Breast cancer vaccines: a clinical reality or fairy tale?

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The characterization of tumor antigens recognized by immune effector cells has opened the perspective of developing therapeutic vaccines in the field of breast cancer. The potential advantages of the vaccines are: (i) the induction of a robust immune response against tumors that are spontaneously weekly immunogenic; (ii) the tumor specificity for some antigens; (iii) the good tolerance and safety profile and (iv) the long-term immune memory, critical to prevent efficiently tumor recurrence. Most trials evaluating breast cancer vaccines have been carried out in patients with extended metastatic breast cancer, characterized by aggressive tumors, resistant to standard cytotoxic treatments, so that clinical efficacy was difficult to achieve. However, some significant immune responses against tumor antigens induced upon vaccinations were recorded. The aim of this review is to analyze the activity of vaccination strategies in current clinical trials. Data of clinical activity have been observed by using vaccines targeting HER2/neu protein, human telomerase reverse transcriptase, carcinoembryonic antigen and carbohydrate antigen given after stem cell rescue. The review discusses possible future directions for vaccine development and applications in the adjuvant setting.

Key words: active immunotherapy, breast cancer, vaccines

Introduction

Traditional cancer treatment regimes provide acceptable response rates and improve survival in patients with breast cancer, but they are generally not selective, inducing cytotoxicity in normal as well as in malignant cells and so they often are not well tolerated. Advances in the understanding of tumor biology have allowed targeted therapies against specific molecular targets to develop. The specificity is aimed at improving tumor targeting, thereby understanding cytotoxicity against normal cells. Recently, this approach has lead to the development of, for example, tyrosine kinase inhibitors or monoclonal antibodies (mAb). Recent results with mAb have validated the immunotherapy approach [1]. An additional improvement may be brought by active immunization with the advantages of a non-toxic therapeutic modality potentially capable of inducing antitumor immune responses in patients with primary tumors and in those with metastases [2–5]. Induction of strong immunity by cancer vaccines is expected to lead to the establishment of immune memory, thereby preventing tumor recurrence. A number of antigens recognized on tumor cells by T cells have been characterized.

Human tumor antigens can be divided into (1) individual (e.g. incidental mutations that occur in CDK4 in melanoma cancer lines); (2) tumor-specific (e.g. common mutations or viral antigens such as HPV16 in cervix cancer or specifically activated in tumors such as MAGE) and tissue-restricted; or (3) differentiation antigens (i.e. PSA in prostatic cancer). Most of the cancer proteins are self-antigens. So the challenge within the field of cancer vaccines is to find the conditions to break a possible immune tolerance towards tumor antigens without inducing substantial autoimmune reactions that are harmful for healthy tissue [6]. Vaccination in patients with breast cancer should induce an expansion of CD8+ cytotoxic T lymphocytes (CTLs) capable of rejecting tumor cells via recognition of tumor-associated antigenic (TAA) epitopes presented on the surface of cancer cells in association with human leukocyte antigen (HLA) class I molecules. However, optimal immunotherapeutic approaches should probably also prime CD4+ helper T cells, given the key role that these cells play in the control of immune responses and in the induction of cytotoxic responses.

The antigens used in breast cancer vaccination strategies can be represented by whole tumor cells (either allogeneic or autologous) or of specific TAAs, which are delivered as DNA (naked or comprised in recombinant viruses), RNA, protein or HLA class I/II restricted peptide epitopes [3]. Antigenic materials can be injected directly, often coupled to immunostimulatory cytokines or adjuvants, or used for ex vivo loading of antigen presenting cells (APCs), usually dendritic cells (DCs). To generate a successful vaccine, this must...
have: a target antigen on tumor cells to direct the immune response; a platform to present the vaccine-derived antigen to immune system; an adjuvant to enhance immune stimulation, and appropriate monitoring techniques [4].

Table 1 summarizes different approaches used in monitoring vaccine therapies activities. There is a great difficulty in comparing the various approaches because monitoring assays have not yet been standardized. Identification of univocal surrogate immunological markers of activity should be a challenge for the future. Ideally, T-cell assays not only need to be sensitive, specific, reliable, reproducible, simple and quick to perform, but they should offer a good correlation with clinical outcome [7]. Several immunological approaches in the treatment of breast cancer showed that it is possible to elicit an antitumor immune response that could potentially destroy tumor cells; however, the clinical activity is still discouraging. Several hypotheses can be postulated to explain these negative results including: the impact of previous oncolytic treatments (chemotherapy and radiotherapy) on immune system; the population of patients treated so far that is characterized by large tumor burden; the ability of large tumors to escape the immune system; and the difficulty to break immune tolerance [8]. Therapeutic cancer vaccines will probably be more active in minimal residual states, but most of the trials so far have been conducted on metastatic patients and limit the success of phase I/II trials [9]. The aim of this review is to analyze the activity of vaccination strategies for patients with breast cancer in current clinical trials, focusing on possible future directions for vaccine development and applications in the adjuvant setting.

**breast cancer vaccines based on characterized antigens**

Many tumor antigens used in breast-cancer immunotherapy are expressed on normal tissues but are overexpressed or mutated on tumor cells: MUC1, HER2, CEA, hTERT, p53 and carbohydrate antigens. Some of these antigens are universal tumor antigen (Ag) hTERT, as they are broadly expressed by most tumors. We will discuss all potential antigens that have been used to construct vaccines for the treatment of breast cancer.

### Table 1. Surrogate markers of immune response in vaccine therapy trials

<table>
<thead>
<tr>
<th>Techniques</th>
<th>Rationale</th>
<th>Advantages</th>
<th>Disadvantages</th>
<th>Correlation with clinical outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>DTH test</td>
<td>Ags are injected intradermally; evaluation of DTH response</td>
<td>Simple, low cost</td>
<td>Not standardized, subjective, not specific</td>
<td>No</td>
</tr>
<tr>
<td>Tetramer staining</td>
<td>Fluorescent MHC/peptide Tetramers flow cytometry</td>
<td>Quantitative cell number evaluation, specific, CD8+ restricted</td>
<td>Text not evaluating activity of T cells, requires specific tetramers, limited to single epitopes</td>
<td>No</td>
</tr>
<tr>
<td>LPA</td>
<td>Measurement of T-cell proliferation in response to Ag stimulation.</td>
<td>Simple</td>
<td>Influenced by non-specific immune response, not suitable for CD8+</td>
<td>No</td>
</tr>
<tr>
<td>ELISA</td>
<td>Measurement of cytokine secretion</td>
<td>Sensitive</td>
<td>Not specific</td>
<td>No</td>
</tr>
<tr>
<td>ELIspot</td>
<td>Direct enumeration of cytokine releasing Ag-specific T cells</td>
<td>Quantitative cell number evaluation, simple and reproducible, suitable for CD4/CD8+ cells</td>
<td>No enumeration of Ag-specific T cells Standardization needs to be attempted</td>
<td>Possible</td>
</tr>
<tr>
<td>Intracellular cytokine measurement by flow cytometry</td>
<td>Flow cytometry of stimulated cells after staining with a mixture of antibodies</td>
<td>Quantitative and functional assay, high specific rapid, simultaneous multiparameter valuation</td>
<td>Requires incubation, unable to obtain live cells, technically complicated</td>
<td>Yes</td>
</tr>
<tr>
<td>qRT-PCR</td>
<td>Molecular method for measuring amplified genes</td>
<td>High accuracy, specific, indirect measurement of function</td>
<td>No subset cells, high costs</td>
<td>No</td>
</tr>
<tr>
<td>Direct cytotoxicity assays</td>
<td>Incubation of CTL with Ag-expressing cells labelled with chromium-51</td>
<td>Suitable for CD8+</td>
<td>Not sensitive, multiple stimulation are request</td>
<td>No</td>
</tr>
<tr>
<td>Limiting dilution analysis</td>
<td>Serial dilutions of T-cells following in vitro stimulation and lysis</td>
<td>Quantitative analysis, functional activity</td>
<td>Expensive, operator-dependent and labor intensive</td>
<td>No</td>
</tr>
</tbody>
</table>

