Table 2 (continued)

Vaccine	Adjuvant	Additional intervention	Condition	Study population	Phase	Status	Clinicaltrials.gov identifier
DC1 vaccine pUMVC3-IGFBP2-HER2-IGF1R (WOKVAC)			Breast cancer	Male and female stage I–III HER2-positive	II	Recruiting	NCT03384914
Dendritic cell vaccine (DC1)	–	(docetaxel), Carboplatin, Herceptin (trastuzumab), Perjeta (pertuzumab)	Breast cancer	Patients with stage II/III HER2-positive breast cancer	Early phase I	Recruiting	NCT03387553
Pembrolizumab		Trastuzumab	Breast cancer	Trastuzumab-resistant, metastatic, HER2-positive breast cancer	1b-2	Closed	NCT02129556
HER2 DC1 vaccine			Breast cancer	Nonmetastatic HER2-positive (IHC 3+), previously treated with DC1 HER2-pulsed vaccine	II	Recruiting	NCT03630809
Allogeneic breast cancer vaccine	GM-CSF	Trastuzumab, Cyclophosphamide	Breast cancer	HER2 (IHC 3+), high risk, metastatic patients	I	Completed	NCT00847171
DNA plasmid based vaccine encoding the HER2/Neu intracellular domain (pNGVL3-hICD vaccine)	GM-CSF		Breast and ovarian cancers	Patients with HER2 (IHC 2+ or 3+) with metastatic stage III or IV breast cancer or stage III or IV ovarian cancer	I	Active, not recruiting	NCT00436254

clinical trial to evaluate the efficacy and tolerability of a HER2 plasmid DNA vaccine in human was performed at the oncology clinic, Radiumhemmet, Karolinska University Hospital (Stockholm, Sweden). This pDNA vaccine encoded the total length of HER2 molecule. Eight patients with advanced/metastatic HER2 expressing breast cancer already on treatment with trastuzumab received the HER2/neu pDNA vaccine with low doses of GM-CSF and IL-2. Six patients (out of eight) completed the three vaccination cycles in this study. The other two patients only received one dose of vaccine and due to complications and rapid development of the cancer no further doses were provided. In fact, one patient developed erysipelas (infection of the upper dermis and superficial lymphatics) and the other patient was withdrawn due to disease progression which might be due to the vaccine. The co-administration of HER2-pDNA vaccine plus GM-CSF, trastuzumab and IL-2 was well-tolerated with no acute toxicity, cardiotoxicity and autoimmunity. The level of specific T cells and antibodies against HER2 were increased significantly after vaccination and long-lasting cellular and humoral immunity were observed in some patients for several years. The median survival time for eight patients was 76 months with a range of 46–96 months (Norell et al. 2010). In another phase I clinical study, the safety and immunogenicity of a dual component human DNA vaccine named V930, and V932 were evaluated. V930 vaccine consisted of two plasmids expressing the ECD and transmembrane domains of HER2 and carcinoembryonic antigen (CEA) fusion B-subunit of *Escherichia coli* heat labile toxin (LTB) (study 1). The V932 used was an adenoviral vaccine vector encoding the CEA fusion LTB and the truncated HER2 (study 2). Patients with grade II, III or IV for breast, colon, ovary, or non-small cell lung cancers that expressed HER2 and/or (CEA) received these DNA vaccines. Both vaccines proved to be safe and tolerable. No cell-mediated responses to CEA or HER2 was detectable in vaccinated patients. Only the immune response against bacterial portion of the vector was detected (Diaz et al. 2013).

Currently two phase I clinical trials using DNA vaccines for HER2 are active. One study is supported by Memorial Sloan Kettering Cancer Center (NCT00393783). The goal of this study is to assess the safety and type of immune responses by the rat HER2/neu vaccination in patients suffering from stage III or metastatic breast cancer (AJCC Stage III and IV) that overexpress HER2. In the second trial (NCT00436254), DNA coding the intracellular domain of HER2 cloned into a pNGVL3-hICD plasmid. Sixty-six patients with HER2 overexpressing cancer were enrolled. The subjects received pNGVL3-hICD vaccine combined with GM-CSF intradermal/month for three consecutive months. The adverse effects, the best dose of DNA vaccine which could elicit HER2 specific immune response was determined. The patients were monitored for up to 15 years by the physicians. Table 2 represents a list of clinical trials for HER2-positive types of cancer.

