

Immunologic approaches to breast cancer treatment

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Despite improvements in the screening and local-regional therapy of breast cancer, 30% of women who are diagnosed with invasive breast cancer will develop metastatic disease during their lifetime. Adjuvant chemotherapy is our best answer to this problem, but this requires the administration of a toxic and expensive course of therapy to many patients to realize the benefit in only a few. For every 10 patients who are node-negative and receive adjuvant chemotherapy, only one breast cancer death will be prevented [1]. New approaches to therapy are needed that can eradicate micrometastatic disease without significant morbidity. The interest in harnessing the power of the immune system for this purpose is not surprising.

Although all researchers like to believe that their approaches are novel and cutting-edge, immunotherapy in its most basic form dates back several millennia to the intratumoral injection of infected purulent materials, as documented in Eastern and Western ancient medical writings [2]. The “modern” age of immunotherapy, however, can be said to start with William Coley, a surgeon at Memorial Hospital in New York. In 1893, he reported that injecting an inoperable sarcoma with streptococcal broth cultures resulted in tumor regression; when he stopped the injections, tumor growth resumed. He followed this with an injection of a new culture that elicited a life-threatening attack of erysipelas. The patient survived this episode, his tumor regressed significantly, and he lived for 8 years. On the basis of these results, Coley began treating patients who had inoperable disease, including patients who had breast cancer, with “Coley’s toxin,” which was prepared from *Streptococcus pyogenes* and *Serratia marcescens* [3].

The successes that were seen with Coley’s toxins were not as significant as advances in surgery, radiation, and chemotherapy. Consequently, interest in

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immunotherapy waned. At the time, however, little was understood of the processes through which the immune system eradicated infectious diseases or tumors. Over the past few decades, our knowledge of the components and mechanisms of the immune system has grown exponentially. Paralleling that growth has been our interest in immunotherapy for cancer, including breast cancer.

How does the immune system fight cancer?

The reason why immunotherapy is so attractive is the specificity; an immune response can be directed against tumor-specific antigens (TAA) through two mechanisms—the humoral response and the cellular response. The humoral response is triggered by the interaction between the variable region of an antibody with specific epitopes on cell-surface molecules. The cellular response involves recognition of antigens by T-cell receptors (TCRs) when they are presented by the cell in conjunction with the major histocompatibility complex (MHC) molecules. Antibodies are not capable of detecting the small processed peptides on MHC molecules on the cell surface, so the nature of the antigens that are recognized by the humoral and cellular arm is different. Whether a humoral or a cell-mediated immune response is more important in generating antitumor immunity still is debated; however, patients who exhibit both responses seem to fare better than those who demonstrate only one type of response [4,5].

The initiation of an antitumor immune response rests with antigen-presenting cells (APCs) to process and present tumor-related antigens. Proteins are phagocytosed by APCs, partially digested into smaller polypeptides, and bound to MHC class II molecules. After the antigen-MHC complexes are transported to the cell surface they can be recognized by naïve T lymphocytes through the TCR. When a naïve helper (CD4⁺) T cell recognizes the antigen, as well as costimulatory molecules that are present on the APC, it becomes activated. Activation results in proliferation and differentiation and the activated helper T cell can then help to promote a cellular response (Th1) or a humoral response (Th2).

A Th2 response ultimately leads to the stimulation of B cells to proliferate and differentiate into plasma cells through the secretion of B-cell stimulatory cytokines (interleukin [IL]-4, IL-5, IL-10). Antibodies that are produced by the plasma cells can recognize breast cancer cell surface antigens and kill tumor cells by a variety of methods. One important method is antibody-dependent cell-mediated cytotoxicity (ADCC), which involves the attachment of tumor-specific antibodies to tumor cells and the subsequent destruction of the tumor cell by immunocompetent cells, most commonly the natural killer (NK) cell. Another way in which antibodies lead to tumor death is through complement-dependent cell-mediated cytotoxicity, where the recognition and attachment of complement-fixing antibodies to tumor-specific surface antigens is followed by complement activation and cell death.

A cellular response occurs when a naïve cytotoxic (CD8⁺) T cell recognizes antigen that is being presented on the surface of an APC. The TCRs on cytolytic T cells recognize antigen that is presented on MHC class I molecules. In the presence of costimulatory molecules on the APC and cytokines that are released from the Th1 helper T cell (IL-12, interferon [IFN]- γ , tumor necrosis factor [TNF]- α), the cytolytic T cell is activated. Once activated, cytolytic T cells destroy tumor cells by way of TCR recognition of tumor-specific antigen that is presented on MHC class I molecules at the tumor cell surface. They bind to the MHC class I receptor–tumor antigen complex and destroy the tumor cell by way of the release of granules that contain granzyme B and perforin and by way of induction of Fas/Fas ligand apoptosis.

Breast cancer-specific antigens

The success of immunotherapy rests upon the presence of breast cancer-specific antigens to which either a humoral or cellular response can be initiated. While breast cancers have been classically categorized as nonimmunogenic, several tumor proteins have been identified to which an immune response can be generated (Table 1) [6]. Mutational antigens are one category of TAA that is encoded by the multiple genetic mutations that are present in malignant cells. The aberrant proteins that result from genetic alterations can deprive the cell of the wildtype functions (and result in malignant transformation) and lead to new proteins that are immunogenic.

Differentiation antigens are antigens that are shared by cancer cells and normal cells; typically, they are overexpressed in cancerous tissue. In breast cancer, the most important of these antigens is the human epidermal growth

Table 1
Potential targets for immunotherapy in breast cancer

Antigen [reference]	Expression (%)	Type	Response
p53 [7,8]	17	Mutational	Cellular
CEA [9]	50	Differentiation	Humoral and cellular
NY-BR-1 [10]	80	Differentiation	Humoral
HER-2/neu	30	Amplified/overexpressed	Humoral and cellular
MUC-1	80	Differentiation/mutational	Humoral
NY-BR-62 [10]	60	Amplified/overexpressed	Humoral
NY-BR-85 [10]	90	Amplified/overexpressed	Humoral
D52 [10]	60	Amplified/overexpressed	Humoral
Mammoglobin [11]	23	Amplified/overexpressed	Humoral and cellular
NY-ESO-1 [12]	24	Cancer-testis antigen	Humoral and cellular
MAGE-3 [12]	14	Cancer-testis antigen	Humoral and cellular
SCP-1 [12]	30	Cancer-testis antigen	Humoral
SSX-1 [13]	12	Cancer-testis antigen	Humoral
SSX-4 [13]	14	Cancer-testis antigen	Humoral
CT-7 [12]	30	Cancer-testis antigen	Humoral

Abbreviations: CEA, carcinoembryonic antigen; CT-7, cancer testis-7; NY-BR, New York Breast; NY-ESO, New York Esophagus; SSX, synovial sarcoma-x.

factor receptor-2 (HER-2/neu). This transmembrane growth factor receptor protein is expressed at low levels on normal tissue but is overexpressed in many types of malignancies, including 30% of breast cancers [14]. This overexpression makes HER-2/neu an appealing molecular target for drug therapy, particularly because its heightened expression is associated with poorer clinical outcome [15].