DTH, delayed-type hypersensitivity; tetramers, peptide MHC-teramers; LPA, lymphoproliferation assays; ELISA, enzyme-linked immunosorbent assay; ELIspot, enzyme-linked immunospot assay; qRT-PCR, quantitative reverse transcriptase polymerase chain reaction; Ag(s), antigen(s); MHC, major histocompatibility complex.
HER2/neu

HER2/neu is a 185-kDa protein receptor with tyrosine kinase activity and extensive homology to epidermal growth factor (EGF) receptor. HER2/neu is expressed in many epithelial tumors and over-expressed in approximately 30% of all primary breast cancer. Overexpression of HER2/neu is associated with a poor prognosis. HER2 is a suitable target because it involves an extracellular domain that can be targeted by antibodies produced by B cells. These antibodies could act either by a functional pathway (i.e. blocking the HER2 signalling pathway) or by an immune mechanism such as ADCC. Moreover, antigens derived from both the extra- or the intracellular domains can be presented by HLA class I or I to CD8+ or CD4+ T cells. Spontaneous T and B cells responses have been observed in patients with HER2/neu-positive tumors, confirming the immunogenicity of HER2/neu [10].

The use of HER2 peptides as a potential target for breast cancer immunotherapy arose from experimental evidence in the rat where it was shown that immunization with a mixture of peptides derived from the extracellular and intracellular domains of human HER2, but not the whole protein, elicited a delayed type hypersensitivity (DTH) [11]. Based on these findings, clinical trials with HER2 peptides have been conducted.

Table 2 summarizes all clinical trials with HER2/neu peptides. Disis [12] reported that nine of 17 patients with breast or ovarian cancer displayed a CD-8 immune response detected as IFN-γ production by positive enzyme-linked immunospot assay (ELIspot) after a vaccination with HER2 peptides mixed with G-CSF. This was associated with minimal toxicity, suggesting that it is possible to generate anti-HER2 responses also in humans without major signs of autoimmunity. In another pilot study, four HLA-A2+ patients with metastatic breast, ovarian or colorectal adenocarcinoma that overexpressed HER2/neu were immunized with the HLA-A2-binding epitope (p369–377) from HER2/neu in Freund’s adjuvant. In three of four patients, peptide-specific CTLs were detected in the blood after one immunization. However, these CTLs failed to lyse HLA-A2+HER2+ tumors cells in vitro [13]. In a following trial, the same HLA-restricted peptide-vaccine with HLA2 peptide and adjuvant GM-CSF has been tested in six patients with either stage III or IV breast or ovarian cancer. This vaccine resulted in the generation of low-level peptide-specific CD8 T-cell immunity that did not persist for an extended time after active immunization. Vaccination with HLA-class I peptides will probably require additional antigen specific or non-specific helper activity to generate long-lived immunity [14]. The same group experimented the vaccine strategy with HLA-restricted helper HER2 peptides (p369–384, p689–703, p971–984) with encompassed HLA A2 epitopes (p369–377, p689–697, p971–979) in 19 patients with stage III or IV breast and ovarian cancer, producing a long lasting immune response (detected by ELISPOT for CD8+) (15). In another series of studies, 18 patients with breast cancer (four with stage III disease and 14 with stage IV disease) were given monthly vaccinations of three 15- amino acid HER2 peptides (369–384, 688–703 and 971–984) that contained the nested CTL epitopes 369–377, 688–696 and 971–979.

The goal of these studies was to overcome the problems associated with using class I epitopes alone, most of which are related to antigen instability or aggregation because of the short length of the peptide. The CD4+ and CD8+ cell responses were long lasting and remained detectable for more than 1 year after the final vaccination [16]. Murray [17] used a vaccine containing E75 (p369–377) plus granulocyte-macrophage colony-stimulating factor (GM-CSF) for a phase I trial in

Table 2. Phase I trials with HER2 peptides-based vaccines

<table>
<thead>
<tr>
<th>Reference</th>
<th>No. and type of patients</th>
<th>Vaccine</th>
<th>Grade 3/4 toxicity rate</th>
<th>No. patients with ELIspot positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Disis, 1999ab</td>
<td>17 with advanced HER2-overexpressing breast or ovarian cancer</td>
<td>HER2 peptides + GM-CSF</td>
<td>No</td>
<td>9/17</td>
</tr>
<tr>
<td>Zaks, 1999</td>
<td>4 advanced HER-overexpressing colorectal, breast or ovarian cancer</td>
<td>HER2 peptides with Freund adjuvant</td>
<td>No</td>
<td>3/4</td>
</tr>
<tr>
<td>Knutson, 2002</td>
<td>6 advanced HER2-overexpressing breast or ovarian cancer</td>
<td>HER2 helper peptides with GM-CSF adjuvant</td>
<td>NR</td>
<td>NR (low levels of CTL)</td>
</tr>
<tr>
<td>Knutson, 2001</td>
<td>19 advanced HER-overexpressing breast or ovarian cancer</td>
<td>HER2 peptides with HLA2 epitopes</td>
<td>NR</td>
<td>5/19</td>
</tr>
<tr>
<td>Murray, 2000</td>
<td>18 advanced HER2-overexpressing breast cancer</td>
<td>HER2 peptides with HLA2 epitopes</td>
<td>NR</td>
<td>NR (CD4+ CD8+ + long lasting response</td>
</tr>
<tr>
<td>Murray, 2002</td>
<td>14 advanced HER-overexpressing breast or ovarian cancer</td>
<td>HER2 E75 plus GM-CSF</td>
<td>No</td>
<td>4/8</td>
</tr>
<tr>
<td>Salazar, 2004</td>
<td>10 advanced HER-overexpressing breast or lung cancer</td>
<td>Four HER2 peptides plus GM-CSF</td>
<td>NR</td>
<td>25%</td>
</tr>
<tr>
<td>Disis, 2004</td>
<td>29 with NED after surgery for HER2-overexpressing breast or ovarian cancer</td>
<td>HER2 ICD peptide</td>
<td>NR</td>
<td>89%</td>
</tr>
</tbody>
</table>

*The trial reports 7/8 minimal response.

No-dose escalation trial.

