Active immunotherapy in HER2 overexpressing breast cancer: current status and future perspectives

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Background: The use of anti-HER2 monoclonal antibodies (mAbs) has improved the clinical outcome of HER2-overexpressing breast cancers (BCs). Unfortunately, often these tumors tend to relapse and, when metastatic, the duration of clinical benefit is limited over time and almost invariably followed by tumor progression. Alternative approaches to this essentially passive immunotherapy are therefore needed in HER2-overexpressing BC patients. As HER2 is one of the most suitable targets for active immunotherapy in BC, manipulating the immune system is a highly attractive approach.

Material and methods: A computer-based literature search was carried out using PubMed (keywords: breast neoplasm, HER2 vaccine, immunology); data reported at international meetings were included.

Results: This review provides a focus on the following active vaccinal approaches under clinical investigation against HER2-overexpressing BC: (i) peptide and protein based; (ii) DNA based; (iii) whole tumor cell based; (iv) dendritic cell based. Moreover, the review discuss future challenges in the field, trying to define the best setting for the development of this innovative strategy, considering both immunological and clinical aspects of HER2 targeting.

Conclusions: Development of effective vaccines for BC remains a distinct challenge but is likely to become a substantial advance for patients with HER2-overexpressing BCs.

Key words: active immunotherapy, breast cancer, HER2, vaccine

introduction

In the last decades, several attempts have been made to develop strategies that could effectively induce potent immune responses against various tumor types. Manipulating the immune system to recognize and eradicate breast tumor cells is a highly attractive possibility in the treatment of epidermal growth factor receptor 2 (HER2)-positive breast cancer (BC).

HER2 is a suitable target for immunotherapy as selectively expressed or overexpressed (HER2 positive) in a subpopulation of BCs [1–3]. At least two different approaches fall into the definition of immunotherapy. The first one is passive immunotherapy, consisting in the adoptive transfer of antigen-specific T lymphocytes expanded ex vivo or the infusion of monoclonal antibodies (mAbs) specific for a given tumor antigen. Passive immunotherapy with anti-HER2 mAbs such as trastuzumab, pertuzumab and Trastuzumab-DM1 (TDM1) is the current mainstay in the treatment of HER2-positive BC. The addition of these antibodies was shown to significantly improve survival as first-line treatment of HER2-positive metastatic BC (MBC) [4–6]. Moreover, TDM1 showed its superiority to the current standard Capecitabine and Lapatinib...
in second-line treatment [7]. Several randomized phase III trials in the adjuvant setting showed that the addition of trastuzumab to chemotherapy significantly reduces recurrences and risk of death [8–11].

In spite of these improvements, clinical practice shows that primary and/or acquired resistance to anti-HER2 mAbs remains a relevant issue.

Another evolving strategy in the context of passive immunotherapy is the functional inhibition or depletion of regulatory T lymphocytes (T\textsubscript{Reg}), elements able to downmodulate antitumor T-cell responses. Blockade of CTLA-4 using mAbs seems to be a particularly promising approach in this context [12]. CTLA-4 is physiologically expressed on both activated CD8 T cell and T\textsubscript{Reg}s, inhibiting the activity of the first and enhancing the inhibitory function of the second. Blockade of CTLA-4 molecules on T\textsubscript{Reg}s may downmodulate their inhibitory activity positively impacting immune responses, either spontaneous or elicited by vaccines [13].

In the setting of solid tumors, a positive synergism may be hypothesized by the association of cancer vaccines with strategies blocking CTLA-4 or other immune-checkpoints [14, 15] (Figure 1). The second approach is active immunotherapy, aiming at activating the patient’s own immune system through the administration of a therapeutic vaccine. This strategy offers multiple theoretical advantages. The first positive feature of vaccines is their tumor specificity, eliciting immune responses directed against antigens selectively expressed by tumor cells, sparing normal tissues with consequent low toxic effect. Besides the first, tumor-specific, activity cancer vaccines may trigger secondary responses by the release of antigens and cytokines following tumor lysis. Such responses may involve T cells, antibodies or elements of the innate system. Even if apparently against the idea of specificity, these events may potentiate the beneficial effects of vaccines, mimicking and restoring the physiologic network of immune responses. Important advantage of vaccines over passive immunotherapy is the potential for a sustained antitumor effect related to the induction of immunologic memory, obviating the requirement for multiple adoptive infusions of immune effectors.