Autologous Tumor Cell Vaccines

Autologous tumor cell vaccine is a patient specific and safe approach for vaccine development by creating a personalized vaccine. Here the tumor cell lysates from a patient's own tumor antigens are exploited to develop an effective immune response. However, the major disadvantages of allogeneic tumor cell vaccine (ATCV) is the inherent poor immunogenicity of tumor cells and inconsistency of the production method (Parvizpour et al. 2018). In addition, due to the presence of endogenous cellular antigens, an autoimmune reaction is possible (Al-Awadhi et al. 2018; Kurtz et al. 2014). In ATCV based breast cancer immunotherapy, an additional antigen, immune modulator or cytokines is combined with the autologous breast tumor cell (Kurtz et al. 2014). Currently, in phase I clinical trials (two different studies), autologous breast cancer cells secreting GM-CSF are being evaluated for activation of immune responses. In one study (NCT00317603) the safety and biological activity of the vaccine is being evaluated in stage IV HER2 metastatic breast cancer patients previously treated with trastuzumab. In the second study (NCT00880464), the vaccine is being investigated in women with operable, stage II, III breast cancer. In another autologous active cellular immunotherapy, 18 patients with metastatic HER2 overexpressing breast cancer have been evaluated for the toxicity and the immune response induced by Lapuleucel-T (APC8024). This vaccine comprised of PBMCs, activated before with the recombinant fusion protein (the intra- and extracellular domains of HER2) plus GM-CSF. Significant anti-HER2 cellular response was observed and the vaccine was very well-tolerated. Three patients presented with disease stabilization for 1 year and partial tumor activity was observed in one patient after 6 months (Park et al. 2007). Early clinical trials of GM-CSF-secreting tumor vaccines demonstrated their safety, bioactivity and clinical benefits in solid tumors. However, vaccination alone is insufficient to induce an immune response (Jaffee et al. 2001; Laheru et al. 2008). Co-administration of some chemotherapeutic agents in proper doses and sequence can augment the immunotherapy. In an experimental work, GM-CSF whole cell vaccine with low doses of cyclophosphamide and doxorubicin induced HER2 specific immune response in HER2/neu (neu-N) transgenic mice (Machiels et al. 2001). Cyclophosphamide seems to inhibit $CD4^+CD25^+$ Treg cells activity and promote the activation of high-avidity specific $CD8^+$ T cells (Ercolini et al. 2005).

Allogeneic Tumor Cell Vaccines

Allogeneic and autologous vaccines are similar in most aspects but also present with some differences. Here tumor antigens are obtained from the same species cancerous cells grown in laboratory. Therefore, they are not patient specific (Srivatsan et al. 2014). Allogeneic vaccines have been studied alone or in combination therapy. An investigation was performed using either allogeneic GM-CSF-secreting breast cancer vaccine alone or in combination with limited doses of cyclophosphamide and doxorubicin. The study was designed to evaluate the adverse effects and immunologic activity of the vaccine and to determine the optimal chemotherapy dose in the combination therapy in HER2-positive breast cancer. This vaccine was made of two cell lines (T47D parent, $HER2^{low}$) and SKBR3 ($HER2^{high}$) which were modified genetically to secrete GM-SCF. Twenty-eight patients were administrated with vaccine alone or with cyclophosphamide and doxorubicin sequentially. The vaccine was safe and no dose limiting toxicity was observed. HER2 specific Th-dependent immunity was induced with vaccine alone or with low doses of chemotherapy agents. It was revealed that doses of 200 mg m^2 and 35 mg m^2 of cyclophosphamide and doxorubicin produced the highest humoral immunity responses. The results of this study suggested that low dose chemotherapy with cyclophosphamide and doxorubicin could break the immune tolerance, with constant immune responses toward specific antigen (Emens et al. 2009). In a phase II trial (NCT00847171), the activity of immune system in combination therapy with trastuzumab, cyclophosphamide and allogeneic GM-SCF secreting cell vaccine in patients with high risk or metastatic breast cancer was evaluated. Based on the vaccination strategy, patients received trastuzumab weekly at the beginning and continued until the completion of vaccination. Patients also received cyclophosphamide and allogeneic breast cancer vaccines expressing GM-CSF. No serious adverse effects were reported.

