

# Recent advances in the development of breast cancer vaccines

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**Abstract:** The manipulation of the immune system through the administration of a vaccine to direct an effective and long-lasting immune response against breast cancer (BC) cells is an attractive strategy. Vaccines would have several theoretical advantages over standard therapies, including low toxicities, high specificity, and long-lasting efficacy due to the establishment of immunological memory. However, BC vaccines have failed to demonstrate meaningful results in clinical trials so far. This reflects the intrinsic difficulty in breaking the complex immune-escaping mechanisms developed by cancer cells. New vaccines should be able to elicit complex immunologic response involving multiple immune effectors such as cytotoxic and antibody-secreting B cells, innate immunity effectors, and memory cells. Moreover, especially in patients with large tumor burdens and metastatic disease, combining vaccines with other strategies, such as systemic BC therapies, passive immunotherapy, or immunomodulatory agents, could increase the effectiveness of each approach. Here, we review recent advances in BC vaccines, focusing on suitable targets and innovative strategies. We report results of most recent trials investigating active immunotherapy in BC and provide possible future perspectives in this field of research.

**Keywords:** breast cancer, cancer vaccines, cancer immunology, HER2, MUC-1, hTERT

## Introduction

Recently, advances in early diagnosis and more effective treatments have reduced the mortality rate due to breast cancer (BC).<sup>1</sup> However, despite this progress, BC remains a leading cause of death in the female population worldwide.<sup>2</sup> In this scenario, manipulating the immune system to direct an effective and long-term immune response against BC cells through the administration of a vaccine is an attractive strategy. Tumor vaccination would have several theoretical advantages over standard therapies. First, the ideal tumor vaccine would induce potent and durable immune reactions against a broad spectrum of tumor antigens. It could be easily administered and manufactured, with modest side effects typical of conventional chemotherapies. Moreover, it would prevent further tumor recurrences, due to the establishment of persistent immune memory. At present, however, active immunotherapeutic strategies against cancer have failed to fulfill the above expectations in clinical trials.<sup>3</sup> This reflects the intrinsic difficulty in finding optimal targets for a cancer vaccine, the most effective type of vaccination, route of administration, and the most immunologically favorable setting of disease (eg, low tumor burden, not heavily pretreated patients). Most importantly, it reflects the difficulty in breaking the complex immune-escaping mechanisms developed by cancer cells.<sup>4</sup> The aim of this review is to summarize recent advances in BC active

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immunotherapy, to address recent results from clinical trials, and to provide possible future perspectives in this field of research.

## Targets and strategies of BC vaccines

It has been well established that the immune system plays a role in controlling tumor growth, and adaptive immunity is the main mediator of “spontaneous” regression of certain types of cancers.<sup>5,6</sup> The immune system has the ability to recognize several types of antigens expressed on tumor cell surfaces, namely the tumor-associated antigens (TAAs).<sup>7</sup> TAAs are presented to immune system effectors such as T-cells by the tumor itself, through the major histocompatibility complex (MHC) or, more likely, by antigen presenting cells (APCs), in particular macrophages and dendritic cells (DCs).<sup>7</sup> These cells are essential in processing antigens into immunogenic peptides and presenting them to naive T-cells through the MHC complex. Through a complex and regulated system of co-activator and inhibitory molecules expressed on the cell surface, these cells play an essential role in priming T lymphocytes and activating an immunogenic response against specific targets.<sup>8</sup> The presence of tumor-infiltrating lymphocytes has been correlated with better prognosis in several types of cancers.<sup>9,10</sup> However, tumor cells often develop the ability to circumvent the surveillance of the immune system.<sup>11</sup> In the tumor microenvironment, molecules such as vascular endothelial growth factor, transforming growth factor (TGF)- $\beta$ , and interleukins are abundant and both actively downregulate the immune function and promote tumor progression, invasion, and metastasis.<sup>12</sup> In addition, tumor cells can directly downregulate T-cell function through expression of transmembrane inhibitory molecules such as FasL or B7-H1/PD-L1 or, indirectly, by promoting functionally suppressive CD4+FoxP3+ T lymphocyte (TReg) function.<sup>13,14</sup> Finally, cancer cells can modulate expression or mask TAAs, reducing their availability and presentation to immune effectors.<sup>15</sup> All these mechanisms can therefore lead to altered DC and T-cell function, and, as final result, to impaired immune response against tumor cells. Immune-escaping mechanisms are particularly active in epithelial cancers such as BC.<sup>16</sup> However, some degree of immune response against TAAs can be demonstrated in BC patients.<sup>17–19</sup> This has prompted researchers to develop active immunotherapies to therapeutically amplify these weak responses against known immunogenic BC antigens. In fact, the aim of an effective therapeutic vaccine is to break peripheral tolerance and activate low-affinity T-cells that