Open issues are the determination of best vaccine-formulation, adjuvant selection, route of administration and strategies to overcome tumor immune-escape mechanisms.

Passive and active strategies should however be considered as dynamic concepts with the possibility to elicit transversal and mutually helpful biologic effects, basis for synergic strategies.

Aim of this article is to review the main features of active immunotherapy strategies against HER2 positive BC, focusing

Figure 1. Overview of different vaccines formulations in HER2 positive breast cancer Anti-HER2 immunization can be elicited using whole HER2 protein or peptides derived from extra- or intracellular domain of HER2, or whole tumor cells genetically modified to express co-stimulatory molecules or immune-activating cytokines. Moreover, DNA, naked or incapsulated in viral vectors, can be used to elicit a specific anti-HER2 immune response. Another type of strategy consists of taking dendritic cells of patients engineered to present HER2-related peptides. Finally, the window explains possible modulatory interventions on inhibitory elements (immune checkpoints), that contribute to immune-escaping of cancer cells. One of the most promising is the blockade of CTLA4, which is expressed at high levels by regulatory T cells (T\textsubscript{Reg}) and exerts inhibitory effects on T-cell activation. Similarly, PD1 and PD1 ligand inhibition, or modulatory intervention on other inhibitory molecules and metabolic enzymes (IDO) are under study. APCs, antigen presenting cells; CTLA-4, T-lymphocytes-associated antigen 4; T\textsubscript{Reg}, regulatory T cells; PD1, programmed cell death protein 1; LAG3, lymphocyte activation gene 3; B7-H3 and B7-H4, B7 family inhibitory ligands; TIM3, T-cell membrane protein 3, IDO, Indoleamine 2,3-dioxygenase.
on their effective clinical translation and providing possible future perspectives in this field of research.

active immune-targeting of HER2 in breast cancer

HER2 as a target for cancer vaccines

The ideal vaccine should be able to induce activation and expansion of specific lymphocyte precursors. These precursors should stimulate both cellular and humoral response promoting immunological clearance of tumor cells. Vaccines should be able to induce immunological memory, be safe, easy to manufacture, administer and effective in all the vaccinated population.

Difficulties in finding suitable tumor antigens, paucity of circulating precursors and complex mechanisms of immune-escape, make this field of research challenging.

Several investigations showed that some patients develop spontaneous anti-HER2-specific immunity with high levels of both cellular and humoral response [2, 3].

This observation supports the hypothesis that HER2 is a suitable target for active immunization and anti-HER2 vaccines are under development.

An overview of active anti-HER2 immunotherapy strategies is presented in Figure 1.

HER2-targeted cancer vaccines

peptide-based vaccines

The largest body of data resulting from anti-HER2 vaccinations derives from clinical trials employing peptide-based vaccines in different clinical settings.

Peptide-based vaccines aim at inducing immune responses (including antibodies, cytotoxic T lymphocytes (CTLs) and helper T cells) using antigenic epitopes derived from tumor-associated antigens. First trials with peptide vaccines started in the 1990s and were based on single-epitope peptides, emulsified with clinical graded adjuvants or pulsed on dendritic cells (DC), restricted by a single HLA class-I type. Recently, multiple or long multivalent peptides are under investigation, aiming at eliciting stronger and more complete responses, including HLA class-II restricted T-helper cells.

New generation trials aims at employing personalized vaccines designed on the basis of a pre-existing, even if weak, immunity against cancer cells. Potential advantages of peptide-based vaccines are their easy manufacturing and capability of eliciting easily usable antigen-specific immune response.