Dendritic Cell Vaccines

Dendritic cells also known as "professional APCs" have a central role in the immune system due to their functions in regulating immune tolerance and initiation of anti-tumor effects. Both during microbial or viral infection and cancer development, DCs can induce tumor-specific CTL responses by presenting the TAA to T lymphocytes through the MHC I and II pathways (Palucka and Banchereau 2012). Moreover, DCs have crucial roles in controlling antibody-based responses. They are capable to interact with B cells directly and helping B cells by inducing the expansion and differentiation of $CD4^+$ T cells, resulting in a raise of specific humoral immunity. Considering all these properties, DCs may be the best candidates in any therapeutic vaccination process leading to a strong immune response against cancer cells (Batista and Harwood 2009; Qi et al. 2006). DC-based vaccines have appeared to be more effective in improving cellular immunity in comparison to peptide-based vaccination approaches (Dissanayake et al. 2014). Growing data in preclinical and clinical experiments reveal that DC-based vaccines are capable to induce strong anti-tumor responses against breast cancer cells (Gelao et al. 2014). Transfection of DCs with specific mRNA, to drive adaptive immunity response, has confirmed to be an effective approach to induce expansion of $CD4^+$ and $CD8^+$ T cells. Bryson et al. (2017) prepared a multifunctional vaccine made from a modified lentivirus, loaded with two breast cancer antigens including alpha lactalbumin, and HER2, which could directly target the resident DCs. Single injections of the DC-targeted lentiviral vectors resulted in tumor self-antigen-specific cellular immunity, decreasing tumor development and rendering an effective immunotherapy for HER2-positive breast cancer (Bryson et al. 2017). Another preclinical study evaluated the efficacy of DCs transfected with an adenovirus expressing the HER2/neu gene (AdNeuTK) and IL-12. Subcutaneous immunization of Friend leukemia virus B (FVB) mice with the DC vaccine could provide anti-tumor immunity in about 60% of the mice under study. The result of in vivo depletion studies also established the role of both $CD4^+$ and $CD8^+$ T cells in inducing anti-tumor immunity (Chen et al. 2001). Xie et al. (2013) engineered DCs with two cancer antigens including the P30 peptide-derived from tetanus toxin (FNNFTVSFWLRVPKVSASHLE) and HER2-derived peptides to generate highly efficient $CD4^+$ and $CD8^+$ T cell responses toward HER2-positive breast cancer. In another report, Viehl et al. (2005) indicated that administration of DCs transfected with HER2 fused to Tat protein led to protection effects against tumor in FVB/N mice challenged with syngeneic HER2 overexpressing breast cancer cells. Authors found that immunization of the mice with Tat-HER2/neu transduced DCs considerably resulting in smaller sizes of the tumor compared to the control group or to mice received DCs transfected with Tat. Both $CD4^+$ and $CD8^+$ T responses were essential to prompt this anti-tumor response (Viehl et al. 2005). Objective clinical responses also have been observed in some patients with HER2-positive breast cancer. In the results of clinical trial reported by Czerniecki et al. (2007) thirteen patients with HER2-positive breast cancer vaccinated with DCs loaded HER2/neu HLA class I and II peptides weekly repeated four times before surgery. Immune response against the peptides observed for both IFN-γ-secreting $CD4^+$ (85%) and $CD8^+$ (80%) T lymphocyte cells (Czerniecki et al. 2007). Although multiple approaches such as selection of the suitable DC subsets, adjuvants and the augmentation of DC functionalities are being assessed to develop the efficiency of DC-based vaccines, but their clinical benefits are still limited.