were not eliminated during selection in thymus.<sup>20</sup> A number of breast TAAs have been recognized and described so far (Table 1). Among them, human epithelial growth factor receptor 2 (HER2), carbohydrate antigens, telomerase reverse transcriptase (hTERT), and mucin-1 (MUC-1) have received the greatest attention for vaccine formulations<sup>7</sup> and have been tested in clinical trials. In order to produce an effective vaccine, an antigen or a pool of antigens (as for whole-tumor-cell vaccines) should be delivered through an appropriate formulation. Activation of the immune system could be enhanced by including adjuvant compounds, and appropriate monitoring techniques should be prompted to assess the immunologic response.<sup>21</sup> Recently, new technologies such as the use of nanoparticles and liposome formulations, which may improve efficacy of vaccines have received great attention, and preclinical studies with interesting results have been published.<sup>22–24</sup> Indeed, various vaccine formulations have been tested so far, but none of these was shown to be superior in all situations. Distinct vaccination platforms engage different aspects of the antitumor immune response and each has its own pros and cons, as briefly summarized in Table 2.

## Peptide-based vaccination

Peptide-based vaccines aim at inducing immune responses (including antibodies, cytotoxic T lymphocytes [CTLs], and helper T-cells) using antigenic epitopes derived from TAAs.

**Table 1** Main breast cancer TAAs

Target	Description
Carcinoembryonic antigen	Glycoprotein involved in cell adhesion, normally expressed during fetal development
Human epithelial growth factor receptor 2	Growth factor receptor belonging to human epithelial growth factor receptor family Overexpressed in 20%–30% of breast cancer cells, correlates with adverse prognosis
MUC-1	Membrane glycoprotein involved in immunologic and cell signaling functions Overexpressed in 70% of breast cancers
hTERT	Component of the telomerase complex, a ribonucleoprotein that maintain chromosome integrity during cell proliferation and division
p53	Tumor suppressor protein, involved in cell cycle regulation and DNA damage repair Mutated in 20% of breast cancers
Mammaglobin-A	Glycoprotein overexpressed in 80% of metastatic breast cancers
Cancer testis antigens (NY-eso-1, MAGE, BAGE, and GAGE)	Proteins expressed in normal germ cells of the testis and embryonic ovaries and in certain types of cancer

**Abbreviations:** TAAs, tumor-associated antigens; hTERT, telomerase reverse transcriptase; MUC-1, mucin-1.

**Table 2** Current vaccine platforms

Vaccine platform	Strengths	Possible limitations
Peptide-based vaccines	Easily produced and delivered Low toxicities Easy to monitor immunologic response	Able to elicit mainly one compartment of immunological response (cytotoxic or humoral) Require boosts inoculation to maintain immunological memory HLA-restricted Require suitable adjuvant Response restricted to one or few epitopes
DC-based vaccines	Not HLA restricted Capable to elicit immunological memory, humoral and cytotoxic response	Technically challenging Requires expansion, maturation, and activation of DCs
DNA-based vaccines	Can be produced on larger scales Can elicit both humoral and cytotoxic compartments	Risk of toxicities Low efficiency of naked DNA or plasmids Difficulties in finding suitable vectors
Whole cell-based vaccines	Can elicit a response against broad spectrum of antigens Not HLA-restricted	Difficult to monitor response Risk of toxicities and autoimmunity Requires cell manipulation to be immunogenic