However, this type of vaccine has potential drawbacks, discussed in a dedicated chapter below.

The most studied HER2-derived peptide in clinical trials is E75 (HER2 aminoacids 369–377), HLA class-I peptide that stimulates CTLs. The peptide is derived from the extracellular domain (ECD) of HER2 and characterized by HLA-A2 restriction [16]; HLA-A2 molecules are expressed in about 40–50% of Caucasian population, and are reported to effectively present suitable targets for effective immunotherapy strategies [17].

Other peptides used in clinical trials [18, 19], were designed for HLA class-II presentation in combination with E75, to elicit a predominant CD4 + T-cell response to strongly sustain immunological memory.

clinical activity in metastatic setting. After characterization of the peptide, a series of small phase I studies were conducted using E75 alone or in combination with immune-adjuvants.

All treated patients had metastatic or locally advanced disease. All trials demonstrated E75 to be safe and capable of inducing peptide-specific CTLs response [19–24]. Results of these studies are summarized in Table 1.

Table 1. Clinical trials with the E75 peptide

<table>
<thead>
<tr>
<th>Vaccine</th>
<th>No. and type of patients</th>
<th>Toxic effects</th>
<th>Results</th>
<th>Reference</th>
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<tbody>
<tr>
<td>Phase I studies</td>
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<tr>
<td>E75 + Freund’s adjuvant</td>
<td>4 patients with colorectal, breast or ovarian cancer</td>
<td>No G3 or G4 toxic effects observed</td>
<td>T-cell response elicited in 75% patients</td>
<td>Zaks et al. (1998) [20]</td>
</tr>
<tr>
<td>E75 and other HLA class II HER2 derivatives peptides</td>
<td>19 patients with breast and ovarian cancer</td>
<td>No G3 or G4 toxic effects observed</td>
<td>T-cell response elicited in 26% patients (FU &gt; 12 months)</td>
<td>Knutson et al. (2001) [24]</td>
</tr>
<tr>
<td>E75 + GM-CSF</td>
<td>6 patients with breast and ovarian cancer</td>
<td>NR</td>
<td>Low level and short-lived T-cell response</td>
<td>Knutson et al. (2002) [22]</td>
</tr>
<tr>
<td>E75 and other HER2 ECD and ICD derivatives HLA class II peptides + GM-CSF</td>
<td>64 patients with breast, ovarian and lung cancer</td>
<td>No G3 or G4 toxic effects observed</td>
<td>T-cell response in 68% of patients (FU &gt; 12 months)</td>
<td>Disis et al. (2002) [19]</td>
</tr>
<tr>
<td>E75 + GM-CSF</td>
<td>14 patients with breast and ovarian cancer</td>
<td>No G3 or G4 toxic effects observed</td>
<td>T-cell response in 50% of patients</td>
<td>Murray et al. (2002) [23]</td>
</tr>
<tr>
<td>E75 incorporated into PLG microspheres</td>
<td>24 patients with breast, ovarian and lung cancer</td>
<td>No G3 or G4 toxic effects observed</td>
<td>T-cell response in 61% of patients</td>
<td>Salazar et al. (2009) [25]</td>
</tr>
</tbody>
</table>

All patients were HER2 overexpressing.

GM-CSF, granulocytes macrophage colony-stimulating factor; HLA, human leukocyte antigen; ECD, extracellular domain; ICD, intracellular domain; PLG, poly-lactide-co-glycolide; G, grade; FU, follow-up.
From a methodological point of view, the majority of patients were vaccinated with escalating doses of vaccine administered monthly, up to six immunizations.

Recently, Salazar [25] presented results of a retrospective analysis aimed at evaluating long-term overall survival (OS) and duration of immunization in patients with BC immunized with anti-HER2 vaccines in these early clinical trials. Fifty-two patients (37 in stage IV, 15 in stage III) were identified and 21 of 52 (12 in stage IV, 9 in stage III) were determined to be alive at the time of analysis [median follow-up (FU) of 112 months]. Six of eight (75%) evaluable patients had persistent T-cell immunity versus immunizing HER2 peptides.