Other Targets for Breast Cancer Vaccine Development

Due to the increasing interest in breast cancer immunotherapy research, in addition to HER2 receptor other molecules such as MUC-1 have been extensively studied shown promising success rates. Mucin or MUC-1 is a transmembrane glycoprotein expressed in the breast, lung, colon, ovary, pancreas and other tissues. The extracellular domain of MUC-1 has a variable number of 20 amino acid tandem repeat units which are highly glycosylated in normal cells, but either hypo-glycosylated or aberrantly glycosylated in cancerous cells. This difference presentation of MUC-1 between normal and abnormal cells makes it an attractive target for immunotherapy (Hossain and Wall 2016). T cells recognizing MUC-1 have been derived from the blood of patients with breast cancer (Disis et al. 1994; Jerome et al. 1993). MUC-1 is overexpressed in more than 90% of breast cancer cells and has a correlation with HER2. In fact, silencing of MUC-1 down-regulate HER2 activation and reverses resistance to trastuzumab (Raina et al. 2014). The most difficult situation for patients with HER2 overexpression is resistance to trastuzumab. Go-203, a MUC-1 inhibitor (Raina et al. 2009) can disrupt MUC-1/HER2 complexes and decrease HER2 phosphorylation. It has been found that a combination therapy with GO-203 and trastuzumab have synergistic effects in HER2-positive breast cancer (Raina et al. 2014). In many clinical studies, the safety and immunologic responses both cellular and humoral responses against MUC-1 in patients with breast carcinoma were demonstrated (Ko et al. 2003).Vaccines designed to target MUC-1 have been used with different carriers such as keyhole limpet hemocyanin (KLH), bovine serum albumin and tetanus toxoid with adjuvants including, monophosphoryl lipid A, Quillaja saponaria extract (QS)-21, Bacillus Calmette-Guerin or incomplete Freund's adjuvant to boost immune responses (Hossain and Wall 2016). In one study, patients with high-risk breast cancer were vaccinated with MUC1-KLH conjugated to QS-21. Patients developed adverse effects such as local skin reactions and mild flu like symptoms. IgG and IgM antibodies titers were increased following the vaccination. Nevertheless, no data about T cell responses was presented (Gilewski et al. 2000). In a phase I/II, nine patients with metastatic breast cancer were inoculated with a recombinant vaccine virus expressing the human MUC-1 plus IL-2 gene (TG1031). Although MUC-1-specific antibody not measured in any of the patients but in two patients MUC-1-specific CTLs was detected. Also, evidence of the presence of T memory cells in tumor biopsies was observed (Scholl et al. 2000). In a phase III, 29 subjects with grade II breast cancer received either placebo or manna-oxidized MUC-1. The patient responses were assessed based on prevention of recurrence or metastatic cancer. No recurrence was reported in the immunotherapy group (0/16) versus 27% (4/15) in the placebo group after more than 5.5 years follow up. Measurable anti-MUC-1 antibody titers and T cell response were determined in vaccinated patients (Apostolopoulos et al. 2006). The follow up of study for 15 years indicated that about 60% and 12.5% recurrence of the disease was possible in placebo and immunized group, respectively (Vassilaros et al. 2007).

Viral Oncotherapy

A minority of HER2-positive breast cancer patients do not respond to targeted mAb therapies because of resistance to HER2 antibodies or due to the inaccessibility of anti-HER2 antibodies (brain metastases). Therefore, in a similar line of research into vaccine development, other new therapeutic modalities are needed (Eager and Nemunaitis 2011).

Recently, an increasing number of oncolytic viruses have been developed for treating different types of cancers (Miest and Cattaneo 2014). Preclinical in vivo experiments with viruses have been carried out for breast cancer HER2. For example, Nanni et al. (2013) prevented the development of HER2 ovarian and breast metastatic tumors using a HER2 targeted oncolytic virus. The first oncolytic herpes simplex virus fully detargetted from both nectin1 and herpes virus entry mediator and retargeted to the human oncoprotein HER2 reported by Menotti et al. (2009). Here the Ig-folded core in the receptor-binding virion glycoprotein gD was replaced with anti-HER2 single-chain antibody. Authors showed that intra-tumoral administration of HSV in nude mice bearing HER2-overexpressing human tumors, stopped the outgrowth of highly progressive tumor (Menotti et al. 2009).

Immune Checkpoint Inhibitors

Cancer immunotherapy through immune checkpoint receptors on T cell surface has already assessed in a range of tumor types, such as breast cancer, head and neck cancer as well as melanoma, some advanced solid and hematological malignancies and non-small cell lung cancer and is quickly changing the practice of medical oncology (Alsaab et al. 2017). The two main immune inhibitory pathways of T cell activation in the context of clinical cancer immunotherapy, PD-1/PD-L1 or PD-L2 and CTLA-4, play unique roles in regulating immunity (Khedri et al. 2011; Yazdian-Robati et al. 2017). Blocking these pathways via mAbs or aptamers provoke significant anti-tumor activities in numerous tumors including breast cancer (Dollins et al. 2008). Kim et al. (2017) evaluated PD-L1 protein expression, the level of PD-L1 mRNA and different histopathologic elements including TILs utilizing fresh and formalin-fixed paraffin embedded HER2-positive breast cancer tissues. They found higher mutational burden, greater numbers of TILs

