# Immune therapy for breast cancer in 2010—hype or hope?

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## ABSTRACT

The identification of numerous breast cancer antigens has generated increasing enthusiasm for the application of immune-based therapies in breast malignancies. Although the use of monoclonal antibodies has revolutionized the "targeted therapy" of breast cancer, and the immunomodulatory effects of bisphosphonates continue to be evaluated, few studies to date have demonstrated widespread utility for other forms of immunotherapy. The present review assesses modern research and explores whether the hopes for immunotherapy can overcome the hype.



## KEY WORDS

Breast cancer, immunotherapy, cancer vaccine, cytokine, monoclonal antibody


## 1. INTRODUCTION

Despite significant advances in the systemic management of breast cancer, metastatic disease (MBC) remains incurable, and in the adjuvant setting, the overall 5-year survival rate for women with node-positive breast cancer has reached a relative plateau. A possible explanation for this situation is that, by the time of breast cancer diagnosis, a substantial number of patients have already developed micrometastases that are never fully eradicated, even with "optimal" adjuvant treatment.

Despite arising from normal host cells, tumour cells can exhibit some degree of immunogenicity, and the immune system has a number of mechanisms by which it acts against them. A possible mechanism for targeting micrometastatic foci or limited disease is to enhance the immune response of the patient to the tumour cells. By enhancing immunogenicity, a robust and lasting immune response might be attained. Renal cell carcinoma and melanoma are the most eloquent examples of the efficacy of immunologic manipulation in eradicating a disseminated cancer [1].

In breast cancer, components of the immune system are thought to play important roles in several parts of the metastatic cascade [2]. Various immune strategies have been tested as therapy for breast cancer: vaccine therapy, administration of exogenous cytokines, monoclonal antibodies, and gene therapy. As a guide to the practicing clinician, the present article reviews current progress and future research into breast cancer immunotherapy.

## 2. METHODS

In April 2010, we searched MEDLINE (1950 to April 2010) for English-language studies on immunotherapy in breast cancer. The American Society of Clinical Oncology (ASCO) database of abstracts was similarly searched for the years 2005–2010. Subject headings used included "immunotherapy," "immunology," "cancer vaccines," "cytokines," and "antibodies, monoclonal," in combination with "breast neoplasm" and "therapeutics." We refined the search by identifying specific categories of immunotherapies: cancer vaccines, cytokine-based therapy, therapeutic monoclonal antibodies, CpG (cytosine and guanine separated by a phosphate) deoxynucleotides, and bisphosphonates.

This search strategy identified 2984 articles, which were screened by title for relevance. In addition, reference lists of other review articles on the topic were screened, and any relevant articles were included.

## 3. RESULTS

### 3.1 Cancer Vaccines

Malignant cells can generate a tumour-specific immune response. This response occurs when an antigen specific to the tumour cells is recognized by the immune system. The antigen is then cleared, neutralized, or destroyed. This innate process may be re-created by the administration of a vaccine containing tumour-specific antigens.

Vaccination strategies are defined as "active specific immunotherapy" because the host immune system is activated *de novo* or is re-stimulated to mount an effective tumour-specific reaction against malignant cells. Theoretically, cancer vaccines have the potential to induce a more robust immune response with minimal toxicity. Multiple antigens have been studied for their ability to stimulate an effective immune response. The ones with the most promise in breast cancer are mucin 1 (MUC1), carcinoembryonic antigen (CEA), the human epidermal growth factor receptor 2 (HER2/*neu*), and alpha-lactalbumin. Table I includes a summary of stage of development and proven utility for various cancer vaccines.