Moreover, seven 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 (ES). ES is one of the hallmarks of endogenous immunity, showing in multivariate analysis, to be an independent predictor of OS.

These data suggest the realistic possibility of inducing durable immunologic responses. However, underpowered studies did not show clinically meaningful improvements.

clinical activity in the adjuvant setting. Two phase II trials were conducted in this setting. In the first, Peoples [26] vaccinated HLA-A2-positive women with node-positive (NP) disease that expressed HER2 (patients with score ≥1 by immunohistochemistry (IHC) were included), after completion of a standard course of surgery, chemoradiotherapy. Hormonal therapy was administered if appropriated. The same authors conducted a similar trial in node-negative (NN) patients. Results of these two trials were published in a combined analysis [27]. A total of 195 women (100 NP, 95 NN) were included, constituting the largest study of adjuvant immunization in BC to date.

Escalating doses of vaccine (ranging from 0.1 to 1 mg) with GM-CSF was administered up to six immunizations. A booster inoculation was administered in 42.5% of patients. Vaccine showed to be safe. At a median follow-up of 18 months, the recurrence rate was 5.6% in the vaccinated group compared with 14.2% in the observation group (P = 0.04), at 24 months the difference was no longer statistically significant (8.3 versus 14.8%) (P = 0.17) [28]. The proportion of patients free from disease [Disease free survival (DFS)] after 18-months was 92.5% and 77% in the vaccinated and control groups, respectively (P = 0.04) [27].

Patients who received optimal dose of E75 had similar toxic effect and enhanced immune responses compared with those who received lower doses. Importantly, there was a trend toward decreased recurrences in optimally dosed patients [29]. Patients expressing low levels of HER2 (IHC 1 or 2+) had more robust immunologic response [30] and the DFS rate was improved at 24 months follow-up (94% versus 79.4% in the control group; P = 0.04) [31].

Interestingly, investigators observed that, among patients who experience recurrent disease, the survival rate was 88% versus 58% (P = 0.3) in the vaccine and control arms, respectively. Although the advantage was not statistically significant, this finding suggests that vaccine may positively affect on the clinical course of the disease in patients who recur [28].

other peptides. GP2 is a 9 aminoacids, HLA-A2 restricted, peptide derived from the transmembrane domain of HER2 (aa 654–662). Preclinical studies have shown that it is as effective as E75 in inducing CTLs response, despite its lower affinity to HLA-A2 [32].

Recently, Carmichael [33] published the results of the first clinical trial of GP2 in 18 HLA-A2 NN BC patients in the adjuvant setting. Patients were eligible if their BC expressed HER2 (from 1+ to 3+ by IHC). The vaccine was tolerable and effectively induced immunologic responses.

In order to elicit a combined CD4 + T and CD8 + T-cell response with peptides vaccination, [34], researchers had modified an immunogenic HER2 (aa 776–779) derived peptide (AE37), with a so called ‘li-Key’ motif to make easier antigenic epitope presentation of the peptide with HLA class II molecules and to augment the CD4 + T-cell response [35]. The vaccine was tested at three different doses with or without GM-CSF in 15 patients with NN BC, in remission after standard therapy. Interestingly, AE37 was able to elicit CD4 + T-cell-specific immunologic response also in absence of adjuvant [36, 37]. A phase II study of AE37 in adjuvant treatment of disease-free HER2-positive BC patients is currently ongoing with promising preliminary results [38].

potential limitations of peptide-based vaccines. Although promising, peptide vaccinations present some objective limitations.

First, peptide vaccines require an adjuvant to elicit efficient immunological response. This is not a limitation per se but certainly a crucial issue. Adjuvants are important for recruitment and stimulation of immune effectors, favoring correct antigen presentation. Identification of the best adjuvant for a given vaccine formulation is still object of intense research.