Another breast cancer antigen that is of interest is mucin (MUC-1), a membrane-bound glycoprotein that consists of a polypeptide core and numerous carbohydrate side chains. The version of MUC-1 that is expressed on breast cancers differs from that in normal tissues in that the carbohydrate side chains are shorter and the peptide backbone is more exposed. As a cell surface glycoprotein, it is most efficient in inducing a humoral response; the presence of a MUC-1 specific IgG and IgM antibody response may be associated with a longer disease-free survival [16]. There also seems to be evidence for a natural immunization against MUC-1 during pregnancy, which has been proposed as one explanation for why breast cancer is less common in multiparous women [17].

Other differentiation antigens are present in the same form on normal epithelial cells and cancer cells. It is possible that an immune response to these antigens could stimulate an autoimmune destruction of the normal tissues that express them. In the treatment of melanoma, for example, this would result in vitiligo, which has been described as a side effect of melanoma immunotherapy and is associated with an improved prognosis [18,19]. Although mastitis may not be an acceptable side effect of therapy, targeting these antigens would be of less concern in the adjuvant setting after a bilateral mastectomy.

Cancer-testis antigens are antigens that present on germ line cells but not on somatic cells. Changes in transcriptional regulation in cancer cells can lead to expression of these antigens. Because germ line cells do not express MHC molecules, these antigens normally are silent; however, when expressed on cancer cells, they are capable of eliciting an immune response. Examples of cancer-testis antigens in breast cancer include MAGE-3 and SSX-expressed proteins.

Techniques, such as CD8⁺ and CD4⁺ T-cell epitope cloning, serum antibody expression cloning (SEREX), and genomic approaches, are making it easier to identify breast cancer antigens. It is reasonable to expect that over the next few years there will be a dramatic increase in the number of identified breast cancer antigens. Which antigens will be the best targets and which methods to elicit a clinically relevant response to those antigens will be the most effective, will remain the core questions in immunotherapy research.

Role of the immune system in breast cancer

When discussing tumor immunology, one cannot help but to think immediately of melanoma, which has emerged as the primary cancer model

for developing immunotherapies. There are several reasons for this, including the ease of studying melanoma in the laboratory (allowing for the identification of multiple melanoma-specific antigens) and the lack of effective adjuvant therapies (resulting in a higher portion of patients who is available for clinical testing). Another reason is the clinical evidence that there is a natural role of the immune system in the development and progression of this melanoma. Melanoma often presents as metastases without evidence of a primary tumor that presumably has undergone immune-mediated regression. Histopathologic evidence of tumor regression is observed frequently within primary melanoma specimens, along with lymphocytic infiltration. It also is not uncommon for melanoma to remain dormant for some time (up to 20 years after diagnosis); this suggests a balance between the tumor and the host immune system.

This begs the question, “Is there a baseline role of the immune system in the natural history of breast cancer?”. Unidentified primary tumors and long intervals between diagnosis and recurrence certainly occur with breast cancer; however, neither is as common as it is with melanoma. If the immune system does suppress the development of a cancer, one would expect that the incidence would increase in patients who do not have a competent immune system. This increase is seen in melanoma; the incidence of melanoma increases dramatically in immunocompromised transplant recipients and these patients often present with multiple lesions [20,21]. This same observation, however, has not been seen with breast cancer [22].

In melanoma, another argument for a prominent role of the immune system is the presence of lymphocytic infiltrate and evidence of tumor regression. Tumor-infiltrating lymphocytes (TILs) also have been identified in breast cancer. One might expect that if the host’s immune system can recognize and impede the growth of breast cancer, then those tumors with a more intense lymphocytic infiltration would be associated with a better prognosis. In one study of more than 1900 breast tumors, evidence of lymphocytic infiltration [23] was an independent predictor of prognosis on multivariate analysis (including tumor size and nodal status) in women who were younger than 40 years of age, but not in women who were older than 40; however, other studies found that lymphocytic infiltration is a poor prognostic sign [24].

Evidence of a natural immune recognition of breast cancer also can be identified systemically. Some patients who have breast cancer have detectable immunity to HER2, antibody responses, and, to a lesser degree, T-cell responses [25–28]. Cytotoxic T lymphocytes that are specific for tumor-associated mucin can be detected in patients who have cancer that have adenocarcinomas that overexpress mucin [29]. Is this immune response clinically relevant? If there is a natural role to the immune system to the progression of breast cancer, the presence of a response should correlate with outcome. In some cases this has been suggested; for example, the presence of a MUC-1–specific antibody response may be associated with

a longer disease-free survival [16]. As with a lymphocytic infiltrate, the evidence for this is scant and conflicting. In the case of HER2, it is difficult to separate any potential impact of a natural HER-2/neu immunity because of multiple interrelated variables. HER-2/neu monoclonal antibody (mAb) and T-cell responses occur when the cancer overexpresses increased levels of HER2/neu, which, in itself, is associated with aggressive disease, response to chemotherapy, and poor outcome. There also are cases where evidence of an immune response seems detrimental. As an illustration, it is well-documented that a percentage of patients exhibit a delayed-type hypersensitivity (DTH) response to a skin test with autologous tumor extracts, including breast cancer [30]. Of 56 patients who had breast cancer who were tested for a DTH response to their tumors and were followed for at least 2.5 years, survival was worse in patients who had a positive response [30]. The evidence remains extremely unclear as to whether the immune system plays any significant role in the development and progression of breast cancer.

Methods of generating an immune response

It would seem, therefore, that researchers who are interested in breast cancer immunotherapy are starting at a disadvantage compared with their colleagues in melanoma. The lack of evidence for a natural role of the immune system in the evolution of breast cancer does not imply necessarily that it is not possible to deliver or generate such a response. One method is passive immunotherapy—the delivery of antibodies or cells that have been sensitized previously to host tumor antigens. With passive immunotherapy, the host need not mount an immune response; the therapeutic agent directly or indirectly mediates tumor killing. One of the best known examples of passive immunotherapy is trastuzumab (Herceptin), a monoclonal antibody to HER-2/neu, and an approved treatment for stage IV breast cancer. The other method for stimulating an immune response is active immunotherapy in which the intervention prompts the host to mount an immune response. Active nonspecific immunotherapy uses agents that stimulate the immune system globally, but do not recruit specific effector cells. Active specific immunotherapy is designed to elicit an immune response to one or more tumor antigens; the prime example is the use of vaccines.