### *3.1.1 MUC1*

The membrane-associated glycoprotein MUC1 is a found on the apical surface of many epithelial cells. It is overexpressed in more than 70% of cancers, making it an attractive antigen for research. Preclinical studies demonstrated that MUC1 could induce a humoral immune response, but not a cellular response [3–6]. Overall, clinical trials using MUC1 have been variable and inconclusive in stimulating an effective immune response. Initial phase I data showed a weak anti-MUC1 response, leading to development of several modifications and adjuvants aimed at enhancing immunogenicity [7–11]. Despite modifications, clinical outcome studies failed to show tumour regression or other important clinical endpoints. Phase II trials also failed to provide convincing evidence of a role for this vaccine in tumour regression [12,13].

One variation of the MUC1 vaccine that met some success in early trials is Theratope (Biomira, Edmonton, AB), designed by linking a synthetic sialyl-Tn (STn) that emulates a unique carbohydrate in human cancer with the protein carrier keyhole limpet hemocyanin (KLH). In a phase II trial in MBC, overall survival (OS) was significantly improved in women treated with Theratope ($n$ = 50; OS: 19.1 months) compared with historical controls ($n$ = 104; OS: 9.2 months) [14]. Following up on this positive trend, Biomira completed

TABLE I Developmental stage and utility of immunotherapies in breast cancer

| *Immunotherapy* | *References* | | | | *Useful?*[a] |
|---|---|---|---|---|---|
| | *Preclinical* | *Phase I* | *Phase II* | *Phase III* | |
| Cancer vaccines | | | | | |
| Mucin 1 | 3–6 | 7–11 | 12–14 | Theratope [15,b] | – |
| | | | | Stimuvax[c] | – |
| | | | | (NCT00925548[d]) | |
| Carcinoembryonic antigen | | 16 | 17 | NCT00217373[d] | – |
| | | | | NCT00003125[d] | * |
| | | | | NCT00085241[d] | * |
| HER2/*neu* | | 18–24 | 25–27 | NCT00854789[d] | + |
| Alpha-lactalbumin | 28 | Trials planned | | | * |
| Cytokine therapy | | | | | |
| Interferon α | 29 | 30 | NCT00227656[b] | | – |
| Tumour necrosis factor α | | 31 | | | – |
| Interferon γ | 32, 33 | | | | – |
| Interleukin 10 | 34–36 | | | | – |
| Interleukin 12 | 37–39 | 40 | | | * |
| Interleukin 2 | 41,42 | | 43 | | – |
| Granulocyte–macrophage colony-stimulating factor | 44–47 | | | | * |
| Flt-3 ligand | 48 | 49 | | | – |

[a] A minus sign (–) means that the therapy is not currently useful, based on available data; a plus sign (+) means that the therapy is useful, with potential to be incorporated into practice, based on available data; and an asterisk (*) means that the data are insufficient to comment on utility (trials still recruiting participants or trials not completed).
[b] Biomira, Edmonton, AB.
[c] Oncothyreon, Seattle, WA, U.S.A.
[d] For details, search using the trial number at www.clinicaltrials.gov/ct2/search.

a multicentre, double-blind phase III trial, randomizing 1038 MBC patients to receive either Theratope or KLH and the adjuvant detox-B stable emulsion [15]. The primary endpoints of that trial, time to disease progression and OS, were not different between the two groups. The only survival benefit was found in *post hoc* analyses for women on hormonal therapy who received Theratope and who developed high antibody titres against naturally clustered STn antigens (OS: 41 months vs. 25 months).

The most developed MUC1 vaccine is Stimuvax (Oncothyreon, Seattle, WA, U.S.A.), which was, until recently, recruiting patients for a phase III trial in hormone-sensitive advanced or metastatic breast cancer (NCT00925548). That trial was suspended in March 2010 when a patient in a phase II trial of Stimuvax acquired encephalitis.

Although MUC1 is an attractive tumour-specific antigen, the evidence available to date for MUC1-based vaccines has not shown altered clinical outcomes in breast cancer.

#### *3.1.2 CEA*

The glycoprotein CEA is expressed in carcinomas of breast, colon, lung, pancreas, and gastrointestinal tract. It has a role in cancer spread, promoting cancer cell adhesion.