Second, HLA restriction is required and most of the vaccines studied so far are HLA-A2 restricted. This is obviously a major drawback, limiting the number of patients that may potentially benefit from such approach. However, a recent study by Patil [39] showed that also HLA-A3 patients can similarly respond to E75 vaccination, increasing the number of potential beneficiaries of E75 vaccine up to 76% of the Caucasian population.

Third, immune response is restricted to one or few epitopes, limiting the magnitude of response and effectiveness in fighting tumor cells.

Fourth, because they mostly stimulate a CD8 + T-cell response, peptide vaccines are not able to generate a strong and sustained immunological memory in the absence of continued antigen exposure and stimulation by antigen presenting cells. A potential strategy to overcome, at least partially, these limitations is to administer multipeptide vaccines or HLA class II restricted peptides and clinical trials are ongoing [40, 41].

Another general issue to be considered is the current lack of knowledge about patient-specific variables on antigen processing and presentation. Pitfalls in these processes may affect vaccine effectiveness.
protein-based vaccines
In order to extend immunological responses to different epitopes of HER2 protein, researchers developed vaccines consisting of the entire or truncated forms of HER2 (e.g. intracellular or extracellular domain).

This type of vaccine has the theoretical advantage to include both HLA class I and II epitopes, avoiding restrictions to specific HLA haplotypes and allowing the elicitation of CD4+ T-cell response.

However, although exogenous soluble protein antigens incorporated by APCs are in general efficient in sensitizing CD4+ T cells, they are less efficient in sensitizing CD8+ T cells. Moreover, with this type of vaccines, it is more difficult to monitor the patient’s response.

Initial clinical trials have been conducted using protein vaccines [42]. Results of these trials are summarized in Table 2 [43, 44].

DNA-based vaccines
The goal of a DNA vaccine is to be taken by APCs, translated into protein and finally processed for presentation.

DNA is usually delivered in the form of expression plasmid, naked or complexed to other molecules, although other types of vectors (such as viral vectors) can also be used [45, 46].

DNA vaccines have the advantage of being simple to construct, produce and administer, co-delivery of cytokine genes can increase their efficacy [47, 48].

Table 2 summarizes results of trials with DNA vaccines [49].

**Table 2. Clinical trials with anti-HER2 vaccines**

<table>
<thead>
<tr>
<th>Vaccine</th>
<th>No. and type of patients</th>
<th>Toxic effects</th>
<th>Results</th>
<th>Reference</th>
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</thead>
<tbody>
<tr>
<td>Protein-based vaccines</td>
<td></td>
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</tr>
<tr>
<td>HER2 ICD + GM-CSF</td>
<td>29 patients with HER2 overexpressing breast or ovarian cancer with no evidence of disease after standard therapy</td>
<td>No G2, G3 or G4 toxic effects reported</td>
<td>Specific T cell and antibody were elicited in 89% and 82% of patients, respectively</td>
<td>Disis et al. (2004) [43]</td>
</tr>
<tr>
<td>dHER2 (truncated recombinant HER2 ECD and ICD) + immunoadjuvant</td>
<td>45 patients with stage II or III BC</td>
<td>One G3 fatigue and one G3 neutropenia</td>
<td>HER2 ECD and ICD antibodies developed after four immunizations. Two patients showed evidence of tumor regression</td>
<td>Limentani et al. (2005) [44]</td>
</tr>
<tr>
<td>DNA vaccines</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Poxviral vector encoding a modified form of the HER2 protein</td>
<td>30 patients with MBC after first- or second-line chemotherapy</td>
<td>No dose-limiting toxic effects were observed</td>
<td>HER2-specific antibodies and T-cell response were detected in 66% patients. Fifteen of 28 evaluable patients had SD after 6 months FU</td>
<td>Guardino et al. (2010) [49]</td>
</tr>
<tr>
<td>Whole tumor cells vaccines</td>
<td></td>
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<tr>
<td>SKBR3 (HER2 overexpressing cell line) genetically modified to secrete GM-CSF administered with Treg depleting doses of CY and DOX</td>
<td>28 patients with MBC</td>
<td>No dose-limiting toxic effects were observed</td>
<td>Induction of efficient immune response, including specific antibody production, enhanced by CY and DOX</td>
<td>Emens et al. (2009) [52]</td>
</tr>
</tbody>
</table>