Active nonspecific immunotherapy

Immunostimulants

Coley's toxin represented the earliest form of active immunotherapy that used a nonspecific immunostimulant. A more familiar approach is the use of bacille Calmette-Guérin (BCG), an attenuated form of the tubercle bacillus. Although first used as a vaccine against tuberculosis, it ultimately was discovered to be a potent immunostimulant that was capable of preventing tumor growth in mice [31]. BCG has been studied in several tumor types and remains an intravesical therapy of early stage bladder cancer. BCG was

studied as an adjuvant to chemotherapy for disseminated breast cancer. Although rates of remission were similar between chemotherapy and BCG compared with chemotherapy alone, remissions seemed to last longer with the addition of BCG [32,33]. In a randomized trial of patients who had stage II breast cancer and were given adjuvant therapy with chemotherapy and tamoxifen with or without BCG, BCG failed to improve overall disease-free survival. The killed vaccine, *Corynebacterium parvum*, is another immunostimulant that has been studied extensively. Again, based on some promising early data [34], *C parvum* was studied in combination with adjuvant chemotherapy for patients who had stage II breast cancer; it failed to improve disease-free survival [35]. Neither BCG nor *C parvum* has a role in breast cancer treatment, although they may serve as adjuvants to tumor-specific vaccines.

Other immunostimulants are not bacterial in nature, but rather are synthetic. Levamisole was developed originally as a veterinary antihelminthic, but was shown to have immunopotentiating properties. Although levamisole is beneficial in colon cancer, its use in breast cancer yielded mixed results with significant toxicities [36–39]. Polyadenylate:polyuridylylate (poly A:U) is another synthetic immunostimulant. This is a polynucleotide polyriboinosinic acid that seems to work primarily through induction of interferon. A randomized trial of poly A:U as adjuvant therapy after mastectomy and radiation resulted in a 4-year relapse-free survival rate of 77% compared with 57% in controls [40].

Cytokines

Cytokines are naturally-occurring soluble proteins that are produced by mononuclear cells of the immune system. They can affect the growth and function of cells through interaction with specific cell-surface receptors. With advancements in molecular biologic techniques, it became possible to clone the genes for these cytokines and mass produce pure forms in large amounts. This allowed researchers to study their function and to explore their use as therapeutics. More than 50 cytokines have been isolated to date and several have been approved by the U.S. Food and Drug Administration (FDA) for the treatment of a variety of malignancies; however, they have had minimal impact on the treatment of breast cancer.

The interferons were described originally as proteins that are produced by virally-infected cells that protect against further viral infection through a variety of effects. These include the increased antigen presentation by way of increased expression of MHC and antigens, enhancement of NK cell function, and the enhancement of ADCC. In addition, the interferons exert direct antiangiogenic, cytotoxic, and cytostatic effects. There are several subtypes of interferons, including IFN- α , IFN- β , and IFN- γ .

IFN- β and IFN- γ have been studied in breast cancer but their potential for cancer therapy remains unclear [41]. Although IFN- γ clearly has immunostimulatory effects, it also has inhibitory effects on antigen presentation,

including the down-regulation of known breast cancer antigens, such as HER2/neu [42,43]. Several hematologic and solid tumors have proved responsive to IFN- α , including chronic myelogenous leukemia, cutaneous T-cell lymphoma, hairy cell leukemia, malignant melanoma, and Kaposi's sarcoma. Few clinical trials have examined IFN- α against breast cancer and demonstrated only minimal responses with considerable toxicity [44,45].

Originally, IL-2 was described as the "T-cell growth factor," because it is required for the differentiation and proliferation of activated T cells. As such, it seems like an ideal choice for immunotherapy. The major drawback of IL-2 is the significant dose-related toxicity. IL-2 leads to significant interstitial edema and lymphoid infiltration into vital organs that can lead to severe hypotension and resultant ischemic damage to the heart, liver, kidneys, and bowel. This limits the use of IL-2 to patients who have excellent performance status, normal pulmonary and cardiac function, and no active infections. IL-2 alone is an effective therapy in patients who have metastatic melanoma and metastatic renal cell carcinoma, but there is minimal evidence to support its use in breast cancer. The possible role of other cytokines with anticancer properties, such as IL-12 or granulocyte macrophage-colony stimulating factor (GM-CSF), is being investigated.

Passive immunotherapy

The potential for therapy with immunostimulants or cytokines rests on the assumption that the immune system has at least a minimal capacity to recognize and ablate tumor cells. Through a global augmentation of the immune system, that baseline anti-tumor response will also be augmented, hopefully to clinically relevant levels. Passive immunotherapy assumes that there is no significant inherent immune response and works through the administration of preformed elements of the immune system. This may be of great benefit in breast cancer, in which a baseline immune response does not seem to be as effective in inhibiting the growth of the cancer.

Monoclonal antibodies

The process by which the body generates an antibody response can be circumvented by the intravenous administration of antigen-specific monoclonal antibodies. In breast cancer, the most well-known is trastuzumab (Herceptin), which is FDA-approved for the treatment of HER-2/neu-expressing tumors. The targeting of other breast cancer antigens, including MUC-1 and epithelial cell adhesion molecule (EpCAM), with monoclonal antibody therapy is under clinical and laboratory investigation. Much of the appeal of this approach is that monoclonal antibodies are easy to mass produce, standardize, and administer. This significantly increases their clinical usefulness as compared with other methods of immunotherapy.

Trastuzumab (Herceptin) is a monoclonal antibody that is directed at HER-2/neu. Because normal body tissues only express HER-2/neu at low levels, trastuzumab is a tissue-selective treatment for breast cancer and was

shown to be efficacious with an overall response rate of 26% as a first-line monotherapy for breast cancer [46]. When used in conjunction with traditional chemotherapy, trastuzumab increased time to disease progression (7.4 versus 4.6 months, $P < .001$), decreased rate of death at 1 year (22% versus 33%, $P = .008$), and increased patient survival compared with chemotherapy alone (median survival, 25.1 months versus 20.3 months $P = .046$) [47].