and significant rates of PD-L1 positivity in HER2-positive cancers (Kim et al. 2017). For the first time, in a preclinical study the effectiveness of PD-L1 immune checkpoint blockade and whole cell vaccination in a HER2-positive mouse model of breast cancer showed complete tumor regression in 50% of the treated mice (Bozeman et al. 2016; Nourbakhsh et al. 2015). Blocking CTLA-4 using ipilimumab has revealed encouraging results in a phase III study (Wolchok et al. 2013). The combination of ado-trastuzumab emtansine with both anti-CTLA4 and anti-PD-1 mAbs, in orthotopic mouse models of HER2 breast cancer, improved innate and adaptive anti-tumor immune responses relative to trastuzumab emtansine (TDM1) or immunotherapy alone and resulted in overcoming the primary resistance to immune checkpoint-blocking antibodies (Müller et al. 2015). In one study reported by Gao et al. (2009) a recombinant oncolytic virus was employed in a combination of a CTLA-4 antibody to preferentially target HER2 breast cancer cells. This combination therapy could cure the majority of the mice, while the virotherapy alone prolonged only the survival time (Gao et al. 2009).

Numerous clinical trials are in progress to assess the addition of PD-1/PD-L1 antibodies to other HER2 based therapies (Table 2). In a clinical phase 1b-2 trial (PANACEA), the safety and clinical outcome of combination therapy of pembrolizumab (PD-1 blocker) plus trastuzumab in women with trastuzumab-resistant, metastatic, HER2-positive breast cancer was assessed. Findings of this study confirmed the clinical benefits of the treatment. No dose limiting toxicities were observed (Loi et al. 2019).

A double blinded phase II trial is now underway to test whether the combination of atezolizumab to TDM1 can further increase clinical effects in participants with metastatic HER2 breast cancer previously treated with trastuzumab and a taxane (NCT02924883). In another randomized clinical trial the activity of three different combination drugs are being evaluated: (1) trastuzumab and vinorelbine combined, (2) trastuzumab, vinorelbine and avelumab (mAb directed against the PD-L1) combined, and (3) trastuzumab, vinorelbine, avelumab and utomilumab combined in progressive HER2-positive breast cancer (NCT03414658).

Conclusions and Future Perspective

Immunotherapy is a promising approach for managing breast cancer, particularly when combined with other standard therapies such as surgery, chemotherapy, radiation and hormonal therapy. Immunotherapy with trastuzumab has provided a proven efficacy in HER2-positive breast cancer patients as monotherapy or in combination with chemotherapeutic agents. Although trastuzumab is well-tolerated, but the acquired resistance and its cardiotoxicity are serious concerns in clinic. Active immunotherapy with several advantages over passive immunotherapy or chemotherapy, can be used as a combination to the other modalities. In vaccination the induction of immune responses is tumor specific and usually well tolerated. The most important aspect of active immunotherapy and anticancer vaccination is the provision of long-lasting immunity against tumor antigens and thus preventing tumor relapse. Many clinical trials have been conducted using HER2 peptide-based vaccines alone or in combination with trastuzumab. NeuVax (E75) is a well-studied HER2 peptide-based vaccine in phase III clinical trial and has been studied in combination with trastuzumab in phase II. Other peptides, GP2 and AE37 are also being studied in clinical trial phase II. Although there are positive reports coming out of these clinical trials, overall no specific immunotherapy approach has been approved for HER2-positive breast cancer yet. Several limitations of HER2 peptide vaccines strategies are noted: (1) E75 peptide is a HLA-A2 and HLA-A3 restricted peptide, thus limited number of patients who are HLA-A2 and HLA-A3-positive will benefit from this type of vaccine; (2) the immunity induced by E75 and GP2 is short-lived and need booster doses to generate long-lasting memory immune cells; (3) developing of immune tolerance against HER2 antigens is possible; and (4) there is an unfavorable impact of chemotherapy and radiation therapy on immune system before vaccination. Strategies using HER2-positive whole tumor vaccine or antigen-encoding DNA vaccine could provide multiple epitopes and stimulate immune response strongly against HER2-positive tumor. However, concerns about serious adverse effects and high risk of autoimmunity hinder their clinical development and investigation about them still is remained in early experimental stage. The ability to overwhelmed T cell anergy using immune checkpoint inhibitors including CTLA-4 or PD-1/PD-L1 has shown promise for HER2-positive breast cancer treatment, demonstrating the potential to harness the immune system but this approach is still relatively nonspecific. It seems that applying vaccination approach integrated with other standard therapies will certainly bring us closer to the final goal of immune-based breast cancer prevention.

Compliance with Ethical Standards

Conflict of interest Javad Behravan is an adjunct professor at University of Waterloo, Waterloo, Ontario, Canada and Co-founder of Theraphage Inc. Kitchener, Ontario, Canada. The authors declare that they have no conflicts of interests.

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