As has been the case with MUC1 vaccines, development of an adequate CEA-based vaccine has involved various modifications. Priming with a recombinant CEA–*Vaccinia* virus and boosting with recombinant avian pox virus CEA was shown to be more effective at inducing CEA-specific immune responses [50]. In another strategy, tricom (triad of co-stimulatory molecules B7.1, intercellular adhesion molecule-1, and lymphocyte function–associated antigen-3) was added.

A clinical trial of CEA–tricom enrolled 58 cancer patients with CEA-overexpressing tumours who were treated with 8 dose levels of CEA–tricom vaccine. After a 4-vaccination schedule, 6 of 33 patients had moderate levels of immunoglobulin G, 1 lung cancer patient had a complete clinical response, and 40% of patients had stable disease [16].

A small phase II trial in 15 MBC patients looked at the benefit of Panvac (Therion Biologics, Cambridge, MA, U.S.A.), a recombinant pox virus vaccine targeting both MUC1 and CEA [17]. Panvac was administered with granulocyte–macrophage colony-stimulating factor (GM-CSF) in 11 patients and with GM-CSF and docetaxel in 3. In the non-chemotherapy arm, 3 of 11 patients had disease stabilization for at least 6 months, 2 of 11 had tumour size reduction not meeting partial response criteria, and 1 had a confirmed partial response. Of patients receiving vaccine and docetaxel, one had a 50% reduction in the size of a chest wall lesion, and another had improvement of disease on bone scan.

Evaluation of anti-CEA vaccines is ongoing in more advanced clinical trials. Scenarios include efficacy in combination with other anticancer therapies such as interferon alfa [IFNα (NCT00217373)], interleukin-2 [IL-2 (NCT00003125)], and radiation therapy (NCT00085241).

#### *3.1.3 HER2/*neu

The tyrosine receptor protein HER2/*neu* is a expressed in epithelial tumours and overexpressed in approximately 20% of primary breast cancers. Several phase I trials of vaccination against HER2/*neu* have demonstrated safety and immunogenicity, with only rare grade 3 toxicities [18,19]. Two small phase I studies have provided some promising data on a HER2/*neu* dendritic cell (DC) vaccine, lapuleucel-T [20,21].

Vaccine optimization remains an issue, with several studies demonstrating short-lived low-magnitude immune responses, especially in the absence of immune adjuvants [22–25]. Clinical outcome studies are more scarce, but promising. A recent study that demonstrated immunogenicity for a HER2/*neu* vaccine in combination with GM-CSF in the adjuvant setting was presented in abstract form at the ASCO annual meeting in 2008. Patients with advanced breast cancer who had been vaccinated with HER2/*neu* vaccines as part of previous phase I/II trials ($n$ = 75) were followed for a median of 2.7 years. In those with HER2/*neu*-specific T-cell immunity, a significant, dose-dependent increase in OS was seen [26].

Peoples *et al.* conducted a trial of HER2/*neu* vaccine in 53 node-positive breast cancer patients treated with standard therapies and free of active disease, but at high risk of recurrence [27]. Women positive for the human leukocyte antigen A2 [HLA-A2+ ($n$ = 24)] were vaccinated against HER2/*neu*; the patients negative for HLA-A2 constituted the control group. The treatment arms were not balanced: tumours in the HER2/*neu*-vaccination arm were larger, more poorly differentiated, and less hormone-sensitive. Despite those differences, outcomes favoured the treatment group: at 22 months' follow-up, disease-free survival in the treatment arm was 86% as compared with 60% in the control arm. Recurrence rates were 8% and 21% respectively; time to recurrence was 11 months and 8 months respectively. This trial has been expanded and is still aiming to enrol a total of 95 patients (NCT00854789).