Although trastuzumab is touted by many investigators as evidence for the potential of immunotherapy in breast cancer, the mechanism by which trastuzumab kills tumor cells has not been defined completely and might not be purely immunogenic. At the molecular level, the binding of trastuzumab to the HER-2/neu protein causes the complex to be internalized and broken down; this decreases the phosphorylation of the neu tyrosine kinase and eliminates its signaling capacity to promote tumor growth. This suggests that trastuzumab acts primarily as an antagonist to the epidermal growth factor receptor [48]. This is not to say that the immune system may not play a role in trastuzumab's mechanism of action. Treatment with trastuzumab increased the killing of HER-2/neu overexpressed cancer cells by HER-2/neu-specific cytotoxic lymphocyte (CTLs) in vitro [49] and enhanced ADCC [50], which may be an important mechanism for trastuzumab-induced responses in vivo [51]. It may be possible to increase the immunologic component by combining Herceptin with systemic cytokines [52,53]. By whatever mechanism trastuzumab kills tumor cells, it is an effective tool against HER-2/neu-overexpressed breast cancers and improves the prognosis of the disease.

Although trastuzumab was effective against HER-2/neu positive breast cancers, it is only expressed on 30% of those malignancies, which excludes most patients from this therapy. MUC-1 is expressed in 80% of breast malignancies, so passive immunotherapy against MUC-1 would be applicable to a much larger fraction of women who has breast cancer. The low level of glycosylation on the aberrant MUC-1 that is present on breast cancer cells unmasks epitopes that are not exposed on normal mucin and creates targets for tumor-specific antibodies [54]. Preclinical research identified several anti-MUC-1 mAbs that are effective in killing breast cancer cells. DF3 is one mAb that is directed at MUC-1; it increased antigen-directed phagocytosis and cytolysis of MUC-1-expressing human breast cancer cells in vitro [55]. Edrecolomab, another monoclonal antibody, is directed against the EpCAM, which is expressed on several types of cancers, including colon and breast. Braun et al [56] measured the efficacy of edrecolomab as a monotherapy in the elimination of tumor cells from bone marrow aspirates in patients who had advanced metastatic breast cancer ($n = 10$). The number of EpCAM-expressing cells decreased in all 10 patients in the study and 4 patients had complete elimination of EpCAM⁺ cancer cells. Another study investigated edrecolomab's efficacy in eliminating bone marrow metastasis of breast cancer following chemotherapy [57]. After completion of chemotherapy, 9 of 14 patients remained positive for

the EpCAM⁺ antigen and were treated. This mAb treatment eradicated all EpCAM⁺ cancer cells in 7 patients and greatly reduced the number in the other 2 patients. Although treatment with anti-MUC-1 or anti-EpCAM mAb are experimental, this line of therapy looks promising, particularly as a complement to other therapies.

Adoptive immunotherapy

Although more complex than delivering monoclonal antibodies, the cellular arm of the immune system also may be used as passive immunotherapy. A cellular immune response to cancer may be more effective than an antibody response; however, attempts to stimulate a cellular response through immunostimulants or vaccines has been difficult. Therefore, the passive administration of cells with antitumor activity to the tumor-bearing host has generated significant interest. This cellular infusion therapy, known as adoptive immunotherapy, involves harvesting cytotoxic T lymphocytes, NK cells, or mononuclear cells from the patient (autologous) or a donor (allogeneic); selecting and expanding them in vitro; and delivering the activated, tumor-specific cellular inoculum to the patient.

Early trials of the adoptive transfer of allogeneic lymphocytes demonstrated little or no clinical effect [58,59]. Slightly more promise has been seen with harvesting autologous lymphocytes, activating them in vitro, and delivering them back to patients. These lymphocytes may be obtained from the peripheral blood or bone marrow of patients who have breast cancer [60,61]. The primary obstacle has been the generation of sufficient numbers of tumor-specific cells for transfer. Minimal benefit is seen when lymphocytes are activated in a nonspecific manner [62,63] because there is a scarcity of tumor-specific T cells in the periphery. Stimulating lymphocytes in the presence of autologous tumor cells [64], genetically-modified tumor cells [65], or tumor-pulsed dendritic cells [66] can increase the specificity of the lymphocytes, and potentially, the clinical response.

A richer source of tumor-specific lymphocytes may be the TILs. Cytotoxic T lymphocytes that are isolated from the breast tumors seem to be tumor-specific and demonstrate cytolytic activity against autologous cell lines [67]. Although TILs seem particularly attractive for adoptive therapy of melanoma, their use in breast cancer shows less potential [68–70]. Adoptive immunotherapy of breast cancer with expanded TILs faces several obstacles, particularly in obtaining adequate numbers. Primary breast tumors often are small (<2 cm); after margin status is determined on the lumpectomy specimen, there is minimal tissue available from which to expand TILs. It also is difficult to establish a cell line in vitro from the tumor, which is needed to test the lytic activity of the T cells before infusion. In stage IV disease, metastatic deposits often are difficult to access (eg, bone, lung, brain) which prevents T-cell harvesting.

An alternate source may be located in the tumor-draining lymph nodes (TDLNs). Tumor-reactive T lymphocytes can be expanded from regional

lymph nodes in patients who have breast cancer; in some patients, these T cells demonstrate a tumor-specific response [71]. Okino et al [72] obtained lymphocytes from regional lymph nodes and peripheral blood from patients who had breast cancer with liver metastases. They saw clinical responses when these cells and OK-432, a biologic response modifier, were administered by way of the intra-arterial route into the liver.

With sentinel node biopsy becoming routine in the management of breast cancer, the lymph nodes that drain the tumor directly are sectioned thinly and scrutinized carefully for the presence of micrometastases. This makes it difficult to obtain significant quantities of T cells from the TDLNs. An alternate approach is to vaccinate the patient at an alternate site and harvest the vaccine-draining lymph nodes (VDLNs). Chang et al [73] demonstrated the feasibility of this approach in patients who had advanced melanoma or renal cell carcinoma. They vaccinated the patients with an autologous tumor vaccine that was admixed with BCG and expanded T lymphocytes *ex vivo* in the presence of IL-2. This approach stimulated complete and partial responses in some patients [74]. Genetic modification of the vaccinating cells to secrete GM-CSF may enhance the T-cell reactivity of the VDLN cells further against breast cancer antigens [75,76]. Although this exact approach would be hampered by the difficulties in creating autologous vaccines in patients who have breast cancer, an alternate approach that uses allogenic cellular or peptide vaccines may be feasible [77,78].