**Abbreviations:** HLA, human leukocyte antigen; DC, dendritic cell.

Many of the first cancer vaccine strategies focused on inducing tumor-specific CD8<sup>+</sup> cells with MHC class I restricted short peptides.<sup>25–27</sup> It is now clear that these CD8<sup>+</sup> T-cell responses are typically weak and short-lived.<sup>25,28</sup> Further studies have clarified that triggering the CD4<sup>+</sup> T-cell response is critical for maximizing tumor immunity, as it both optimizes the CD8<sup>+</sup> T-cell response and supports the humoral antitumor immune response.<sup>29</sup> Thus, researchers have focused on studying peptide-based vaccines that are able to trigger both CD4<sup>+</sup> and CD8<sup>+</sup> responses, using longer peptides or mixtures of epitopes.<sup>30</sup> Peptide vaccines have several potential advantages, which include easy manufacturing, easily evaluable immunological response, and low expected toxicities. These advantages have made the peptide-based vaccination widely studied and employed in clinical trials. However, this strategy presents some objective limitations. First, to be effective, peptide vaccines often require co-administration of an immunological adjuvant. Adjuvants play an important role in favoring recruitment and efficient stimulation of immune effectors. Identification of an even more efficient adjuvant for a given vaccine is crucial for the effectiveness of the formulation and has been the object of intense research. Second, most of the peptide-based vaccines tested are restricted to HLA-A2. This limits the number of potentially benefiting patients. Third, although easily monitored, immune response is directed against one or a few epitopes, possibly reducing the effectiveness of response and favoring mechanisms of immune escape. Finally, we should consider population- and patient-specific variability in antigen processing and presentation, which could affect the effectiveness of such a strategy.<sup>31</sup>

## DNA-based vaccination

The principle of this approach is based on the assumption that the DNA encoding for a given TAA can be taken by APCs, translated into protein, and finally processed for presentation. DNA can be delivered naked or complexed with other molecules. Frequently, the most used vectors are viruses that are able to efficiently transfect target cells.<sup>32,33</sup> Recently, new technologies such as nanoparticles and liposome preparations have been successfully employed to deliver DNA vaccines.<sup>34–36</sup> A large body of evidence supports the idea that stimulating a coordinated immune response, involving cellular, humoral, and innate immune effectors (natural killer cells and macrophages), most effectively mediates tumor rejection.<sup>37,38</sup> DNA vaccines, because of their unique mechanisms of action, could stimulate a more “physiologic” immune response against antigens and could be produced on a larger scale. However, finding an effective vector can be challenging.

## Dendritic cells-based vaccination

DCs are the most important APCs. They naturally express high levels of MHC molecules, co-stimulatory proteins, and cytokines.<sup>39,40</sup> Autologous DCs can be modified by fusion with cancer cells by pulsing with peptides or by transfection to express tumor antigens.<sup>41</sup> DC vaccination represents one of the most intriguing platforms in cancer vaccines. In fact, DCs are able to stimulate both class I and class II responses and can be further modified in order to co-express co-stimulatory molecules, and responses can be directed against multiple targets. This type of platform has been successfully employed and approved for clinical use in castration-resistant prostate cancer.<sup>42,43</sup> However, this

vaccine platform remains technically challenging due to the uncertainty related to the optimal route of administration and expansion, maturation, and/or activation of DC cells, which is not easily achievable *ex vivo* and, as a result, this limits larger scale manufacturing.