In another trial, the same group of investigators enrolled 186 women in remission from a previously treated breast cancer. Of those patients, 101 (HLA-A2+ and -A3+) were given a HER2/*neu* vaccine and GM-CSF as an adjunct. The remaining 85 patients constituted a control group ($n$ = 85). Recurrence rates at a median follow-up of 20 months were 5.6% in the vaccinated group and 14.2% in the control group [51].

Based on these limited data, HER2/*neu* vaccines hold some promise for the future.

#### *3.1.4 Alpha-Lactalbumin*

Alpha-lactalbumin is a breast-specific differentiation protein expressed in high amounts in most human

breast carcinomas and in mammary epithelial cells only during lactation. Recent data show that immunoreactivity against alpha-lactalbumin provides substantial protection against growth of autochthonous tumours in transgenic mouse models of breast cancer [28]. It was concluded that alpha-lactalbumin vaccination has the potential to provide safe and effective primary protection against the development of breast cancer for women in their post-childbearing, premenopausal years, when lactation is avoidable and the risk for developing breast cancer is high. Clinical studies are currently being planned.

### 3.2 Cytokine Therapy

Cytokines are regulatory molecules secreted by lymphocytes and macrophages. They alter the function of target cells by binding to cell-surface receptors. Cytokines provide important intercellular signals in inflammation, immunity, and the biology of tumour and endothelial cells.

*In vivo* data have shown that, despite the presence of adequate infiltration by lymphocytes into breast carcinomas, the immune response appears to be downregulated—a circumstance thought to result from the release of "inhibitory" cytokines into the tumour microenvironment [2]. Furthermore, breast cancer is weakly immunogenic and poorly recognized by the immune system [52]. Some available data demonstrate that certain cytokines are able to promote the generation or activation of antitumour effector cells, including DCs or lymphokine-activated killer (LAK) cells. Once activated, these cells can result in inhibition and, in certain instances, regression of tumour growth [53].

The foregoing mechanisms of action have led to numerous hypotheses about clinical activity for cytokines in breast cancer when delivered alone or in combination with chemotherapy or other biologic agents. Those hypotheses have included a reduction in the immunosuppressive effects on the microenvironment, enhanced innate or adaptive immunity, and potentially direct cytotoxicity. In Table I, the stage of development is outlined for various cytokines; Table II lists the roles of cytokines that have been studied in breast cancer.

#### *3.2.1 IFNα*

Laboratory data show that IFNα has antiproliferative effects on the growth of MCF-7 cell lines. In addition,

TABLE II Roles of cytokines in breast cancer

| *Cytokine* | *Role* | *Reference* |
|---|---|---|
| Interferon α | Antiproliferative effects | Kamamura *et al.,* 1998 [33] |
| Tumour necrosis factor α | Induces apoptosis and necrosis | Purohit *et al.,* 2002 [53] |
| | Activates lymphokine activated killer (LAK) cells | Wang *et al.,* 2003 [54] |
| Interferon γ | Activates macrophages/monocytes | Pulaski and Ostrand–Rosenberg, 2002 [32] |
| | Differentiation of naïve T-helper cells | Schreiber and Schreiber, 2003 [55] |
| | Increases activity of LAK cells | |
| Interleukin 10 | Modulates monocyte/macrophage function | Kundu and Fulton, 1997 [36] |
| | | Venetsanakos *et al.,* 1997 [34] |
| | | Dorsey *et al.,* 2002 [35] |
| Interleukin 12 | Stimulates T cells, natural killer (NK) cells | Cavallo *et al.,* 1999 [37] |
| Interleukin 2 | Proliferation of LAK, NK, T cells | Rosenberg *et al.,* 1985 [56] |
| | | Mule *et al.,* 1987 [57] |
| | | Addison *et al.,* 1995 [41] |
| | | Stewart *et al.,* 1999 [42] |
| Granulocyte–macrophage colony-stimulating factor | Growth and differentiation of dendritic cells (DCs) | Caux *et al.,* 1992 [58] |
| | | Dranoff *et al.,* 1993 [46] |
| | | Disis *et al.,* 1996 [45] |
| Flt-3 ligand | Stimulates early progenitor DCs | Brasel *et al.,* 1996 [48] |
| | | Maraskovsky *et al.,* 1996 [59] |