In addition to T cells, NK cells may have the potential for adoptive immunotherapy of cancer because their natural role in the body is fighting virally-infected cells and cancerous cells [79]. The genetically-modified NK cell line, NK-92-scFv(FRP5)- ζ , expresses a chimeric antigen receptor that is specific for HER2/neu [79]. *In vitro*, NK-92-scFv(FRP5)- ζ cells were effective at inducing apoptosis in cells that overexpressed HER2/neu and could eliminate them completely with prolonged incubation in culture [79]. NK cells plus IL-2 also were examined as an adoptive treatment for metastatic breast cancer following an autologous blood stem cell infusion [80]. Non-MHC restricted cell-based therapies also have been examined. Lymphokine activated killer (LAK) cells are generated *in vitro* by incubating the patient's peripheral blood mononuclear cells (PBMC) with high-dose IL-2 and are capable of killing cells in a non-MHC-restricted fashion. Adoptive immunotherapy with LAK cells and high-dose IL-2 had minimal effect in patients who had breast cancer [81]. The human leukemic cell line, TALL-104, also killed cancer cells in a non-MHC-restricted fashion and induced tumor regression in breast tumor-bearing mice [82]. In a phase I clinical trial, 15 patients who had metastatic breast cancer received the adoptive transfer of TALL-104 cells [83]. Specific CTL activity developed in 7 patients and 5 patients had stable disease for 2 to 6 months.

Adoptive immunotherapy is at an early stage in its development and the cost, complexity, and variability of these strategies limit their use to investigational studies. In addition, the lack of available tumor in many

patients who have breast cancer restricts the applicability of some approaches. Additional research and increased understanding of the complex interactions that are involved will define better the potential of adoptive cellular therapy in the treatment of breast cancer.

Active specific immunotherapy (vaccines)

The goal of active specific immunotherapy is to generate a host immune response to known or unknown tumor-associated antigens. Generally, these are referred to as cancer vaccines, although this term can be misleading. We are used to referring to vaccines as protective against infectious agents (ie, they are meant to stimulate the immune system against antigens that it has not seen). In contrast, cancer vaccines are designed to stimulate an immune response against antigens on cells to which the immune system already has been exposed.

Many different vaccine strategies are under investigation, each with advantages and disadvantages in regard to clinical feasibility and cost, the number of antigens that is available, and the mechanism of response (cellular, humoral, or both; Table 2) [84]. Some vaccine strategies use specific peptide antigens. These are highly purified and, therefore, are less likely to contain potentially irrelevant material. This has important implications regarding standardization and quality control; however, single antigens are less immunogenic and can be vulnerable to antigen modulation. Several other approaches use whole cells that contain many antigens. These

Table 2
Breast cancer vaccine therapies

Vaccine type	Advantages	Disadvantages
Peptide ganglioside vaccines	Standardization and quality control.	Low immunogenicity. Single antigen, easy for tumor to escape recognition.
Antiidiotype vaccines	Standardization and quality control. Increased immunogenicity (breaks tolerance).	Single antigen, easy for tumor to escape recognition.
Allogeneic cellular vaccines	Does not require autologous tumor. Standardization and quality control.	May not share antigens with patient.
Autologous cellular vaccines	Highly patient-specific. Do not need to know tumor-specific antigens.	Requires harvesting tumor and creating cell lines. Costly and labor-intensive.
Dendritic cell vaccines	Strongly immunogenic.	Either need known antigens or may require harvesting tumor and creating cell lines. Costly and labor-intensive.

cells can be allogeneic or autologous and can be used intact or lysed. Generally, these vaccines are more difficult to standardize and analyze; theoretically, this approach has the greatest potential for eliciting an immune response that is directed against multiple antigenic targets.

Breast cancer antigen vaccines

For a known, breast cancer-specific antigen, it is possible to use that protein as a vaccine—either the entire protein, which allows the patient's own immune system to cleave and bind peptides—or just selected immunogenic peptides. Immunizing the patient with the protein probably would not be sufficient because most of these antigens are weakly immunogenic. To improve the immunogenicity, the peptides often are delivered to the patient with an immune adjuvant that is meant to induce inflammation and push the immune process toward immunity, rather than tolerance. BCG or DETOX (“detoxified Freund's adjuvant,” composed of monophosphoryl lipid A and a purified mycobacterial cell-wall skeleton) are examples of adjuvants that are meant to cause a generalized or nonspecific inflammatory response that will increase the likelihood of recognizing the vaccinating antigens. Cytokines also have been given simultaneously with the vaccines to stimulate the immune response further.

Even when combined with adjuvants or cytokines, peptide vaccines are easy to standardize, mass produce, and administer. This makes it simple to test the vaccine effectively and has important implications regarding quality control, cost-effectiveness, and clinical usefulness, particularly in the adjuvant setting. Conversely, these vaccines are limited by the fact that even if a peptide vaccine is immunogenic, it may not be the “right” vaccine for many patients. Commonly-expressed tumor antigens are not present on all patients' tumors or may be present in varying degrees (see Table 1). In addition, the T cell's recognition of an antigen depends on the presentation of that antigen on a specific MHC molecule. Only certain HLA phenotypes can present any given peptide to induce an immune response, so they only will function on a limited subset of patients. A classic example is that of the MART-1/Melan-A antigen in melanoma. The antigen is expressed by 80% of melanomas, but the peptide only binds to HLA-A2. Because approximately 45% of whites have HLA-A2, only 36% (80% of 45%) of patients who have melanoma and are given a MART-1/Melan-A vaccine would see a benefit.

There is tremendous interest in MUC-1 vaccines because MUC-1 is expressed on 80% of breast cancers and higher circulating antibodies to MUC-1 in women who have early breast cancer and is associated with an improved disease-free and overall survival [16]. Early data on MUC-1 vaccines demonstrated promising results [85]. Sixteen patients who had metastatic breast carcinoma were immunized with a MUC-1 peptide plus keyhole limpet hemocyanin (KLH) and DETOX. This resulted in anti-MUC-1 IgG antibodies in 3 of 16 patients and anti-MUC-1 CTL activity in 7 of 11 patients [86]. A mannan-MUC-1 fusion protein was given to

25 patients who had metastatic breast, gastric, or colorectal cancer which resulted in anti-MUC-1 IgG antibodies in 13 of 25 patients and MUC-1-specific T-cell proliferation in 4 of 15 patients [87]. Ongoing research will determine the clinical impact of MUC-1 vaccines.

Given the success of Herceptin, there has been considerable work in creating Her-2/neu vaccines [88]. Immunization with a HER-2/neu vaccine can result in DTH peptide-specific responses in skin and specific T-cell responses in peripheral blood lymphocytes [89,90]. Phase I studies of HER-2/neu vaccines showed low levels of antitumor activity despite a detectable immune response [86,91–94]. HER-2/neu is a 185-kd protein with no clear consensus as to which part is the optimal target for vaccination. Attempts to identify the optimal oligopeptide within the HER-2/neu protein and the use of different adjuvants led to new vaccine strategies, including prevention of disease and treatment of patients who are of a specific HLA subtype [95–101].