NR, not reported; NED, no evidence of disease; IDC, intracellular domain ECD, extracellular domain; GM-CSF, granulocyte-macrophage colony stimulating factor; ELIspot, enzyme-linked immunospot assay; ELISA, enzyme-linked immunosorbent assay; CTL, cytotoxic lymphocytes.
14 patients with metastatic breast (n = 13) or ovarian (n = 1) cancer. These patients were vaccinated with escalating doses (500–1000 μg) of E75 (p369–377) mixed with 250 μg of GM-CSF. No grade 3 toxic reactions to the vaccine were noted. Of eight patients tested for CTL induction, four had a CTL response after in vitro stimulation with autologous dendritic cells that had not been pulsed with peptide, consistent with the presence of activated/memory cells ex vivo. Four patients also had an E75-specific CTL response after in vitro stimulation with E75. In addition, CTLs from three patients specifically recognized E75 on indicator tumors, as demonstrated by cold target inhibition of tumor lysates. E75-specific tumor-lytic CTLs were present in some patients for more than 1 year after vaccination [17].

Four putative class II HER2/neu peptides, which were found to generate detectable specific T-cell responses (stimulation index >2) in a majority of patients in a previous study, were used to formulate a single vaccine. The multipeptide vaccine was administered intradermally with GM-CSF as an adjuvant in 10 patients with HER2/neu overexpressing breast or lung cancer. Twenty-five percent of patients developed HER2/neu peptide-specific T-cell immunity and 50% developed HER2/neu peptide-specific antibody immunity. No patient developed HER2/neu entire protein-specific T-cell or antibody immunity. The majority of peptides exhibited high binding affinity, in vitro, to more than three of the 14 HLA-DR alleles analyzed. The group of peptides used in this study demonstrated high binding affinity to multiple DR alleles suggesting that in vitro binding affinity may be able to predict the in vivo immunogenicity of class II peptides. However, only a minority of patients immunized with the multipeptide vaccine developed HER2/neu peptide-specific T-cell or antibody immunity, and none developed HER2/neu protein-specific immunity [18].

Interestingly, in a first study in the adjuvant setting, 29 patients with no evidence of disease after surgery for HER2/neu-overexpressing breast or ovarian cancer received three level doses (low 25 μg, intermediate 150 μg or high 900 μg) HER2/neu (intracellular domain) ICD protein vaccine. The vaccine was administered intradermally, monthly for 6 months, with GM-CSF as an adjuvant. The vaccine was well tolerated. The majority of patients (89%) developed HER2/neu ICD-specific T-cell immunity. The dose of vaccine did not predict the magnitude of the T-cell response. The majority of patients (82%) also developed HER2/neu-specific immunoglobulin G antibody immunity. Vaccine dose did not predict magnitude or avidity of the HER2/neu-specific humoral immune response. Time to development of detectable HER2/neu-specific immunity, however, was significantly earlier for the high- versus low-dose vaccine group (P = 0.003). Over half the patients retained HER2/neu-specific T-cell immunity 9–12 months after immunizations had ended. So, although the dose of vaccine did not impact on the magnitude of T cell or antibody immunity elicited, patients receiving the highest dose developed HER2/neu-specific immunity more rapidly than those who received the lowest dose. Clearly, we are waiting for the clinical outcome report, which needs a long-term follow-up [19].

Another study is ongoing in the adjuvant setting [20]. The vaccine formulation contained a truncated recombinant HER2 protein (dHER2) combined with a new potent immunological adjuvant. dHER2 includes the extracellular domain (ECD) and a part of the ICD of the HER2 protein. The trial’s objective was to evaluate safety and immunogenicity. Three dose levels of dHER2 protein were tested: 20, 100 and 500 μg. Three cohorts of 15 patients with stage II or III breast cancer were enrolled sequentially. Patients received six vaccinations over 14 weeks. All patients receiving the 20 and 100 μg doses have completed the course of six vaccinations, and those in the 500 μg cohort will have done so by March 2005. To date, the vaccine has been well tolerated overall and has shown minimal toxicity. Grade 3 fatigue and grade 3 neutropenia were recorded once each in different patients who continued treatment. No symptomatic cardiac dysfunction was observed. Ab responses against dHER2, ICD and ECD were elicited. After four vaccinations, two of 12 patients in the 20 μg and nine of 14 in the 100 μg cohort responded to the ECD. Results in the 500 μg cohort are pending. The dHER2 vaccine appears to be safe in the small number of patients tested. Ab to the ECD and the ICD are induced in a dose-dependent manner, suggesting that the higher-dose vaccine may be required for future phase II and III studies.

Altogether, these results suggest that in order to have a maximal induction of the immune response against HER2 in terms of antibody and T cell responses, both the extracellular and intracellular domains of the protein should be included in the vaccine formulation. This should also guarantee the induction of a long-lasting immunological memory.

**MUC-1**

MUC-1 is a membrane-associated glycoprotein expressed by many types of ductal epithelia, including pancreas, breast, lung and gastrointestinal tract. It is overexpressed and aberrantly glycosilated by malignant cells. It is a multifunctional protein involved in the protection of mucous membranes, signal transduction, and modulation of immune system. More than 70% of cancers overexpress MUC1, making this antigen a potential target for immunotherapy [21, 22]. MUC1 is sufficiently immunogenic to elicit strong antitumor immunity as a tumor antigen [7]. Preclinical studies, using tumor cells that expressed MUC-1 protein or peptide Ag, concluded that MUC-1 could induce humoral response without inducing cellular response [23–26].

In a first phase I clinical trial, a 105-amino acid synthetic MUC-1 peptide with five repeated immunodominant epitopes mixed with Bacillus Calmette-Guerin (BCG) was tested in 63 patients with adenocarcinomas (including nine breast cancer patients) three times at 3-week intervals. The vaccine was well tolerated. Only three patients showed a strong immune response detected by DTH. In addition, the examination of 55 biopsies showed intense T-cell infiltration in 37 patients and lesser infiltration in seven patients, but only seven of 22 patients had a two- to four-fold increase in mucin-specific CTL precursors after vaccination. None of the breast cancer patients showed a disease control [27].

An alternative approach was used in a following trial, where 16 patients with metastatic breast carcinoma were treated with a vaccine consisting of 5 μg of the 16-amino acid MUC-1 peptide (plus keyhole limpet hemocyanin and DETOX as adjuvants). All patients generated strong anti-keyhole limpet
vaccines (rVV) that contain in their genome the CEA gene, molecules (B7.1). CEA-vaccines viruses are recombinant virus response), especially if administered with co-stimulatory vaccines that have shown safety and efficacy (immune system commonly becomes tolerant to it. So the CEA cancer cells promotes their adhesion and the metastatic process. A member of adhesion molecules and its overexpression in expressed in certain tissues and most carcinomas of the colon, carcinoembryonic antigen-based vaccines reported.

of inducing this level of MUC-1 immunity have not been patients also had high anti-MUC-1 IgG titers. The clinical effects obtained in seven of the 11 tested patients. Five of these seven developed a weak anti-MUCIN IgG response. Evidence for class I-restricted killing of MUC1-expressing tumor cell lines was found. In the adjuvant setting nine patients after breast treatment with mannan-MUC1 in a phase I dose escalation study. High titer of high-affinity anti-MUC1 IgG Ab were produced in 13/25 patients, where the levels of Ab directly correlated with the amount of immunogen given [32]. The amounts of Ag seemed to produce higher antibodies levels with respect to other studies conducted in other neoplasms with MUC1 carbohydrate immunogens [33–36].