induction of a number of IFN-inducible genes was shown to be a primary effect of IFNα [29]. A clinical study to determine the effectiveness of IFNα and IL-2 recruited women who had previously received 1–2 prior chemotherapy regimens for measurable inoperable, recurrent, or metastatic breast cancer. Of 40 patients accrued to the study, 32 were evaluable for response. Toxicities were frequent but manageable. The most common grades 3 and 4 toxicities were lymphopenia (17%) and malaise or fatigue (24%). No complete responses were observed, but 1 patient had a partial response (3%), and 6 patients had stable disease (19%) for an undetermined duration. It was therefore concluded that IFNα and IL-2 were ineffective [30]. A further clinical study exploring the combination of IFNα and capecitabine has been completed, but results are not yet available (NCT00227656).

#### *3.2.2 TNFα*

The multifunctional cytokine tumour necrosis factor α (TNFα) plays a key role in apoptosis, cell survival, and oncogenesis [60]. It is able to induce apoptosis and necrosis, activate cytolytic effector cells, and upregulate the expression of intercellular adhesion molecule 1 on tumour cells, an important event in the interaction with LAK cells [54]. The interaction of TNFα with the TNF receptors 1 and 2 activates several signal transduction pathways, leading to the diverse functions of TNFα. The TNF receptor 1 signalling molecules have been elucidated reasonably well, but regulation of their signalling remains unclear.

The anti-neoplastic effect of TNFα has been shown to be substantially augmented by co-administration of IL-12 [31]. In one study, investigators activated human peripheral blood DCs to produce TNFα, which had a direct antitumour effect against breast cancer cell lines *in vitro,* leading to growth inhibition and apoptosis [57]. Those observations led to the hypothesis that TNFα should be included as part of immunotherapeutic treatment regimens. However, caution should be used in adding TNFα, because potential side effects include the induction of an autoimmune response from generalized T-cell activation [55].

#### *3.2.3 Interferon γ*

Interferon γ (IFNγ) is a pro-inflammatory mediator that has an important function in the activation of the monocyte–macrophage lineage. It has been demonstrated that IFNγ is a critical component in regulating an innate phagocytic response against MBC [32]. In addition, IFNγ has been shown to increase the activity of LAK cells in patients with breast cancer [33]. The use of IFNγ is currently limited because of the need for systemic delivery, which is associated with significant side effects and toxicity, including fever, fatigue, nausea, vomiting, and neurotoxicity. For this agent to be feasible in breast cancer, an alternative delivery system such as intratumoral instillation or conjugation to a cancer-specific antibody needs to be developed, allowing for cytokine release to be more targeted. Development of the required drug delivery system is ongoing.

#### *3.2.4 IL-10*

The pleiotropic cytokine IL-10 can be immunosuppressive or immunostimulatory, depending on its relative concentration in the local microenvironment. Its principal action is to modulate monocyte and macrophage function. Laboratory studies using human breast cancer cells have shown that tumour tissue contains high levels of IL-10 messenger RNA. Interestingly, normal breast tissue showed little-to-no messenger RNA expression [34]. Conversely, however, animal studies showed that systemic administration of recombinant human IL-10 to animals bearing malignant mammary tumours led to significant inhibition of tumour growth and increased inflammatory cellular infiltration [35]. Kundu and Fulton [36] showed that IL-10 expression downregulates class I major histocompatibility complex expression in tumour cells, leading to enhanced natural killer (NK) cell–mediated tumour cell lysis. In the tumour microenvironment, IL-10 is therefore considered to have concentration-dependent activity, with low concentrations facilitating tumour growth, but higher concentrations having considerable antitumour effects. As in the case of IFNγ, a method of targeted delivery of these agents is needed before they can be further investigated in breast cancer.