Besides cell surface proteins, carbohydrate tumor-associated antigens may be targets of vaccines [102,103]. Sialyl-TN (STn) is a carbohydrate that associates with the MUC-1 mucin. The cancer vaccine, Theratope (Biomira Inc., Alberta, Canada), consists of STn conjugated to KLH as a carrier protein and DETOX as an adjuvant [104,105]. Seven of 12 patients who had metastatic breast cancer and were vaccinated with Theratope had a partial clinical response or stabilization of their disease [106]. A follow-up prospective trial randomized patients to receive Theratope with or without intravenous (IV) or oral low-dose cyclophosphamide. A survival advantage was seen in patients who received the vaccine with IV chemotherapy. A phase III trial randomized 1028 women who had metastatic breast cancer that was stable or in remission to receive IV cyclophosphamide followed by Theratope or cyclophosphamide that was followed by a vaccine of KLH and DETOX. Early reports demonstrated no overall difference in time-to-disease progression or survival; however, analysis of the subset of patients that received hormonal therapy showed a trend toward increased time-to-disease progression. It is possible that patients who were not on hormonal therapy progressed and were taken off Theratope before their immune systems had time to obtain a significant immune response. Further research into the potential use of Theratope is ongoing.

Anti-idiotypic vaccines

Cell surface gangliosides may be weakly immunogenic as a result of exposure to the immune system and the development of tolerance. A novel vaccination method to overcome this tolerance is the administration of anti-idiotypic antibodies. Anti-idiotypic antibodies exploit the fact that the immune system uses a network of interacting antibodies to mount a response against a specific antigen [107,108]. According to this “network hypothesis,” an external antigen is recognized by specific idiotypes that are expressed by antibodies and TCRs. Antibodies that are produced against an antigen can generate a series of anti-idiotypic antibodies that mimic the

three-dimensional structure of the original antigen. This would be like making a mold of the inside of a lock to reproduce the structure of the key that fits it. Thus these anti-idiotypic antibodies can induce specific immune responses that are similar to the responses that are induced by the original antigen [109,110]. For use as immunotherapy, antibodies are raised against anti-ganglioside antibodies so that the variable region of the antibodies essentially are mirror-images of the ganglioside itself, only composed of protein. When an immune response occurs to this mirror-image protein, it also is cross-reactive against the original nonprotein antigen [111,112]. Several advantages to anti-idiotypic vaccines are related to the fact that they do not depend on the availability of large amounts of pure antigen, which often is a limiting economic factor in vaccine production. Also, any acquired tolerance to the original antigen can be broken by using a different molecular form of the same antigenic moiety (in this case, the protein antibody instead of the nonprotein ganglioside); this improves upon the immunogenicity of standard antigen vaccines. Anti-idiotypic vaccines are under investigation in breast cancer [113–115].

Autologous cellular vaccines

Peptide vaccines are applicable only if one has a target antigen in mind. Presumably, any patient's cancer may have multiple antigens to which an immune response may be directed. Using the patient's cancer as the vaccine precludes the need to identify these antigens specifically. Theoretically, this approach ensures that all biologically-relevant antigens are presented to the immune system. Autologous tumor cells are harvested from the patient, irradiated, and returned to the patient to stimulate a tumor-specific immune response along with adjuvants to increase the immunogenicity. There are several drawbacks to autologous cellular vaccines. First and foremost, the approach is limited to individuals who have sufficient tumor to prepare a vaccine. This has restricted trials to patients who had adequate tumor from which cell lines could be created. This is extremely difficult in patients who have breast cancer because they rarely have a large amount of harvestable tumor, and in whom establishment of cell lines is difficult. Dillman et al [116] attempted to establish cell lines from patients who had breast cancer for autologous vaccines but were successful in only 8 of 115 samples (7%).

Despite the difficulties, several investigators have examined the use of autologous cellular vaccines in breast cancer. Wood and Baynes [117] administered autologous irradiated breast cancer cells intradermally with GM-CSF to patients who had stage IV breast cancer. Immune responses were seen in 11 of 12 patients but clinical outcome was not reported. Ahlert et al [118] vaccinated 90 women who had primary or metastatic breast cancer with a Newcastle disease virus-modified, irradiated tumor cell vaccine. In the patients in whom a vaccine could be established with maximum viability, there was a trend toward improved survival which

highlights the potential and the frustrations of this approach. The technical complexities that are inherent in procuring tumor and preparing a vaccine, have, to date, precluded widespread conduct of multi-institutional trials to test formally the efficacy of these vaccines. Treatment would be extremely costly and is feasible at a limited number of academic institutions.

Allogeneic tumor cell vaccines

Several breast cancer-associated antigens are shared among a large number of patients. Therefore, it is reasonable to expect that one could create a vaccine from cultured cell lines that would stimulate an anti-tumor immune response in any patient who shared some of those antigens. This is the principle behind allogeneic tumor cell vaccines. This approach offers several advantages over autologous vaccines in breast cancer. Specifically, allogeneic vaccines are readily available for patients who lack sufficient tumor to produce an autologous tumor cell vaccine and can be standardized, preserved, and distributed in a manner akin to any other therapeutic agent. Allogeneic cellular vaccines are being investigated as adjuvant therapy in melanoma. These include Canvaxin (Cancer Vax, Carlsbad, California), an allogeneic melanoma vaccine that is composed of three viable irradiated melanoma cell lines that are chosen specifically for their high content of immunogenic melanoma- and tumor-associated antigens [119], and Melacine (Corixa, Seattle, Washington), a lysate of two homogenized melanoma cell lines that are combined with the adjuvant, DETOX [120].

To augment the immunogenicity of allogeneic cellular vaccines, cells can be modified genetically *ex vivo*. One approach is to modify the cells genetically to produce cytokines which results in a sustained release of those cytokines at the site of the vaccine. Locally-secreted cytokines can recruit antigen-presenting cells at the vaccine site and enhance *in vivo* lymphocyte activation and expansion, thereby augmenting the immune response. Another method for increasing the immunogenicity of cellular vaccines is to transfect the cells with CD80 (B7-1), a costimulatory that is present on antigen-presenting cells. Essentially, this converts the tumor cell into a tumor-antigen-presenting cell that is capable of activating naïve T cells [121,122]. The use of an allogeneic breast cancer vaccine that is genetically modified to express CD80 is under clinical investigation [123–125].