In another trial six patients were vaccinated four times with a 106-amino acid-long MUC1 peptide conjugated with KLH plus immune adjuvant QS-21. In this study the T-cell response to MUC-1 was minimal; no evidence of autoimmune reaction was found [37]. In the adjuvant setting nine patients after breast surgery were treated with MUC1-KLH conjugate plus QS-21. High IgM and IgG antibody titers against synthetic MUC1 were detected. From the preliminary results of efficacy MUC1, the vaccine has been shown to be able to elicit a humoral response but not a cell mediated response [38].

These studies suggest that it is possible to elicit a CTL response against MUC-1 antigen, but the antibody response is very variable. Moreover, it is important to emphasize that the MUC1-1 peptides used for vaccination do not resemble the form of antigen that is found on tumor cells. These cells in fact bear a molecule that most of the time is underglycosilated compared with its normal counterpart and could not be detected by antibodies elicited towards a non-glycosilated peptide.

carcinoembryonic antigen-based vaccines
Carcinoembryonic antigen (CEA) is a glycoprotein that is expressed in certain tissues and most carcinomas of the colon, rectum, breast, lung, pancreas and gastrointestinal tract. It is a member of adhesion molecules and its overexpression in cancer cells promotes their adhesion and the metastatic process. However, because CEA is normally expressed in the body, the immune system commonly becomes tolerant to it. So the CEA peptide-based vaccines must first break this tolerance. CEA-vaccine viruses and recombinant canarypox (avianpox) virus-CEA vaccines (ALVAC-CEA) are recombinant CEA-based vaccines that have shown safety and efficacy (immune response), especially if administered with co-stimulatory molecules (B7.1). CEA-vaccines viruses are recombinant virus vaccines (rVV) that contain in their genome the CEA gene, while the ALVAC-CEA is a non-replicating avianpox virus that contains the human gene [39]. The ‘prime and boost technique’ with prime inoculation of replicating virus and subsequent boost doses with the ALVAC, seems in vitro to be more effective with respect to vaccination with single procedures [40].

Clinical trials have confirmed the more valid antitumor response with the combination of the two vaccines rather than the single one. In 18 patients with CEA-positive metastatic tumors (two with breast cancer), Marshall et al. [41], despite the absence of any objective responses, demonstrated that cell-mediated immune response is higher with the sequence VAAA (one inoculation with rVV followed by three with ALVAC) than AAAC (three doses of ALVAC followed by one with rVV). Indeed, the co-stimulation with B7.1 (but not with GM-CSF) was related with disease stabilization [41, 42].

A new development in targeting CEA approaches is the introduction of TRICOM (a triad of the costimulatory molecules B7.1, intercellular adhesion molecule 1 and lymphocyte function-associated antigen-3). This method has been developed with two different vaccines; a replicating rVV and a recombinant avipox vector (rF). Preclinical and clinical trials (in colorectal cancers) have shown an initially efficacy of this strategy [43]. Similar studies in breast cancer are ongoing: a phase II study (rVV given before chemotherapy and a rVF given after induction and high-dose chemotherapy) in patients with untreated metastatic breast cancer; a phase II randomized pilot study of sequential rV-CEA-TRICOM vaccine, rF-CEA-TRICOM vaccine, and GM-CSF with standard adjuvant chemotherapy for women with high-risk stage II–III breast cancer. The results from these trials are still pending [44]. In a phase I study conducted in 58 patients with advanced CEA-expressing carcinoma (three with breast cancers), subjects were treated with eight dose levels of fowlpox-CEA-TRICOM alone or in combination with vaccinia-CEA-TRICOM and G-CSF. The toxicity was light or moderate and consisted of grade 1 local skin reactions, regional adenopathy, fatigue and mild flu-like syndrome. After four vaccinations, six out 32 patients had only a modest increase of IgG levels, while in the cohorts receiving the sequential strategies only two of 14 patients were IgG ELISA positive. Regarding the clinical outcome, one patient (lung cancer) obtained a complete response and 23 (40%) patients had stable disease, with 14 of these having prolonged stable disease (>6 months) [45].

hTERT
As a potential molecular therapeutic target for cancer, the telomerase reverse transcriptase (hTERT) has been intensively scrutinized because of its near universal expression in human cancer cells, and its critical functional role in tumor growth and development. One proposed clinical strategy is hTERT-directed immunotherapy, supported by the identification of immunogenic hTERT epitopes that trigger tumor-lytic T cells in preclinical in vitro human studies. Telomerase maintains chromosomal integrity by protecting telomeric DNA that would otherwise be lost during successive rounds of cell division in rapidly dividing cells such as tumor cells. hTERT is a common and immunogen tumor antigen and is expressed in about 85% of all human cancers.
Clinical trials have shown that hTERT peptides or DCs-based vaccines elicit an immune response in various tumors [46, 47]. A phase I clinical trial was performed to evaluate the clinical and immunological impact of vaccinating advanced cancer patients with the HLA-A2-restricted hTERT I540 peptide presented with keyhole limpet hemocyanin by ex vivo generated autologous dendritic cells. As measured by peptide/MHC tetramer, enzyme-linked immunospot, and cytotoxicity assays, hTERT-specific T lymphocytes were induced in four of seven patients with advanced breast or prostate carcinoma after vaccination with dendritic cells pulsed with hTERT peptide. Tetramer-guided high-speed sorting and polyclonal expansion achieved highly enriched populations of hTERT-specific cells that killed tumor cells in an MHC-restricted fashion. Despite concerns of telomerase activity in rare normal cells, no significant toxicity was observed. Partial tumor regression in one patient was associated with the induction of CD8+ tumor infiltrating lymphocytes. These results demonstrate the immunological feasibility of vaccinating patients against telomerase and provide rationale for targeting self-antigens with critical roles in oncogenesis [48].

**sialyl-Tn**
The Tn, TF and sialyl-Tn antigen represent the immature glycosylation products of serine and threonine of the protein core and are naturally masked by the complete glycosylate chain. All three epitopes are strongly expressed on cancer cells and may be associated with disease progression and metastasis. Sialyl-Tn is a core-region carbohydrate antigen that is formed by the premature 2–6 sialylation of N-acetylgalactosamine, the expression of which has been associated with some human malignancies. All vaccines procedures have been developed with the pretreatment with intravenous or low dose orally cyclophosphamide. Chemotherapy can induce lymphocytopenia with a potential benefit from a reduction in T regulators.

In a randomized phase II trial 23 patients with metastatic breast cancer were randomized to receive 100 μg STn linked to KLH with DETOX-B adjuvant with or without low-dose cyclophosphamide (intravenously or orally). All patients developed IgG and IgM responses to sialyl-Tn. Two patients reported minor response, while five patients experienced stable disease. Patients pretreated with cyclophosphamide had a higher antibody titer and a longer survival (P = 0.0176 with respect to historical control) [49, 50]. Out of a total of 40 patients, 33 with high risk or metastatic breast cancer received theratope STn-KLH vaccination following by high-dose chemotherapy and stem cell rescue. Out of 26 evaluable patients the authors described a positive ELIspot for IFN-γ in 11 patients. The vaccine was well tolerated. Patients with greatest specific lytic activity of peripheral blood lymphocytes had a longer remission compared with patients who displayed less specific immune activity [51, 52].