#### *3.2.5 IL-12*

As a pivotal factor for the initiation of cellular immunity, IL-12 has multiple stimulatory effects on T cells and NK cells. In mice harbouring spontaneously metastasizing mammary adenocarcinomas, antitumour effects have been demonstrated for local and systemic recombinant murine IL-12 [37]. Recently, in a murine model of breast cancer, intratumoral administration of polylactic acid–encapsulated microspheres containing IL-12, TNFα, and GM-CSF, was shown to enhance T-cell infiltration, resulting in tumour regression [36]. Tumour-specific T cells in the lymph nodes and spleen were also activated by this process, allowing for an additional long-term immune response to be activated.

Studies combining IL-12, chemotherapy, and trastuzumab have also been undertaken. Sequential (but not concurrent) use of paclitaxel and IL-12 reduced tumour burden in mice [38,39]. A phase I study in 21 patients with metastatic HER2/*neu*-positive tumours administered paclitaxel and trastuzumab followed by IL-12 [40]. Clinical response was observed in 10 of 21 patients: 1 complete response, 4 partial responses, and 5 stable disease at 1 year of follow-up. These data are promising, but results from more advanced trials are needed.

#### *3.2.6 IL-2*

Because of its proliferative effect on LAK, NK, and T cells, IL-2 also plays an important role in immunomodulation.

When administered alone or in combination with LAK cells, IL-2 has led to regression of a variety of established tumours [34,56]. In a murine model, intratumoral injection of an adenovirus expressing the IL-2 gene resulted in regression of established murine breast cancer [41]. Studies exploring the efficacy of the antitumour effects of IL-2 have not produced similar results. Attempts use gene therapy to produce IL-2 in human breast tumours (by depositing an adenoviral vector encoding IL-2 into subcutaneous tumour) did not result in clinical responses despite measureable levels of IL-2 protein being detected in tumour biopsies [42]. A recent phase II study of trastuzumab with pulsed intermediate-dose IL-2 did not result in any objective responses [43].

#### *3.2.7 GM-CSF*

Granulocyte–macrophage colony-stimulating factor is a growth and differentiation factor for human DCs [58]. These specialized antigen-presenting cells are believed to be responsible for stimulating naïve T cells. In experimental models, DCs have also been shown to augment secondary immune responses better than other antigen-presenting cells do [44]. In rodent models, GM-CSF can, when mixed with a soluble antigen, be used as a vaccine adjuvant to induce both an antibody and a T-cell antigen-specific immune response [45]. Transfection of tumour cells with DNA encoding GM-CSF, or direct injection of GM-CSF into established tumours, both resulted in antitumour immune responses in several murine models [46,47]. Administration of GM-CSF as a vaccine adjuvant has also been shown to result in the mounting of an effective immune response, but the extent to which GM-CSF augmented the activity of the vaccine was unclear. Clinical data are pending.

#### *3.2.8 Flt-3 Ligand*

Flt-3 ligand is a potent stimulator of early progenitor DCs [59]. Animal studies have demonstrated that, when administered systemically to mice, Flt-3 ligand increased circulating progenitor DCs that retain an antigen-presenting function and the capacity to stimulate proliferation of antigen-specific T cells [48]. Flt-3 ligand is therefore currently under investigation as an anticancer agent and a possible vaccine adjuvant. In a phase I trial of 10 patients with HER2/*neu*-overexpressing cancer, participants received HER2/*neu* peptide–based vaccine that targeted the intracellular domain of the HER2/*neu* protein and Flt-3 ligand. In 5 patients, the HER2/*neu* peptide–based vaccine was given alone, and in 5 patients, the vaccine was admixed with GM-CSF on day 7 of the Flt-3 ligand cycle. Proliferative responses by T cells to the HER2/*neu* peptides and intracellular domain protein suggested that vaccine regimens including Flt-3 ligand as an adjuvant were not effective in eliciting a significant HER2/*neu* protein–specific proliferative response from the T-cells [49].