## Whole cells-based vaccination

Another potential approach is immunizing the patient with whole tumor cells, derived from the patient herself (autologous) or from cell-line cultures (allogeneic). These vaccines have been shown to induce antigen-specific T-cell responses. However, more frequently, tumor cells act as antigenic pool for *in vivo* or *ex vivo* APCs presentation. To enhance immunological response, tumor cells can be genetically modified to express co-stimulatory molecules or cytokines.<sup>44</sup> Theoretical advantages of such approach comprise providing a pool of tumor antigens, generating immune responses to more than one antigen, and thereby possibly overcoming the tumor antigen loss.<sup>45</sup> Moreover, this could lead to a more “complex” response, involving both CD4+ and CD8+ T-cells, against different antigens. Potential drawbacks may be the triggering of autoimmunity and difficulties in monitoring the consequent immunologic response that may be directed against unknown TAAs.<sup>46</sup>

## Anti-HER2 vaccines

HER2 is a protein receptor with tyrosine kinase activity belonging to the human epidermal growth factor receptor family overexpressed in 25%–30% of BC.<sup>47</sup> HER2 is effectively targeted by a number of new-generation therapies, such as the monoclonal antibodies trastuzumab, pertuzumab, or T-DM1,<sup>48–50</sup> and small tyrosine kinase inhibitors such as Lapatinib, Neratinib, and Afatinib.<sup>51–53</sup> HER2 is a suitable target for BC vaccines, and several investigations have shown that some patients develop spontaneous anti-HER2-specific immunity with high levels of both cellular and humoral response.<sup>17,54</sup>

The largest body of data resulting from anti-HER2 vaccinations derives from clinical trials employing peptide-based vaccines in different clinical settings. The most studied HER2-derived peptide in clinical trials is E75 (HER2 amino acids [aa] 369–377) derived from the extracellular domain of HER2 and characterized by HLA-A2 restriction.<sup>55</sup> In the initial studies, E75 was demonstrated to be safe and capable of inducing peptide-specific CD8+ response. The majority of patients enrolled in these pivotal studies had breast or ovarian cancer and were vaccinated with increasing doses of vaccine

administered monthly, with up to six immunizations.<sup>17,25,26,56,57</sup> In a retrospective analysis aimed at evaluating long-term outcome of BC patients enrolled in these early trials, it was shown that 21 out of 52 (40%) identified patients were alive at the time of analysis (median follow-up [FU] of 112 months).<sup>58</sup> Six out of eight (75%) evaluable patients had persistent T-cell immunity versus immunizing HER2 peptides. Moreover, seven out of eight evaluable patients (88%) developed T-cell-specific immunity against HER2 epitopes other than the one used for immunization, likely due to epitope spreading. These data showed that E75 was able to induce effective and long-lasting immunologic responses. Given the encouraging results, E75 was tested in two larger Phase II clinical trials in an adjuvant setting.<sup>59</sup> A total of 195 women with HER2 positive (with score  $\geq 1$  by immunohistochemistry) were included (100 lymph-node positive, 95 lymph-node negative) after completion of a standard course of surgery, chemotherapy, and radiotherapy.<sup>60</sup> The final results of this study have been very recently reported.<sup>61</sup> The vaccine was administered with granulocyte-macrophage colony-stimulating factor (GM-CSF) for up to six immunizations, and 49% of patients received booster inoculations. At 5 years, the disease-free survival (DFS) rate for vaccinated patients was 89.7% versus 80.2% for controls ( $P=0.08$ ).<sup>62</sup> Although not statistically significant, vaccination was associated with a 48% reduction of relative risk of recurrence. Interestingly, among the node-positive population, benefits of vaccination were shown to be larger. In fact, the 24-month DFS rate was 90.2% for vaccinated patients and 79.1% for controls ( $P=0.13$ ), with a relative reduction of risk for recurrence of 53%. Interestingly, patients expressing low levels of HER2 (immunohistochemistry 1 or 2+) had more robust immunologic response,<sup>63</sup> and in this population the DFS rate was improved at 5 years of FU,<sup>64</sup> with the DFS rate of 88.1% in vaccinated patients versus 77.5% in controls ( $P=0.16$ ). In contrast, in patients whose tumors overexpressed HER2, the DFS rate was 90.3% for vaccinated patients versus 83.3% for controls ( $P=0.44$ ). Of 30 vaccinated patients who had HER2-overexpressing tumors, only 12 patients received trastuzumab before vaccination, and none of these patients had recurrences. Because of the trial design, not all patients received the vaccine dose that was subsequently determined to be optimal. Moreover, a booster inoculation was administered in 53 patients (49%). Intriguingly, among the patients who received booster inoculations the DFS rate at 5 years was 95.2% ( $P=0.11$  vs controls).