In a large prospective phase III randomized trial, 1028 patients with metastatic breast cancer and no evidence or progressive disease after first-line chemotherapy, were randomized to teratope or control (administration of the KLH only) during concomitant endocrine therapy. The patients with evidence of immuno response had a benefit on survival; patients included in the teratope arm had an improvement on the progression-free survival (PFS) (PFS being 8.3 versus 5.8 months, respectively [53–55]). A randomized, double blind, phase III trial on 1030 women with MBC failed to demonstrate a significant improvement in TTP or OS. Analysis of a pre-stratified subset of patients receiving hormonotherapy showed a difference in OS (36.5 months for the teratope arm versus 30.7 months for the control arm; P = 0.39).

**p53**
p53 is the most commonly mutated gene in human cancers and is mutated in 20% of breast cancers [56]. The mutated p53 product gene is associated with tumor progression. Anti-p53 antibodies have been found in sera of patients with several types of cancers including breast cancer. A study describes a T-cell immune response in three patients with breast tumors who had mutated p53 gene and accumulated p53 protein. All showed a humoral response to p53 protein and the T cells of these patients recognized the wild-type p53 protein and proliferated in response to it. The data reported are relevant to the immune processes leading to autoimmunity and have a bearing on anti-p53 vaccine development in tumor immunology [57].

Two phase I dose escalation studies have been conducted mainly in colorectal cancers with a minimal toxicity and an induction of immune response [58, 59]. A recent study explored vaccination with p53 pulsed dendritic cells [60]. In this phase I pilot study, the toxicity and efficacy of autologus dendritic cells (DCs) loaded with a cocktail of three wild-type and three modified p53 peptides have been analysed in six HLA-A2+ patients with progressive advanced breast cancer. Vaccinations were well tolerated and no toxicity was observed. Disease stabilisation was seen in two of six patients, one patient had a transient regression of a single lymph node and one had a mixed response. ELIspot analyses showed that the p53-peptide-loaded DCs were able to induce specific T-cell responses against modified and unmodified p53 peptides in three patients, including two of the patients with a possible clinical benefit from the treatment. The strategy for p53-DC vaccination seems safe and without toxicity. Furthermore, indications of both immunologic and clinical effect were found in heavily pretreated patients with advanced breast cancer. An independent clinical effect of repeated administration of DCs and IL-2 cannot, of course, be excluded; further studies are necessary to answer these questions.

Table 3 summarizes all peptide-based vaccine approaches.

**breast cancer vaccines based on whole tumor cells**
Clinical studies on tumor cell-based vaccines are based on the concept that autologous or allogeneic tumor cells express many tumor-associated antigens, and that their inoculation with strong adjuvant or cytokine could activate the immune response. Earlier studies of human cancer vaccination were based on the hypothesis that because autologous or allogeneic tumor cells express many TAA, presentation of those TAA in the presence of a strong adjuvant (e.g. BCG, influenza virus, avian influenza virus or NDV) or cytokines known to promote...
T- and B-cell proliferation will activate innate immunity that will be sufficient to maintain an immune response against tumor, and that that immune response will lead to a clinical response. Potential concerns arose from the unknown amounts of tumor Ag that were administered, the unknown immunodominance of the tumor Ag, and the safety of using tumor DNA.

Freedman et al. [61, 62] used viral oncolysates to treat advanced ovarian cancer and high-risk untreated squamous cell carcinoma of the uterine cervix. These oncolysates were prepared from lysates of cultured ovarian and cervical lines infected with the influenza A/PR8/34 virus. The encouraging results (namely, the disappearance of ascites in several patients, the disappearance of pleural effusions in one patient, and the shrinkage of tumor masses in two patients without ascites) provided strong support for the hypothesis that active immunotherapy with cancer cells given in combination with a viral helper or immunomodulator could induce clinical responses. Ahlert et al. conducted a trial on 27 patients with previously treated metastatic breast cancer plus 31 patients with ovarian cancer. These patients were treated with autologous cells infected with Newcastle disease virus. The major responses were noted in the inoculation of the vaccine, which had a minimal dead cell contamination and maximal viability [63].

A different approach consisted in autologous fibroblasts/tumor cells-gene modifiers, by cytokine-gene transfection as adjuvant stimulation of immune response [64]. Allogeneic vaccines (human breast cancer lines) were experimented in two separate trials in metastatic breast cancer patients, but the clinical outcome is still pending. A mixture of autologous/allogeneic vaccine was tried on 42 patients with early breast cancer, in whom two had a disease improvement. It was observed that the vaccine induced in vitro an immune response to autologous vaccine but not to allogeneic tumor [65, 66]. In another study published by Dols et al. [67], 30 HLA-A2 women with metastatic breast cancer received up to 14 vaccinations with MDA-MB-231-CD80, an HLA-A2 allogeneic breast cancer cell line, which had been lipofected with cDNA for the CD80 costimulatory molecule. Tumor cells were administered with BCG or GM-CSF as an adjuvant. Sera obtained before and after vaccination were analyzed for antibodies to tumor cell lysate, MUC1, HER2/neu and p53. Since the cell line was grown in fetal bovine serum (FBS), sera were also analyzed for antibodies to FBS. Eighteen of 24 patients for whom sera were available exhibited anti-FBS activity at baseline. Eleven of these 18 patients and all six patients without baseline anti-FBS activity showed an increased titer after vaccination. A two-fold increase in the titer of IgG specific to tumor cell lysate was observed in six patients. Of 24 patients, eight made an antibody response to HER2/neu, four to MUC1 and one to p53. Although antibody production to a variety of tumor cell-associated antigens was detected, the results of this study suggest that a whole cell vaccine comprising a CD80-transfected allogeneic breast cancer cell line with adjuvant BCG or GM-CSF was not a reliable method to induce significant antibody responses in women with advanced breast cancer [67].

Wiseman [68] reported a 10-year follow-up analysis of 13 patients with inflammatory breast carcinoma treated with surgery, chemotherapy and allogeneic tumor cell/BCG immunotherapy. In that study, patients had been given chemotherapy followed by surgery and continued chemotherapy for 24 months, after which radiation was delivered to the chest wall and regional nodal basins. All patients were given 10^7 irradiated viable tumor cells pooled

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Table 3. Clinical trials with tumor associated antigens-based vaccines