### 3.3 Trastuzumab

The HER2/*neu* proto-oncogene is a member of the human epidermal growth factor receptor family [61,62]. Its overexpression is associated with more aggressive disease and worse prognosis. Trastuzumab (Herceptin: Hoffman–La Roche, Mississauga, ON) is a monoclonal antibody targeting the HER2/*neu* extracellular receptor, thereby inhibiting growth signals mediated by that pathway.

After clinical trials showed improved disease-free survival with trastuzumab treatment, trastuzumab was approved by the U.S. Food and Drug Administration in 1998 for the treatment of HER2/*neu*-positive metastatic breast cancer. An overview of the trastuzumab clinical program is beyond the scope of this article, but a detailed review has been published by Shak [63].

#### *3.3.1 Single-Agent Trastuzumab Therapy*

In women with MBC who progress on standard chemotherapy, single-agent trastuzumab was shown to produce a 15% response rate, with a median duration of response of 9.1 months [64]. Other trials have also demonstrated improved efficacy with trastuzumab monotherapy. Vogel *et al.* randomized 114 women to two-weekly dose regimens of trastuzumab [65]. The overall objective response rate was 26%, ranging from 35% in tumours with heavy immunohistochemical staining for HER2/*neu* to no response in weakly-to-moderately staining tumours. Based on that study, trastuzumab demonstrated safety and efficacy as first-line monotherapy in MBC overexpressing the HER2/*neu* oncogene.

#### *3.3.2 Trastuzumab in Combination with Chemotherapy: the Metastatic Setting*

Preclinical data suggested a synergistic effect between trastuzumab and several chemotherapeutic agents, including platinum agents, taxanes, cyclophosphamide, and anthracyclines [66–70]. Positive phase II data have been complemented by strong phase III data showing that trastuzumab has a synergistic effect in combination with platinum-based chemotherapy [71].

#### *3.3.3 Trastuzumab in Combination with Chemotherapy in Early or Locally Advanced Breast Cancer*

Evidence for the use of trastuzumab in combination with chemotherapy in early-stage breast cancer comes from four large trials—HERA (the Herceptin Adjuvant trial) [72,73], the National Surgical Adjuvant Breast and Bowel Project B31 trial, the North Central Cancer Treatment Group N9831 trial, and the Breast Cancer International Research Group BCIRG006 study [74]—which all concluded that trastuzumab taken for 1 year after primary treatment reduces recurrence and improves survival in early-stage HER2/*neu*-overexpressing breast cancer.

#### *3.3.4 Trastuzumab and IL-12*

Preclinical studies have shown that NK cells secrete potent immunostimulatory cytokines in response to dual stimulation with trastuzumab-coated tumour cells and IL-12 [75]. Co-administration of these agents in mice resulted in enhanced NK cell activity. In a phase I trial of IL-12 and trastuzumab in 15 patients with HER2/*neu*-overexpressing malignancies, 1 complete response was reported in a woman with MBC, and 2 patients experienced stabilization of bone disease lasting 10 and 12 months respectively [76]. Antibody-dependent cellular cytotoxicity against tumour targets did not correlate with clinical response or IL-12 dose. Unfortunately, the addition of IL-12 did not enhance the activity of trastuzumab.

### 3.4 CpG Oligodeoxynucleotides

Unmethylated CpG oligodeoxynucleotides (CpG ODNS) are characteristic of bacterial DNA and have established immunostimulatory properties [77]. CpG ODNS have been studied in combination with monoclonal antibodies and with cytotoxic chemotherapy in epithelial malignancies. Wang *et al.* [78] evaluated CpG ODNS in several human and murine breast cancer models, showing tumour inhibition of at least 40% in all models. Addition of trastuzumab led to enhanced antitumour activity, resulting in more than 96% inhibition of tumour growth. It was suggested that the potentiating effects of CpG ODNS on monoclonal antibodies may be mediated by activation of NK cells, which mediate antibody-dependent cellular cytotoxicity.