AE37 is an HER2 (aa 776–779)-derived peptide modified with a “li-Key” motif in order to elicit a combined CD4+ T- and CD8+ T-cell response.<sup>28</sup> This vaccine formulation was tested

at three different doses with or without GM-CSF in 15 patients with node-negative BC, in remission after standard therapy. Intriguingly, without adjuvants, AE37 was able to effectively elicit a specific combined response.<sup>65–67</sup>

Another HER2-derived peptide tested in clinical trials is GP2. This is a nine-aa, HLA-A2-restricted peptide derived from the transmembrane domain of HER2 (aa 654–662), with a lower affinity to HLA-A2.<sup>68</sup> Preliminary results of a Phase II trial of the GP2 vaccine with GM-CSF were recently presented.<sup>69</sup> In this trial, 172 HLA-A2-positive newly diagnosed women with node-positive or high-risk node-negative disease after standard therapy were enrolled. GP2 was shown to be able to elicit strong immunologic response in the vaccinated group, and, although not statistically significant, the immune response correlated with a >50% reduction in BC recurrences (4.3% vs 11.6% in the vaccine and control group, respectively;  $P=0.41$ ).

Results of the clinical trials of anti-HER2 vaccines, using different types of platforms, are summarized in Table 3.<sup>70–73</sup>

## Anti-MUC-I and anti-CEA vaccines

MUC-1 is a membrane glycoprotein expressed by many types of epithelial cells, including breast, lung, and the gastrointestinal tract.<sup>74</sup> In cancer cells, it is overexpressed and aberrantly glycosylated, and involved in cell-to-cell and cell-to-matrix adhesion, in signal transduction, and in modulation of the immune system. Nearly 70% of cancers overexpress MUC-1.<sup>74,75</sup> It has been demonstrated that MUC-1 can be immunogenic and could be a suitable target for cancer immunotherapy.<sup>76–78</sup>

A series of pilot studies using anti-unglycosylated MUC-1 peptide-based vaccination were undertaken, with conflicting results.<sup>79–83</sup> Further studies showed that directing immunologic response against glycosylated form of MUC-1 could translate into stronger humoral responses. An anti-MUC-1 vaccination conjugated with keyhole limpet hemocyanin (KLH) was shown to elicit strong humoral response and to be safe.<sup>84–86</sup> Based on these results, Miles et al conducted a Phase III trial in 1,028 women with metastatic BC who had previously received chemotherapy and had had either a complete or a partial response, or no disease progression.<sup>86</sup> All women received T-Reg-depleting doses of cyclophosphamide (300 mg/m<sup>2</sup>) 3 days before vaccine injection. Although the treatment was well tolerated and able to induce strong humoral response, no DFS or overall survival (OS) benefit emerged.<sup>3</sup> However, in patients treated with concomitant hormone therapy (34%), a trend toward improved time to progression and OS was observed. This observation suggests that combined therapy with anti-estrogens and anti-MUC-1 could be beneficial.