Dendritic cell vaccines

When exposed to tumor-associated antigens and inflammatory cytokines, dendritic cells (DCs) take up, process, and present these antigens to naïve CD8⁺ and CD4⁺ lymphocytes, and thus, initiate cellular and humoral responses against the tumor in question [126]. DCs are believed by many investigators to be the optimal avenue through which to initiate active anti-tumor immunity and presumably are involved somewhere in the immune response pathway for most of the vaccines that were described above. DC vaccines seek to bypass the need for the recruitment and processing of

antigens by DCs. This allows the clinician to deliver DCs that already express tumor antigens to the patient [127]. This approach is being investigated in breast cancer [128,129].

After dendritic cells are obtained from the patient through leukapheresis, the best method of priming the DC with the appropriate antigens is still being investigated. Two approaches are to expose immature DCs to tumor lysates or to fuse DCs with tumor cells. This allows for MHC class I and class II epitopes and the diversification of immune responses. This approach is attractive in that immune responses can be generated without the need for the characterization of tumor-specific antigens. Like autologous cellular vaccines, it only is applicable to the minority of patients who have breast cancer in whom enough tumor may be harvested. Additionally, breast tumors typically have dense connective tissue stroma from which it can be difficult to release the tumor cells without affecting their viability [130]. Another method is to load exogenous peptides onto the empty MHC class I molecules of mature DCs. DCs that are pulsed with HER-2/neu or MUC-1 [131], carcinoembryonic antigen (CEA) [132], or mammaglobin-A [133] are capable of stimulating antigen-specific immune responses.

In situ vaccination

Many of the methods for stimulating an immune response require the harvesting of tumor, including harvesting TILs for adoptive immunotherapy or tumor cells for autologous or DC vaccines. Because these methods use autologous tissue, they can be the most immunogenic; however, the impediments to obtaining this tissue limits these approaches for immunotherapy of breast cancer. One promising approach for breast cancer may be that of *in situ* vaccination. Although most vaccines are created *ex vivo* and delivered to the patient to stimulate an immune response, *in situ* vaccination uses immunostimulatory agents to generate an immune response to the tumor *in vivo*. This can be done as a treatment for stage IV disease and circumvents the need to generate cell lines from resected metastatic deposits or can be done before surgical resection as a form of neoadjuvant immunotherapy.

In situ vaccination centers primarily on the use of cytokines to generate the immune response. Because local cytokine production is involved in inflammation and local immune reactions, the goal of using systemic cytokines is to achieve clinically-relevant levels at the site of the tumor. To do this, high systemic doses are required; IV administration has been associated with significant systemic toxicity and only limited effects on the tumors. Local delivery may reflect more physiologically-relevant effects than does systemic cytokine administration. Direct injection of GM-CSF [134], IL-12 [135], or interferons [136] into the tumor induced lesion regression; however, this approach is limited by the short half-life of most cytokines and the need for frequent injections. By delivering low doses of cytokines in a sustained fashion into the microenvironment of the tumor mass it may be

possible to achieve physiologically-relevant therapeutic levels of cytokines without the systemic toxicities. Several studies demonstrated that the cytokine microenvironment at the tumor site has a significant impact on the outcome of the immune response, including the use of IL-2 [137,138], IL-4 [139,140], or IL-12 [141]. An improved ability of IL-12 to induce long-term immunity was observed following peri-tumoral injections [142].

In situ vaccination would overcome the significant toxicities that result from the high systemic doses of cytokines that are required to achieve therapeutic dose levels within the tumor. It does not require the identification of specific tumor antigens, which have been limited in breast cancer. Most importantly, most of these approaches do not require the harvesting of tumor cells from the patient, which has been a limiting factor in the translation of several immunotherapeutic approaches to the treatment of breast cancer. Several methods have been used to obtain high levels of cytokines in the tumor microenvironment.

Gene-modified cells

One popular vaccine strategy is to use autologous tumor cells that are transfected with the gene for a cytokine *ex vivo*, but it requires the establishment of a primary culture. This may not be applicable in breast cancer, where the ability to develop a primary culture is more challenging. In addition, selection of transfected cells may require prolonged culture, which may alter expression of nominal tumor antigens. This approach, however, can be used as an alternative method for *in situ* vaccination. Instead of transfecting autologous tumor cells, nontumor cells may be modified genetically to secrete cytokines and can be injected at the site of a tumor to obtain paracrine secretion into the tumor microenvironment. Direct injection of IL-12–transduced fibroblasts effectively eliminated established tumors in a murine model [143]. This approach also resulted in the induction of effective systemic immunity. In a phase I trial, IL-12–secreting fibroblasts led to partial responses in patients who had melanoma, breast cancer, and head and neck tumors; some persisted for up to 2 years [144].

The intratumoral delivery of DCs also may stimulate systemic immunity. To prime DCs with whole tumor cell lysates would be difficult in breast cancer. Immature DCs can acquire antigen from apoptotic cells and stimulate antigen-specific CTL [145] and the presence of increased DCs within solid tumor masses may correlate with an improved prognosis [146,147]. The intratumoral delivery of DCs can inhibit established breast tumor growth in a CD8⁺-dependent fashion and shows potential for a DC-based immunotherapy strategy [148].

In vivo transfection

An alternate approach to injecting transfected cells at the tumor site is the transfection of the gene directly into the tumor cells *in vivo*. This can be

accomplished through the use of viral vectors. The use of adenoviral vectors for gene therapy for cancer has been investigated for several tumors [149–151]. Based on the observation that tumor cells that were transduced ex vivo with retroviruses that expressed cytokines enhanced tumor immunotherapy [152,153], interest turned toward transducing tumor cells in vivo. This approach initiated the development of systemic antitumor immunity when vectors that contained the genes for IL-12 [154–156], GM-CSF [157], and IL-2 [158] were used. Intratumoral delivery of IL-2 by adenoviral infection resulted in the regression of local and distant sites in murine breast cancer models [159–161]. In addition, animals that showed a complete response developed protective immunity to rechallenge. These results led to a phase I clinical trial in patients who had metastatic breast cancer and melanoma [162]. At the site of injection, incomplete local tumor regression was seen in 24% of patients; postinjection biopsies demonstrated tumor necrosis and lymphocytic infiltration of CD8⁺ cells.