GM-CSF, granulocytes macrophage colony-stimulating factor; ECD, extracellular domain; ICD, intracellular domain; G, grade; BC, breast cancer; MBC, metastatic breast cancer; FU, follow-up; SD, stable disease; Treg, regulatory T lymphocytes; CY, cyclophosphamide; DOX, doxorubicin.
However, several difficulties are associated with DCs vaccines. Ex vivo expansion, maturation and/or activation of DCs are crucial for optimal antigen presentation and stimulation of T cells but also technically challenging, as well as many concerns remain about the optimal route of administration.

Brossart [55] conducted a pilot study in 10 women with advanced ovarian and breast carcinoma. Patients were vaccinated subcutaneously with mature DCs pulsed with HLA-A2–restricted HER2 or MUC1 peptides. After three immunizations, 5 of 10 patients developed tumor-specific CTLs, which efficiently inhibited HER2 overexpressing cancer cell lines in vitro.

More recently, Czerniecki [56] reported of 27 patients with HER2 overexpressing ductal in situ carcinoma receiving lymph node injection of mature DCs loaded with a mixture containing HER2, HLA class I and II restricted, peptides before surgery. Immunized patients developed specific immune response against the peptides and presented high levels of specific CD4+ and CD8+ T cells. Six of 13 evaluable women presented reduction in tumor size. Notably, three (11%) patients developed transient asymptomatic decrements in cardiac ejection fraction >15% after vaccination; this was the first report of HER2 vaccination associated with decreased cardiac function [57].

Morse [58] conducted a pilot study in seven women with high-risk stage II, III or resected stage IV HER2-positive BC who were disease free after surgery or adjuvant therapy. Vaccine consisted of both mature and immature autologous DC loaded with HER2 ICD peptide. Patients continued adjuvant hormonal therapy after immunization. No relevant toxic effects were reported. Six patients developed immunological T cell response against HER2 ICD and specific antibodies. All patients were alive and disease free at 5 years FU.

Peethambaram [59] reported results of a pilot trial with lapuleucel-T (APC8024). Lapuleucel-T consists of autologous APCs loaded with a recombinant antigen including the extensive HER2 sequence linked to GM-CSF domain. Eighteen patients with metastatic ovarian, colorectal and BC were treated. No relevant toxic effects were reported, three patients with BC had SD at 12 (\(n=2\)) and 21 (\(n=1\)) months, respectively.

Overall, the use of DC vaccines seems promising but results are too preliminary and further improvements are needed.