It was shown that most of the immunogenicity of MUC-1 resided in a repeated 20-aa peptide domain in the extracellular portion of the protein. This peptide, fused with oxidized mannan (M-FP) in an attempt to improve APC presentation, was able to induce both cytotoxic and humoral responses.<sup>87–90</sup> Hence, a pilot Phase III study in early stage BC patients using oxidized mannan–MUC-1 was undertaken.<sup>91</sup> In this small, placebo-controlled, randomized study, 31 patients with stage II and no more than four lymph nodes involved were injected with the vaccine after surgery, radiotherapy,

**Table 3** Clinical trials with anti-HER2 vaccinations

Platform	Vaccine	Setting and number of patients	Adverse events	Results
Protein-based vaccine	HER2 ICD + GM-CSF	Adjuvant, 29 pts	No G2, G3, or G4 toxicities reported	Specific T-cell and antibody were elicited in 89% and 82% of pts, respectively <sup>70</sup>
Protein-based vaccine	dHER2 (truncated recombinant HER2 ECD and ICD) + immunoadjuvant	Adjuvant, 45 pts	One G3 fatigue and one G3 neutropenia	HER2 ECD and ICD antibodies developed after four immunizations Two pts showed evidence of tumor regression <sup>72</sup>
DNA-based vaccine	Poxviral vector encoding a modified form of the HER2 protein	Metastatic, 30 pts	No G3 or G4 toxicities reported	HER2-specific antibodies and T-cell response were detected in 66% pts (15 out of 28) evaluable patients had SD after 6 months FU <sup>71</sup>
Whole cell-based vaccine	SKBR3 (HER2 overexpressing cell line) genetically modified to secrete GM-CSF administered with TReg depleting doses of CY and DOX	Metastatic, 28 pts	No G3 or G4 toxicities reported	Induction of efficient immune response, including specific antibody production, enhanced by CY and DOX <sup>73</sup>

**Abbreviations:** GM-CSF, granulocyte-macrophage colony-stimulating factor; ECD, extracellular domain; HER2, human epithelial growth factor receptor 2; ICD, intracellular domain; pts, patients; G, grade; BC, breast cancer; MBC, metastatic breast cancer; FU, follow-up; SD, stable disease; TReg, regulatory T lymphocytes; CY, cyclophosphamide; DOX, doxorubicin; SKBR3, human HER2 overexpressing breast cancer cell line.



and commencement of the adjuvant Tamoxifen. At 15 years of FU, the recurrence rate in patients receiving placebo was 60% (9 out of 15), whereas in those receiving immunotherapy it was 12.5% (2 out of 16) ( $P=0.002$ ).<sup>92</sup> All patients injected with M-FP showed no evidence of toxic effects or signs of autoimmunity during the 12–15-year FU. No response to MUC-1 was seen in the patients treated with placebo, whereas 9 of 13 patients immunized with MUC-1 had developed MUC-1-specific antibodies and 4 of 10 patients MUC-1-specific T-cell responses. The clinical results obtained with this strategy are very promising, and further larger studies are warranted to confirm these results.

Carcinoembryonic antigen (CEA) is another glycoprotein aberrantly expressed in a multitude of cancer cell types, such as colorectal, lung, or breast cancer.<sup>93</sup> It is a member of adhesion molecules, and its overexpression in cancer cells promotes their adhesion and the metastatic process.<sup>94</sup> PANVAC™ is a new cancer vaccine targeting both CEA and MUC-1. The vaccine is delivered through two sequential viral vectors, recombinant vaccinia (rV–) and recombinant fowlpox (rF–) – given in sequence, containing transgenes for the targets and for three human T-cell co-stimulatory molecules (TRICOM [TRIad of COstimulatory Molecules]) to enhance immune system activation. The PANVAC vaccines are injected subcutaneously and processed by APCs.<sup>95</sup> In a pilot study, 26 heavily pretreated patients with ovarian cancer or BC were enrolled. Side effects were largely limited to mild injection-site reactions. For the 12 BC patients enrolled, the median time to progression was 2.5 months and the median OS was 13.7 months. One patient had a complete response and remained on study for 37 months and another had partial response, whereas four patients had stable disease. Patient with fewer previous lines of treatment or low tumor burden were more likely to respond to the vaccine.<sup>96</sup> In another pilot study, 25 patients with advanced metastatic carcinoma were treated with the vaccine. Two patients with BC were enrolled, one had a confirmed decrease of >20% in the size of large liver metastasis, whereas both had long-duration disease stabilization.<sup>97</sup> These preliminary results show promising activity of this vaccine in BC, and further larger studies are currently going on.