Biodegradable controlled-release polymers

Although the use of in vivo gene transfer and gene-modified cells have had some encouraging results, the current gene transfer technologies may lack the versatility that is required for clinical application [163,164]. The development of more clinically-feasible and less expensive alternative technologies for the delivery and sustained release of cytokines to the tumor microenvironment can enhance significantly the clinical implementation of cytokine-based breast cancer immunotherapies. Local and sustained in vivo delivery of therapeutic agents also can be achieved with biodegradable controlled-release polymers [165]. Biodegradable polymer microspheres have been used for in vivo drug delivery [166], vaccination with antigenic peptides [167], systemic protein delivery [168], and cancer immunotherapy [169,170]. Recent advances in encapsulation technologies led to the successful encapsulation of immunostimulatory cytokines [168]. These encapsulated cytokines stimulated antitumor responses using GM-CSF [169] and IL-1 α [170] in murine models.

Recently, a novel technology for highly-efficient encapsulation of biologically-active molecules into poly-lactic acid microspheres (PLAMs) was described. Phase inversion nano-encapsulation results in the efficient encapsulation of labile proteins without significant denaturation or losses of bioactivity. Human cytokine-loaded PLAMs release physiologically-relevant quantities of bioactive cytokines for extended periods of time in vivo. Intratumoral injections of IL-2-loaded microspheres provoked an NK cell-mediated suppression of human tumor xenografts in severe combined immunodeficiency (SCID) mice [171]. Treatment of tumor-bearing mice with a single intratumoral injection of PLAM that was loaded with recombinant IL-12 promoted complete regression of the primary tumor and prevented the metastatic spread to the lung [172]. This technique was superior to tumor vaccination, bolus injection of free IL-12, and other routes of administration.

This approach shows potential as a neoadjuvant immunotherapy in tumors where adjuvant postoperative vaccines may not be feasible (eg, breast cancer). In murine models, preoperative intratumoral injection of IL-12–loaded PLAM 1 week before surgical resection resulted in significantly reduced local recurrence and distant metastases [173]. Cytokine cocktails of IL-12 with GM-CSF or TNF- α further improved upon these results and resulted in reduced recurrence of primary tumors, eradicated established metastatic disease, and improving postoperative survival [174,175] This approach shows strong potential as neoadjuvant immunotherapy for breast cancer [175].

Obstacles to immunotherapy in breast cancer

Much of the emphasis in this avenue of research has centered on the creation of an immune response to breast cancer–specific antigens and success is determined by the presence of serum antibodies that recognize tumor antigens or cytolytic activity demonstrated in PBMC. This is only half of the story because a clinically-relevant immune response is two-tiered. It begins with the generation of antibodies or cytotoxic T cells that can recognize breast cancer–specific antigens. There is a second tier to that response—the ability of those antibodies or T cells to recognize cells that express those antigens and to kill the malignant cells.

Breast cancers are not passive bystanders to the immune response; they develop mechanisms to evade immune recognition. Breast cancers have been present in the body for a considerable time before being diagnosed and have reached this point despite ongoing immune surveillance. Humoral responses are limited by the fact that monoclonal antibodies have poor penetration into solid tumors which limits their effectiveness in stage IV disease. Cancers can escape immune recognition by way of single-antigen vaccines simply through antigenic modulation. Because a population of tumor cells develops resistance to a chemotherapeutic agent, the chemotherapy will not cure the cancer. Likewise, if a subpopulation of cancer cells no longer expresses the protein or ganglioside it is vaccinated against, it is no longer susceptible to that immunotherapy. Patients who have progressive disease after immunotherapy often exhibit “antigen loss.” In the case of T-cell–based therapies, tumor cells that lose the ability to bind antigen to MHC and express the MHC on the cell surface also would escape immune recognition. Many primary and metastatic breast cancers have lost this ability [176,177].

In breast cancer, the potential success of immunotherapy may be hampered by the prospect that the immune system might be facilitating tumor growth. A lymphocytic infiltrate was shown to be a poor prognostic finding [24]; immunosuppression does not increase the incidence of breast cancer and it may decrease it [22]. Nonspecific immunostimulants may worsen outcome [178], whereas immunosuppressive therapies (eg, corticosteroids) may give positive results [179]. How could the immune system, intuitively believed to be “on our side” in fighting cancer, betray us in this

manner? Tumor-infiltrating lymphocytes may be producing cytokines and other factors that are favorable to breast cancer cells. Several of these factors are listed in Table 3.

There are many approaches to breast cancer immunotherapy. It will not be possible to test every new approach in large randomized trials, especially considering the multiple adjuvant therapies that already have proven effective in breast cancer. Another area of research that has lagged behind the ability to stimulate an immune response is identifying more clinically-relevant surrogate markers for the generation of an immune response. The methods for *in vitro* monitoring of immune responses remain crude compared with the methods for stimulating those responses. The relevance of this is clear when one looks at the number of studies in breast cancer in which the immunotherapy was able to stimulate antigen-specific antibodies to T cells, but there was no evidence of a clinical response. It is imperative to design better methods for defining “success” in a phase II trial before we start accruing the thousands of patients that is necessary to demonstrate an improved survival in a phase III trial.

The evidence is overwhelming that it is possible to generate an immune response to breast cancer in some, if not all, patients and that early successes must be built upon aggressively. It is important that we focus that effort on: (1) the design of new methods of generating an immune response; (2) overcoming the immunosuppressive factors that prevent clinical success; and (3) improving monitoring strategies to allow us to identify effective therapies. We also must center our efforts toward patients who have breast cancer patients on strategies that can be integrated into a multi-modality approach. It is important to realize that methods that are costly and labor-intensive are going to be difficult to use in the adjuvant setting; this is where immunotherapy is most likely to have an impact. Additionally, strategies that are dependent upon autologous tumor are going to be limited severely in women who have breast cancer. The application of immunotherapy to the treatment of breast cancer faces several obstacles; however, the prospect of

Table 3
Factors produced by tumor-infiltrating lymphocytes that may favor breast cancer

Factor	Benefit to breast cancer	Reference
IL-6	Enhanced motility and increased cell-cell separation	[180]
IL-8	Increases invasiveness of breast cancer	[181,182]
IL-10	Suppresses cellular immune responses	[183]
CSF-1	Mediator of breast cancer invasion and metastases	[184]
HB-EGF	Stimulates breast cancer proliferation	[185]
bFGF	Stimulates proliferation, angiogenic	[185]
VEGF	Stimulates breast cancer proliferation	[186]
TNF- α	Stimulates proliferation, motility	[187,188]
Prolactin	Stimulates proliferation	[189]

Abbreviations: bFGF, basic fibroblast growth factor; HB-EGF, heparin-binding epidermal growth factor; VEGF, vascular endothelial growth factor.

engendering lifelong immunity to a patient's cancer with minimal toxicity is sufficiently realistic to justify the extensive research effort that is necessary to make this a reality.

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