### Table 3. Ongoing clinical trials on combination of HER2 vaccines and Trastuzumab or Lapatinib

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Disease condition</th>
<th>Trial status</th>
<th>Trial number and PI</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Phase I studies</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>dHER2 + AS15(a truncated form of HER2 + adjuvant AS15) and Lapatinib</td>
<td>HER2 overexpressing stage IV breast cancer</td>
<td>Ongoing but not recruiting</td>
<td>NCT00952692 Michael A. Morse</td>
</tr>
<tr>
<td>HER2 peptide-based vaccine + ex vivo T-cell expansion + Trastuzumab or Lapatinib</td>
<td>HER2 overexpressing stage IV breast cancer</td>
<td>Recruiting</td>
<td>NCT00791037 Mary Disis</td>
</tr>
<tr>
<td><strong>Phase II studies</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GM-CSF secreting cancer cell + Trastuzumab and cyclophosphamide</td>
<td>HER2 overexpressing stage IV breast cancer</td>
<td>Completed</td>
<td>NCT00399529 Leisha A. Emens</td>
</tr>
<tr>
<td>GM-CSF secreting cancer cell + Trastuzumab and Cyclophosphamide with no evidence of disease</td>
<td>HER2 overexpressing high-risk breast cancer</td>
<td>Recruiting</td>
<td>NCT00847171 Leisha A. Emens</td>
</tr>
<tr>
<td>GM-CSF secreting cancer cell + Trastuzumab and Cyclophosphamide</td>
<td>HER2-negative stage IV breast cancer</td>
<td>Recruiting</td>
<td>NCT00971737 Leisha A. Emens</td>
</tr>
<tr>
<td>DC vaccine + Trastuzumab and Vinorelbine</td>
<td>HER2 overexpressing stage IV breast cancer</td>
<td>Recruiting</td>
<td>NCT00266110 Jonathan S. Serody, Lineberger</td>
</tr>
<tr>
<td>HER2 ICD vaccine + Trastuzumab</td>
<td>HER2 overexpressing locally advanced of stage IV breast cancer</td>
<td>Ongoing but not recruiting</td>
<td>NCT00343109 Mary Disis</td>
</tr>
</tbody>
</table>

More details on clinical trials are available at [http://www.clinicaltrials.gov](http://www.clinicaltrials.gov)

GM-CSF, granulocytes macrophage colony-stimulating factor; DC, dendritic cells; ICD, intracellular domain.
the median PFS was 17.7 months, and the Kaplan–Meier estimate of PFS was 33% at 3 years; these findings favorably compare with results obtained in similar settings by conventional treatments. Immunologic evaluation confirmed a boosted and maintained T-cell response against HER2, including phenomenon of ES, that inversely correlated with serum levels of TGF-Beta.

In line with these findings, Benavides [30] showed that, within the E75 trial, 24% (n = 7) of patients among HER2 (IHC 3+) population received trastuzumab and sequentially the E75 vaccine. This therapeutic association was confirmed safe and immunologically beneficial. Interestingly, there were four recurrences and one death among vaccine-alone patients (n = 22) and no recurrences or deaths in the combination group (n = 7).

Emens [61] conducted a phase I study exploring feasibility and safety of standard trastuzumab in combination with HER2-positive, GM-CSF secreting, allogeneic breast tumor-cell vaccine. Twenty-two patients with HER-2 positive metastatic BC were enrolled. No dose-limiting toxic effects were observed. Clinical benefit rates (complete responses + partial responses + SD) at 6 and 12 months were 50% (95% CI 27% to 72%) and 35% (95% CI 15% to 59%), respectively.

Norell [62] conducted a pilot trial with a DNA vaccine, with low doses IL-2 and GM-CSF and concurrent trastuzumab. Eight patients with metastatic HER2-positive BC, pretreated with trastuzumab were enrolled. No relevant toxic effects were observed. Treatment-induced immediate, strong antibody production along with long-lasting T-cell response. Notably, two of six patients who completed all three vaccination cycles, two were long-term survivors, still alive more than 4 years after last vaccination.

Disis [63] conducted a phase II study of HER2 ICD-vaccine concomitant with trastuzumab in HER2-positive advanced or metastatic BC patients. Data on the first 25 patients are available. Vaccine was well tolerated without unexpected adverse events. The majority of patients (75%) developed robust CD8+ and CD4 + T-cell response versus HER2 protein. Data on clinical activity are expected.

Combinations of HER2 vaccines with other anti-HER2 therapies are feasible.