<table>
<thead>
<tr>
<th>Reference</th>
<th>Phase</th>
<th>No. and type patients (no. with breast)</th>
<th>Vaccine</th>
<th>Grade 3/4 toxicity rate</th>
<th>No. patients with ELIspot or ELISA positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reddish, 1998</td>
<td>I</td>
<td>16 advanced breast cancer (8)</td>
<td>MUC1 peptide plus KLH and DETOX Mannan-MUC1</td>
<td>No</td>
<td>3/11 weak</td>
</tr>
<tr>
<td>Karanikas, 1997</td>
<td>I</td>
<td>25 advanced epithelial cancer (9)</td>
<td>Five MUC1 epitopes plus BCG</td>
<td>No</td>
<td>3/63 DTH strong + 37/37 skin biopsy +</td>
</tr>
<tr>
<td>Goydos, 1996</td>
<td>I</td>
<td>63 advanced epithelial cancers (9)</td>
<td>MUC1 long peptide plus HLK and QS21</td>
<td>NR</td>
<td>7/9 IgM ELISA + 9/9</td>
</tr>
<tr>
<td>Musselli, 2002</td>
<td>I</td>
<td>6 breast cancer</td>
<td>MUC1 plus KLH QS21</td>
<td>1 G3 vomiting</td>
<td></td>
</tr>
<tr>
<td>Gilewski, 2000</td>
<td>I</td>
<td>9 breast cancer</td>
<td>VAAA versus AAAV</td>
<td>No</td>
<td>VAAA &gt; response</td>
</tr>
<tr>
<td>Marshall, 2004</td>
<td>I</td>
<td>18 advanced CEA-expressing carcinomas</td>
<td>rF-CEA-Tricom +/- rV-CEA-Tricom</td>
<td>No</td>
<td>10/13</td>
</tr>
<tr>
<td>Miles, 1996 - MacLean, 1996</td>
<td>II</td>
<td>18 advanced breast cancer</td>
<td>Theratope with KLH and DETOX-B: CTX versus no-CTX</td>
<td>No</td>
<td>All</td>
</tr>
<tr>
<td>Sandmaier, 1999 - Holmberg, 2000</td>
<td>II</td>
<td>33 high risk or advanced breast cancers</td>
<td>Theratope following HDCHT</td>
<td>No</td>
<td>11/26</td>
</tr>
<tr>
<td>Mayordom, 2004</td>
<td>III</td>
<td>1028 advanced breast cancers</td>
<td>Theratope vs KHL with concomitant HT following first-line therapy</td>
<td>NR</td>
<td>NR</td>
</tr>
<tr>
<td>Gilewski, 2001</td>
<td>I</td>
<td>27 metastatic breast cancers</td>
<td>Globo-H conjugate</td>
<td>Two G3</td>
<td>16/27</td>
</tr>
</tbody>
</table>

VAAA, sequential ‘prime and boost’ vaccination with rV-CEA followed by three avipox-CEA; ELIspot, enzyme-linked immunospot assay; ELISA, enzyme-linked immunosorbent assay; KLH, keyhole limpet haemocyanin; HDCHT, high-dose chemotherapy; HT, hormone therapy.
from three cultured cell lines admixed with $10^7$ Connaught BCG organisms. At 10-years follow-up, four of the 13 patients (31%) were alive and free of disease. The results also indicated an apparent plateau in the survival curve at about 5 years. The authors suggested that a multimodality approach is not only feasible in such high-risk populations but may also lead to long-term survival.

Adjuvant autologous tumor cells vaccines have been demonstrated to be effective in the treatment of other cancers such as renal cell cancer. An important study has been completed on 558 patients randomized after radical nephrectomy to autologous renal tumor cell vaccine (six intradermal applications at 4-week intervals postoperatively; vaccine group) or no adjuvant treatment (control group). At 5-years and 70-months follow-up, the hazard ratios for tumor progression were 1.58 (95% CI 1.05–2.37) and 1.59 (1.07–2.36), respectively, in favour of the vaccine group ($P = 0.0204$, log-rank test). Five years and 70 months progression-free survival rates were 77.4% and 72%, respectively, in the vaccine group and 67.8% and 59.3%, respectively, in the control group. This study demonstrated that adjuvant treatment with autologous renal tumor cell vaccine in patients with renal-cell carcinoma after radical nephrectomy seems to be beneficial and can be considered in patients undergoing radical nephrectomy due to organ-confined renal-cell carcinoma of more than 2.5 cm in diameter [69].

The potential advantage of using tumor cell-based vaccines in breast cancer is that these comprise the complete antigen pool of an individual tumor for activating polyclonal immune responses. However, the induction of antigen-specific immune responses is impaired by the fact that T-cell activation is dependent on additional non-specific costimulatory signals provided by the antigen-presenting cell. The majority of solid human tumors does not express costimulatory molecules and is unable to deliver all signals required for T-cell activation. In contrast, tumors often induce immunologic tolerance. Therefore, the introduction of genes encoding costimulatory molecules, such as CD80 or cytokines in the studies in breast cancer is that these comprise the complete antigen response to antigen stimulation. Dendritic cells (DC) express high levels of HLA complexes and of costimulatory proteins (B7.1 also defined as CD80, B7.2 'CD86', CD 40, ICAM 1, and LFA-3) and produce some cytokines (IL-12) that are necessary for the activation of T cells. DC represent the most potent antigen-presenting cells. They are capable of stimulating both naive and memory T cells. The extraction procedure is facilitated by mobilization of bone-marrow precursors with growth factors (GM-CSF) or flt-3L, since the DC peripheral blood concentration is very low (0.5%). Furthermore, dendritic cells can be generated by peripheral blood monocytes.

Animal models have demonstrated that tumor antigen-loaded DC could activate specific CTL and even regression of established tumors in cancer-bearing hosts. Clinical trials have experimented DC-based vaccines in immunogeneic tumors (such as non-Hodgkin lymphoma, myeloma, melanoma) and non-immunogeneic tumors (non-small-cell lung carcinoma, prostatic cancer, colorectal cancer) [70, 71]. These early promising data have led to multiple research endeavors to also employ DC immunotherapy for poorly immunogeneic breast cancer. The vaccination with HER2/neu-adenoviruses transfected DCs in BALB-neuT mice prevented or delayed the onset of mammary carcinomas, with respect to the inoculation of immodified DCs or adenovirus transfected DCs [67]. Chen and Chen had already demonstrated that vaccination with DCs modified with a recombinant adenovirus (rAd) to express HER2/neu and IL-12 offered partial protection in a transplantable HER2/neu-expressing tumor model [72, 73].

Preclinical and phase I clinical trial have already been conducted with MUC-1 pulsed DCs vaccines with an induction of a immune response and a minimal toxicity [74, 75]. Since CEA peptide-based vaccines seem to be weak immunogens, a novel dendritic cell-CEA peptide vaccine has just been developed and tested in a phase I trial for lung or colorectal cancer patients with a valid immune response detected by tetramer stainings and LAP [76]. In another study Pecher et al. [77] performed a phase I/I clinical trial using human autologous DC transfected with CDNA of the human tumor antigen mucin (MUC1) as a vaccine in 10 patients with advanced breast, pancreatic or papillary cancer. After liposomal transfection, flow cytometry testing showed that 2%–53% of the DC expressed mucin epitopes. Patients were immunized two or three times with 1 million transfected DC injected subcutaneously (s.c.). A vaccine-specific delayed-type hypersensitivity (DTH) reaction was observed in three of 10 patients. After vaccination, four patients showed a two- to 10-fold increase in the frequency of mucin-specific interferon-γ (IFN-γ)-secreting CD8+ T cells. The authors demonstrated the feasibility and safety of a vaccine consisting of autologous gene-transfected DC, and that immunologic responses could be induced even in patients with pretreated and advanced disease [77].