Clinical data supporting CpG use are sparse in the breast cancer literature. A phase I trial was initiated by Pfizer (NCT00031278), aiming to characterize the safety and efficacy of antisense oligodeoxynucleotide CpG 7909 in combination with trastuzumab in MBC. The trial was terminated because of a modification to the route of administration. A continuation study was initiated for those previously treated with CpG 7909, and results of the latter study are awaited.

### 3.5 Bisphosphonates

The γδ T cells are a small subset of peripheral T cells that recognize unique phosphoantigens lacking the requirements of classical antigen-presenting molecules. They have been shown to have potent antitumour activity *in vitro* [79–82].

Bisphosphonates are potent activators of γδ T cells, inducing cytokine secretion and cell-mediated cytotoxicity by inhibition of farnesyl pyrophosphate synthase of the mevalonate pathway, leading to accumulation of isoprenoids [83].

Kunzmann *et al.* [84] showed that bisphosphonates can induce expansion of γδ T cells and thereby have antiproliferative and antitumour functions. Compared with untreated cultures, pamidronate-treated bone marrow cultures from 24 patients with multiple myeloma showed reduced survival. In metastatic renal carcinoma, a synthetic phosphoantigen (bromohydrin pyrophosphate) amplified γδ T cells in 11 of 15 patients [85].

Wilhelm *et al.* [86] treated 19 patients with non-Hodgkin lymphoma or multiple myeloma with a combination of pamidronate and IL-2. Significant activation and proliferation of γδ T cells was seen in 5 of 9 patients, and objective responses were observed in 3 of 9 patients. Only patients with significant proliferation of γδ T cells responded to treatment.

Mattarollo *et al.* [87] demonstrated synergy with a combination of chemotherapy, bisphosphonates, and γδ T cells. Chemotherapy and bisphosphonates both sensitize tumour cell lines to γδ T cell–mediated cytotoxicity, but the combination was shown to enhance the effect. Similar results were seen in the ABCSG-12 study (Austrian Breast and Colorectal Cancer Study Group), which showed improved disease-free survival by 36% (hazard ratio: 0.64; $p = 0.01$) in hormone-positive premenopausal patients receiving zoledronic acid with endocrine treatment. The mechanism could again partly reflect induction of γδ T cells.

## 4. CONCLUSIONS

The application of immunotherapeutic principles to the treatment and prevention of breast cancer has been ongoing for many decades. Although cytokines, cancer vaccines, and other host factors have been extensively studied in breast cancer, the therapeutic efficacy of these approaches remains unproven. The recent identification of tumour-specific immunity and of several breast cancer antigens has generated enthusiasm for the application of immune-based therapies. Although monoclonal antibodies, cytokines, and vaccines have all individually shown some promise, and although the immunomodulatory effects of bisphosphonates have taken a front seat in the treatment of breast cancer, it is likely that the best strategy to combat breast cancer will be a multimodality strategy. Clearly, different strategies demonstrate benefit in different patient populations. It may be that the best results will be obtained from vaccines in combination with a variety of antigens, or from vaccine and antibody combinations. Nonspecific and specific immunotherapy combinations may be another potent strategy. The effect of any of the aforementioned strategies in combination with more traditional cancer therapies is another avenue, given that some duration benefits have been seen with cytokines and chemotherapy. Given the mechanisms of immunotherapy, these treatments are most likely to work in the adjuvant setting and not in the setting in which they are usually tested: the heavily treated patient with MBC.

It is hoped that ongoing studies will yield the breakthrough needed to establish immunotherapy as a viable option in the treatment of breast cancer.

## 5. CONFLICT OF INTEREST DISCLOSURES

The authors declare that no financial conflict of interest exists.

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