Hamilton [64] recently published results of a small pilot trial using recombinant protein vaccine (ICD and ECD portions of HER2) with concurrent lapatinib in 12 women with trastuzumab refractory HER2 overexpressing metastatic BC. Treatment was well tolerated. Specific anti-HER2 Abs were induced in all patients, whereas specific anti-HER2 T cells were detected in one patient. Interestingly, OS at 300 days was 92% (95% CI 77% to 100%).

conclusions and future perspectives

HER2 inhibition is the mainstay for treatment of HER2-positive BC. Trastuzumab and, more recently, pertuzumab and TDM1 have radically changed the prognosis, improving survival rates of these patients. Other antibodies are under investigation and will be soon part of the clinical armamentarium. In this thrilling and competitive scenario, it is difficult to identify a role for active immunotherapy. However, two important considerations need to be done.

First of all, clinical trials with anti-HER2 therapies indicate that, at least considering the unselected overall population of HER2-positive BCs, an approach combining various chemotherapeutic drugs with anti-HER2 agents is more efficient [4, 65, 66]. This obviously leads to significant increase in toxic effects.

Moreover, we cannot ignore that all the above mentioned treatments have limited efficacy over time. In fact, the best first-line available treatment of HER2-positive BC (docetaxel, trastuzumab and pertuzumab) leads to a median PFS of 18.5 months [4]. Sparing toxic effects and prolonging survival are important goals that could be achieved with active immunotherapy.

Unfortunately, although safe and highly tolerable, none of the active available immunotherapies produced relevant clinical responses by means of improved survival in comparison to standard treatments in HER2-positive BCs.

Moreover, it should be considered that even the advocated high safety and tolerability of cancer vaccines may be challenged by concerns raised by some authors about the risk of severe events associated with the induction of elevated numbers of HER2-specific CTLs. A possible problem could be given by the expression of HER2 in some normal tissues and in a recent clinical trial with the adoptive infusion of gene-modified HER2-specific CTLs, an extremely severe event was reported [67].

Several mechanisms may explain the low clinical effectiveness reported. The main barrier against vaccination is probably due to complex immuno-escaping mechanisms developed by cancer cells. Regulatory cells like TRegs and molecular immune-checkpoints (e.g. 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, have been developed to block such modulatory checkpoints and unleash the immune response, with promising initial translation into clinical setting. One of the most intriguing perspective of these strategies is obviously their synergism with immunotherapy approaches like cancer vaccines.

Moreover, the paucity of specific T-cell precursors and difficulty to elicit both an efficient humoral and cellular response against tumor antigens hinder the development of effective vaccines.

Another obstacle is the emerging difficulty to translate the immunological into satisfactory clinical responses. On one hand, this is the expression of the difficulty of the immune system per se in breaking the barriers of the immune-tolerance of cancer cells; on the other hand, this probably reflects the difficulty of conducting clinical trials in more ‘immunologically favorable’ settings. Efforts should be made to conduct clinical trials in patients with low tumor burden, not heavily pretreated, probably the best setting for anticancer vaccine.

Research on newer type of vaccines, more potent immune-adjvant and best route of administration could overcome some limitations of first generation anti-HER2 vaccines.
As previously said, concepts of active or passive immunotherapies have to be intended as strictly connected and potentially synergic. For example blockade of negative regulatory elements (e.g. anti-CTLA-4; TRegs depletion) may fall in the ‘passive’ group of interventions, it may positively impact as advautant of ‘active’ vaccinations, while the passive adoptive infusion of antitumor immune effectors may create favorable conditions to break tumor tolerance and elicit spontaneous immune responses. On the other end, active vaccination could be a suitable strategy to favor the homeostatic proliferation of adoptively transferred tumor-specific T lymphocytes.

It is important to point out that the few available clinical trials confirm the potential of associating different immunological strategies, showing promising results in HER2-positive BC to overcome barriers of immune-escaping.

In conclusion, development of effective vaccines for HER2-positive BC remains a distinct challenge and the way toward the addition/integration of active immunotherapy to the already available armamentarium is still far away. A better understanding of the complexity of tumor progression, an improvement in technology and identification of the optimal clinical setting (probably advuant or low tumor burden) will certainly help in developing effective immune-based treatments.

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references