## Anti-hTERT vaccines

hTERT is another potential target for cancer vaccination. Telomerase is a ribonucleoprotein complex that maintains chromosomal integrity by protecting telomeric DNA for continuous cell proliferation.<sup>98</sup> The complex contains hTERT and an RNA template. hTERT has broad expression in cancer

cells, with little or no expression in normal somatic cells. A first pivotal study in 7 HLA-A2 advanced breast or prostate cancer patients showed good tolerability and promising clinical efficacy. Patients enrolled in this study were vaccinated with autologous monocyte-derived DCs pulsed ex vivo with hTERT I540 peptide and KLH as adjuvant. Among the two patients with BC, one showed a mixed response of skin lesions, previously progressed despite chemo, hormonal, and radiotherapy.<sup>99</sup> Another study was conducted on 19 HLA-A2-positive patients with metastatic BC who were refractory to conventional therapy. Following vaccination, a specific CD8+ T-cell response was seen in more than 50% of the patients. Despite this, no objective responses were shown. However, in a post hoc analysis, it was shown that the median OS was significantly greater among patients with high CD8+ T-cell response than among low or no responders (32.2 months vs 17.5 months,  $P=0.03$ ).<sup>100</sup>

## Combining BC vaccines with other strategies

Despite the encouraging preliminary results and excellent profile of tolerability, BC vaccines still show limited clinical efficacy. Antigen variability and mechanisms of tumor immune-escaping can impair the effectiveness of active immunization. Moreover, possible difficulties of the immune effectors to reach poorly vascularized tumors and high tumor burdens may contribute to limit the efficiency of vaccines. For patients with larger burdens of tumor and disseminated disease, it is fairly clear that vaccines alone are not able to outmatch the immune tolerance mechanisms of cancer cells; moreover, these become progressively more complex with tumor progression. Thus, a possible way to overcome the known limits of active immunotherapy may be combining BC vaccines with other strategies, such as systemic BC therapies, passive immunotherapy, or immunomodulatory agents. Interaction of BC vaccines and systemic therapy could be complex and poorly predictable. Thus, any combinatorial strategy requires strong biologic rationale. For example, target-immune checkpoints and reducing activity of T-Regs could overcome immune tolerance and increase the effectiveness of vaccination. Especially for patients with advanced disease, incorporating drugs that target BC-biology-inhibiting key intracellular signaling pathway may be required to enhance the activity of vaccines. In this field, results of first pivotal studies incorporating BC vaccines with targeted therapies have already been reported. Finally, disruption of tumor cells by conventional therapies could lead to the release of

tumor fragments/antigens that are otherwise not accessible for presentation and processing and proinflammatory cytokines, with the final result of an increased immune response.

The humanized anti-HER2 monoclonal antibody trastuzumab has been combined with an HER2 peptide-based vaccine in a Phase I/II study in 22 pretreated patients with HER2 overexpressing stage IV BC.<sup>101</sup> The association treatment was well tolerated and did not result in additional toxic effects. At a median FU of 36 months, the median progression free survival was 17.7 months (33% proportion of patients without events at 3 years). This finding favorably compares with the results obtained in similar settings by conventional treatments.

Patil et al<sup>102</sup> recently reported preliminary results of a Phase I study of trastuzumab in combination with GP2 vaccine plus GM-CSF in an adjuvant setting. Nineteen patients were treated. The combination was shown to be safe, with only mild reaction at the site of injection. No adverse cardiac events were reported. The vaccine was shown to be highly immunogenic, and further studies on this combination are warranted.