Various approaches of different modified DC-based vaccines have been tested in breast cancer patients and have been demonstrated to be safe, even if their efficacy needs to be proven in long-term follow-up large controlled trials. In another report, Svane et al. [78] conducted a phase I pilot study with the aim of evaluating the toxicity and efficacy of autologous DCs loaded with a cocktail of three wild-type and three modified p53 peptides in six HLA-A2+ patients with progressive advanced breast cancer. Vaccinations were well tolerated and no toxicity was observed. Disease stabilization was seen in two of six patients, one patient had a transient regression of a single lymph node and one had a mixed response. ELISPOT analyses showed that the p53-peptide-loaded DCs were able to induce specific T-cell responses against modified and unmodified p53 peptides in three patients, including two of the patients with a possible clinical benefit from the treatment. The strategy for p53-DC vaccination seems safe and without toxicity. Furthermore, indications of both immunologic and clinical effect were found in heavily pretreated patients with advanced breast cancer. An
independent clinical effect of repeated administration of DCs and IL-2 cannot, of course, be excluded.

Another study by Kontany et al. [79] evaluated the ability of DCs to elicit tumor-specific immunity and clinical effects of DC vaccine immunotherapy targeting MUC1 tumor antigens. DCs from 14 patients with advanced or metastatic breast or lung cancer (nine positive for MUC1 and five negative for MUC1) were loaded with MUC1 antigens or tumor lysate and used for therapeutic vaccination. After vaccination, all the MUC1-positive patients acquired antigen-specific immunity whereas only one case with MUC1-negative cancer showed the specific immunity. Clinically, marked effects such as reduction in tumor sizes or tumor marker levels or disappearance of malignant pleural effusion were observed in seven of the nine MUC1-positive cases. However, the MUC1-negative patients did not respond to DC vaccines. Survival of MUC1-positive patients was significantly prolonged in comparison with MUC1-negative patients (mean survival: 16.75 versus 3.80 months, \( P = 0.0101 \)).

These data suggest that MUC1 is sufficiently immunogenic to elicit strong anti-tumor immunity as a tumor antigen and that DC vaccines targeting MUC1 are useful for immunotherapy of cancer. DCs are an attractive target for the therapeutic manipulation of the immune system to increase otherwise insufficient immune responses to tumor antigens. However, the complexity of the DC system requires rational manipulation of DCs to achieve protective or therapeutic immunity. Further research is needed to analyse the immune responses induced in patients by distinct ex vivo-generated DC subsets that are activated through different pathways. The ultimate ex vivo-generated DC vaccine will be heterogeneous and composed of several subsets, each of which will target a specific immune effector. These ex vivo strategies should help to identify the parameters for in vivo targeting of DCs, which is the next step in the development of DC-based vaccination. Table 4 summarizes the principal results from DC-based vaccines in breast cancer patients [80–88].

**Table 4.** Phase I/II trials of modified DCs vaccines for metastatic breast cancer patients

<table>
<thead>
<tr>
<th>Reference</th>
<th>Phase</th>
<th>No. and type patients</th>
<th>Vaccine</th>
<th>Grade 3/4 toxicity rate</th>
<th>No. patients with ELIspot positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brossart, 2000</td>
<td>I</td>
<td>10 advanced breast and ovarian cancer treated with HDC/ASCT</td>
<td>HER2E75 and MUC1</td>
<td>NR</td>
<td>5/10</td>
</tr>
<tr>
<td>Chui, 2003</td>
<td>I</td>
<td>9 high risk or advanced breast cancer (12 evaluable)</td>
<td>Her2E75 (4); CEAmRNA (3); HER2ICD (1); CEAmRNA-HER2ICD (1)</td>
<td>No</td>
<td>NR</td>
</tr>
<tr>
<td>Melisko, 2003</td>
<td>II</td>
<td>17 advanced HER2 positive breast cancer (12 evaluable) treated with HDC/ASCT</td>
<td>APC8024 (HER2-GMCSF)</td>
<td>No</td>
<td>7/12</td>
</tr>
<tr>
<td>Kyistra, 2003</td>
<td>I/II</td>
<td>16 advanced HER2 positive breast cancer (3 cohorts)</td>
<td>3 level doses APC8024 (HER2-GMCSF)</td>
<td>No</td>
<td>All</td>
</tr>
<tr>
<td>Peethambaram, 2004</td>
<td>I</td>
<td>10 advanced solid tumors (3 breast)</td>
<td>APC8024 (HER2-GMCSF)</td>
<td>No</td>
<td>All</td>
</tr>
<tr>
<td>Svane, 2003</td>
<td>I</td>
<td>6 advanced breast cancer</td>
<td>wt-p53 + IL2</td>
<td>No</td>
<td>All</td>
</tr>
<tr>
<td>Pecher, 2002</td>
<td>I/II</td>
<td>10 advanced solid tumors</td>
<td>MUC1 transfected DC</td>
<td>No</td>
<td>All</td>
</tr>
</tbody>
</table>

HDC/ASCT, high dose chemotherapy/autologous stem cell cell transplant; pos or +, positive; Ab, antibody response; LPA, lymphoproliferation assay; ELIspot, enzyme-linked immunospot assay; wt-p53, wild type p53.

**other approaches of immunization**

**polyvalent vaccines**

To increase the efficacy in immunological response, a polyvalent antigens-based vaccine has been developed. Gilewski et al. [91] determined the safety and immunogenicity of a vaccine containing three synthetic Globo H (carbohydrate antigen common on carcinomas)-KLH conjugates combined with the adjuvant QS-21 in 27 patients with metastatic breast cancer (15 without evidence of disease). The vaccine was well tolerated. An induction of immune response was detected by IgM ELISA in 16 of 27 patients, while only three patients demonstrated a significantly IgG reactivity. Nine of 27 patients reported an increase of complement-dependent cytotoxicity (CDC) [89]. In a following trial, the same group treated 10 patients with high risk or advanced breast cancer with a heptavalent glycoprotein-based antigen (GM-2, GloboH, Lewis-\( \gamma \), TF, sTn clustered and MUC-1) with evidence of low/moderate toxicity and a good immune activation detected by both Ig-ELISA and flow cytometric analysis [90]. The whole cell preparations of MCF-7 have shown more immunologic activity than lysed cell preparations in 6 of 9 patients with breast cancer. Further investigations are needed to test if the addition of lysed cells to the vaccine could significantly increase the immunological response [91–93].

**anti-idiotypic vaccines**

The potential of immunological therapies with antibodies have been illustrated by experience with anti-HER2 monoclonal antibody trastuzumab. Traditionally, antitumor antibodies were expected to mediate their effects by antibody- or complement-dependent cytotoxicity, by receptor internalization or by interference with receptor re-expression. Thereby anti-idiotypic antibodies hold high promise, thanks also to their high specificity, flexibility in design and potential amplification by Ab–Ab complexes. In 15 patients with high-risk breast cancer...
(with three or more lymph nodes involved) an anti-idiotype monoclonal antibody vaccine (1E10) has been tested, which mimics the neu-glicolyl-GM3 antigen. This study demonstrated that this strategy was feasible and active in immune response induction [94].

Prime–boost approaches

The optimization of vaccine components for selective activation of effector and memory responses has been developed. The ‘prime and boost technique’ is an example of activation with a second and different vector-infected cells vaccine. Similarly, distinct classes of oligodeoxyribonucleotides containing CpG motifs can be combined with vaccines to stimulate a specific effector of immune system [95].