Trastuzumab has also been safely combined with an HER2-positive, GM-CSF-secreting, allogeneic breast tumor-cell vaccine. Twenty-two patients with HER2-positive metastatic BC were enrolled in this study. No dose-limiting toxic effects were observed. Clinical benefit rates (complete responses + partial responses + stable disease) at 6 and 12 months were 50% (95% CI, 27%–72%) and 35% (95% CI, 15%–59%), respectively.<sup>103</sup>

A plasmid DNA encoding HER-2/neu together with low doses of GM-CSF and IL-2 and concurrent trastuzumab has been tested in eight patients with metastatic HER2-positive BC. The study was conducted by Norell et al.<sup>104</sup> No relevant toxic effects were observed. The treatment was shown to induce immediate and strong antibody production alongside long-lasting T-cell response. Notably, two out of six patients who completed all three vaccination cycles were long-term survivors, still alive more than 4 years after the last vaccination.

Finally, Hamilton et al<sup>105</sup> published interesting results of a small pilot trial using recombinant anti-HER2 protein vaccine with concurrent Lapatinib in 12 women with trastuzumab-refractory HER2 overexpressing metastatic BC. No unexpected adverse events were shown. Specific anti-HER2 humoral response was induced in all patients. Interestingly, OS at 300 days was 92% (95% CI, 77%–100%).

## Conclusion and future perspectives

In recent years, outstanding progress has been achieved toward the cure of BC. More personalized therapies, molecularly targeted drugs, and a deeper understanding of the mechanisms of disease have allowed improving the prognosis of certain subtypes of tumor. In this rapidly changing scenario, there is a growing interest in developing an effective cancer vaccine. Unfortunately, none of the vaccine tested so far in clinical trials has turned out to be “practice changing.” Nevertheless, three important lessons can be drawn.

First, many vaccines elicit a measurable immunologic response, such as specific antibodies or specific CD8+ T-cells, but this response often has little or no impact on tumor growth. Engaging only one compartment of the immune system (eg, only cytotoxic response or humoral response) is probably not sufficient for an effective therapeutic vaccine. New vaccination strategies should therefore aim at eliciting a wide response, involving multiple immune effectors such as cytotoxic and antibody-secreting B-cells, innate immunity effectors, and memory cells. The underlying concept would be that a “complete” immunologic response may promote increased release of tumor cell fragments/antigens and proinflammatory cytokines, resulting in an immunologic virtuous cycle.

Second, the main barrier against vaccination is probably due to complex immuno-escaping mechanisms developed by cancer cells. Regulatory cells such as T-Regs and molecular immune checkpoints (eg, CTLA-4, PD1/PD1L) play crucial roles in maintaining self-tolerance, and tumors are able to exploit these elements to get protection from immune system's attack. New strategies based on blocking antibodies, recombinant forms of ligands, or receptors should be implemented to block such modulatory checkpoints and strengthen the immune response, with promising initial translation into clinical setting. One of the most intriguing perspectives of these strategies is obviously their synergism with immunotherapy approaches such as cancer vaccines.

Third, patients with large tumor burden and very advanced disease, enrolled in most of the clinical trials on cancer vaccines, are also those who benefit less from cancer vaccines alone. In fact, as cancer progresses and spreads in spite of multiple lines of treatment, immune-tolerance mechanisms become more intricate and the immune system is less likely to counteract the tumor. Therefore, in these patients, combining vaccine with established drugs targeting cancer biology, such as endocrine therapy, tyrosine receptor inhibitors, or chemotherapy, is required to achieve satisfactory

clinical results. In conclusion, in order to maximize the likelihood of success, new BC vaccines should be developed by integrating a thorough understanding of immune tolerance mechanisms and tested in well-designed clinical trials conducted in immunologically favorable settings. Moreover, an additional effort should be made to improve immunotherapy in the specific basal-like subtype, which requires novel therapeutic strategies more than luminal and HER2-positive types.

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## Disclosure

The authors have no conflicts of interest to declare.

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