Finally, two different tailored approaches could be tested for amplifying the CTL effector status. An approach is to use modified antigens that forms low-affinity T cell receptor (TCR)/foreign-peptide MHC I complexes to select high affinity memory CTL effectors. A limitation of this approach could be small cell division (similar to homeostatic proliferation) and thus the clonal bursts could be small. This approach could be useful in the adjuvant setting. In patients with advanced disease superagonist modified antigens could be used to elicit a large burden of CTL effectors, in order to overactivate certain response pathways but not others (e.g. IFN secretion) [96, 97].

discussion

Active immunotherapy is an investigational approach in breast cancer treatment. Cancer vaccines are designed to be specific in order to target only cancer cells preserving normal tissues. Data available on clinical trials demonstrated that they are safe and with low toxicity, that is a major advantage over conventional treatments. In particular, the postulated risk of autoimmune diseases induced by cancer vaccines is reported to be almost absent in the clinical setting [98]. Vaccination strategies are still on early testing phase. Several issues should be discussed in this section.

First, the area of immunomonitoring generates several questions linked to the use of non-defined antigen sources (tumor cells, apoptotic bodies, heat shock proteins, tumor lysates). Immunomonitoring is an essential step in the development of evidence-based immunotherapy. Prediction of clinical efficacy based on immunologic monitoring is crucial for the rational design of cancer vaccination studies as well as for defining the correlates of protection. Several vaccination studies in cancer patients have reported T-cell responses in the peripheral blood but usually in a minority of patients or only after prolonged re-stimulation with antigens in culture. One of the arguable values of vaccine therapy is the ability to enrich or to deplete the cellular reagents and to define the specificity of the T cells used for therapy. With vaccines it is not possible to select particular lymphocyte populations from the patient directly. However, the compartments of the immune system are natural environments for optimal expansion of T cells and for the complex interplay among innate and adaptive immune mechanisms. It is presumptuous to believe that our understanding of this complexity and our technologies are adequate to allow us to recreate optimal immune effectors in vitro and to expect them to perform as we desire upon re-infusion. However, it is possible in patients on clinical trials to enrich for specific effectors by vaccination with defined antigens, and to measure their responses to each antigen simultaneously, in various compartments (e.g. lymph node, blood and tumor). We would like to point out that surrogate end points for vaccine efficacy should be re-emphasized, despite some current sentiment to the contrary. For the development of new generation vaccines, we must rely on knowledge derived from basic research. In infectious diseases, it is well established that antigen specific lymphocytes must be activated substantially for successful (i.e. protective) vaccination. Consequently, assessing responses of antigen specific lymphocytes is an important step in the evaluation of novel vaccines. There are a number of new techniques permitting investigators to dissect T-cell responses in vivo. It is now possible to determine molecular features of human T-cell responses in great detail, going much beyond what is usually done to assess T cells in animal models.

Secondly, in the phase I/II trials no current cancer vaccine used alone has induced significant objective responses in patients with breast cancer. We have to consider that the standard approach for assessment of clinical response cannot be applied to vaccine therapy. Using conventional oncologic criteria for clinical tumor response, objective response rate in cancer vaccines trials was only 2.6%, which is similar to the overall response rate we determined in a detailed analysis of cancer vaccine trials performed by others. This low clinical effectiveness raises important questions about the appropriate directions for future clinical immunotherapy efforts, especially at a time when alternate approaches such as cell transfer studies confirm the powerful potential of immunotherapy to mediate the regression of large volumes of metastatic disease in experimental models and in humans [99]. Hundreds of vaccine clinical trials in patients with metastatic cancer have been published. Some trials do not specify the exact criteria used to determine clinical response; some trials use very ‘soft’ criteria that make the incidence of cancer regression difficult to evaluate. Examples include ‘temporary growth cessation in some individual metastases or symptoms disappeared’ or ‘tumor necrosis or ‘stable disease’ or ‘unexpectedly long survival’. Another analysis included as a partial response ‘any measurable response in any lesion’. Soft criteria of this sort cause considerable confusion in the analysis of clinical trials because they can occur in the natural course of tumor growth. For future trials it should be essential to standardize methods of response evaluations in vaccination strategies.

Thirdly, the use of a few defined antigens, insufficient to determine an effective clinical response, is another important issue. Several experimental evidences showed that tumor metastases (in melanoma models) likely to respond to immunotherapy have a different genetic profile than those unlikely to respond to therapy. This genetic profile differs particularly in the expression of immunologically relevant genes, suggesting that melanoma metastases that respond to therapy are conditioned to respond even before therapy by an immunologically active environment. Several signatures were identified that were descriptive of immune or other biological functions that might be relevant to immune responsiveness...
No extensive data are available in breast cancer vaccines studies such as in melanoma or renal cell carcinoma trials.

Finally, recent research has emphasized the importance of active suppressor mechanisms arising both from the tumor and from the immune system itself that can inhibit antitumor immune reactions in vivo. Perhaps the most important of these regulatory effects are mediated by CD4\(^+\)CD25\(^+\) lymphocytes with the ability to suppress both the proliferation and effector functions of immune cells. A major advantage of cell transfer therapies is the ability to deplete host lymphocytes, including these regulatory cells, before cell transfer, and this preparation is critical to the success of many preclinical cell transfer immunotherapies. For cancer vaccines to be effective, it may require the elimination of these regulatory T cells, and although reagents to selectively eliminate these cells in vivo are being developed, their clinical efficacy has yet to be established. Chemotherapy- or radiation-induced lymphodepletion can eliminate regulatory cells but cannot be used in conjunction with cancer vaccines because the needed effector cells are also eliminated [101–103]. Even if breast cancer is not considered a high immunogenic property, preliminary immunological results are encouraging: the vaccines are able to break the immune tolerance versus the self-antigen expressed in tissues.

In order to validate a vaccination strategy it is important to define the best and most appropriate population in which to test the vaccine, taking into account current therapies in this population to combine with the vaccine approach. Greatest expectations in the area of cancer vaccines regard the use of such biotherapy in the adjuvant setting. In this group of patients, the immunosuppressive effect of bulky disease does not overwhelm the immune system and the effector–target ratio is then favorable. Only a limited number of trials are actually ongoing in the adjuvant setting. Limited data on the follow-up of these patients are available. We need to establish the best surrogate markers to optimize vaccine activity and to address questions about the best treatment schedules and the best antigen. It is also important to move on from laboratory surrogate markers to clinical end points, such as demonstration of increased survival rates, tumor eradication or tumor control. Future clinical trials should be designed specifically in patients with high-risk breast cancers. In this population we need to demonstrate that the level of immune response is a positive predictor of survival improvement outcomes. Vaccine schedules that produce high rates of immune response should be the ones that produce higher rates of survival. Other end points include doses, immunization schedules, methods of administration, timing of vaccinations and of following boosts to maintain a durable immune response. Integrating targeted vaccine therapy after inducing a major response with a monoclonal antibody (e.g. trastuzumab therapy) could be considered as another setting of therapeutic approach. Finally, to the future directions, a better understanding of the relation between innate and adaptive immune responses, and of the immune escape mechanisms employed by tumor cells, the discovery of mechanisms underlying immunological tolerance, and acknowledgment of the importance of both cell-mediated and humoral adaptive immunity for the control of tumor growth are necessary for leading to a more comprehensive immunotherapeutic approach.

**references**


