Novel Immunologic and Biologic Therapies for Breast Cancer

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The treatment of breast cancer has benefited substantially from the introduction of trastuzumab in 1998. Yet trastuzumab only represents the first of a series of newer biologic therapies that will change the manner in which patients with breast cancer are treated. Initially, biologic therapies will be used in combination with existing chemotherapeutic agents. However, as biologic therapies improve, chemotherapeutic agents are likely to be replaced with biologic agents that are more effective, less toxic, and more patient- and tumor-specific. Promising classes of agents include monoclonal antibodies and cancer vaccines.

Introduction

The US Food and Drug Administration (FDA) approval of trastuzumab in September 1998 signaled a fundamental change in the way that oncologists approach patients with breast cancer. Although chemotherapy remains the predominant systemic therapy, treatments aimed at recruiting the immune system into the battle against cancer increasingly command the attention of scientists, physicians, and the public. Breast cancer is evolving into a disease in which traditional therapies such as surgery, radiation, and chemotherapy will be followed by or used in conjunction with biologic therapies that augment the immune response of patients to their own tumor. With further development, biologically directed or immune-based therapies may supplant traditional therapies as front-line treatment for breast cancer. Consequently, physicians will need a solid understanding of the role of the immune system in controlling cancer, and they will be asked to choose among an increasing number of novel therapies available to patients with breast cancer.

Antibody Therapy for Breast Cancer

Early studies made use of polyclonal, non-human antibodies harvested from the sera of animals immunized with the antigen of interest. As such, limited antibody availability, poorly defined antigenic targets, and rapid clearance of non-human antibodies from the circulation hampered the development of effective therapies. Issues surrounding antibody availability and poorly defined antigenic targets were solved with the discovery of monoclonal antibodies in 1975 by Kohler and Milstein [1]. These early monoclonal antibodies allowed for an endless supply of well-characterized antibodies. However, they were generated in non-human systems, and were thus viewed by the patient's immune system as a foreign, non-human protein. Consequently, patients often developed humoral immune responses (*ie*, antibodies) against the foreign immunoglobulin. The use of non-human monoclonal antibodies in humans is therefore generally limited to short-term or single-dose administration [2].

The development of chimeric or humanized monoclonal antibodies allowed patients to be treated repeatedly with the same monoclonal agent for extended periods of time [3]. With such antibodies, the likelihood of developing an immune response to repeated administration in humans is extremely low [4••]. Indeed, today, the major remaining obstacle to developing additional effective antibodies for the treatment of cancer is identification of appropriate antigen targets.

The bioengineering of therapeutic antibodies is not limited to the humanization of non-human monoclonal antibodies [5]. The same techniques that have been used to develop less immunogenic monoclonal antibodies have also been used to alter the basic structure of antibodies. Examples include antibody fragments, bispecific antibodies, and antibody conjugates with toxins, chemotherapeutic agents, or radiopharmaceuticals. Each of these antibody constructs retains most or all of the native ability of the antibody to bind to specific antigens with high affinity, and in addition has altered pharmacologic or immune properties better suited for a defined task. Examples of several are discussed in the following sections.

Trastuzumab

Trastuzumab was engineered from a mouse monoclonal antibody (4D5) directed against the HER2 transmembrane protein. Trastuzumab differs from the parent mouse monoclonal in that the majority of non-antigen binding sequences have been replaced with human sequences. The HER2 protein was chosen as a target for therapy based on its overexpression in approximately 25% of women with

breast cancer and its correlation with poor outcome in these patients [4••].

Trastuzumab received FDA approval for use in the United States in 1998 based on the results of two large trials. The first evaluated use of trastuzumab was as a single agent in 222 patients with recurrent or refractory metastatic breast cancer. This phase II trial demonstrated a 15% response rate (partial response [PR] + complete response [CR]) and a 9-month median duration of response. As expected, the incidence of human anti-mouse antibodies (HAMA) following use of this humanized monoclonal antibody was low, with only one of 211 patients (0.5%) demonstrating antibodies against trastuzumab. Unexpectedly, there was a high incidence of cardiac dysfunction, with 10 patents (4.7%) experiencing clinical congestive heart failure, cardiomyopathy, or a decrease in ejection fraction of greater than 10% [4••].

The second trial evaluated the use of trastuzumab in combination with chemotherapy in the setting of first-line metastatic disease. Patients with prior exposure to anthracyclines received paclitaxel at 175 mg/m^2 every 3 weeks. Patients without prior anthracycline exposure received doxorubicin (60 mg/m^2) and cyclophosphamide (600 mg/m^2) every 3 weeks. In addition, patients were randomized to either no additional therapy or combination therapy with trastuzumab (4 mg/kg loading followed by 2 mg/kg/wk). In the preliminary report of the study, the response rate to chemotherapy was significantly improved with the addition of trastuzumab [6]. This effect was most pronounced for those patients treated with paclitaxel, in whom the response rate increased from 15% with paclitaxel alone to 38% with paclitaxel and trastuzumab. Responses in patients receiving trastuzumab were also more durable. The median duration of response increased from 4 months in the group receiving paclitaxel alone to 8 months for the group receiving paclitaxel with trastuzumab [6].

Many questions remain concerning the optimal dose, schedule, and setting in which to use trastuzumab. The currently approved dose and schedule for trastuzumab was chosen based on the assumption that plasma levels of trastuzumab needed to mimic levels known to be active in vitro (10 μg/mL) [4••]. Consequently, plasma trough levels generally exceed 10 μg/mL shortly after the initiation of weekly therapy at 2 mg/kg. Nonetheless, it is possible that doses and schedules exist other than those currently approved by the FDA that are more effective or more convenient for the patient.

The use of trastuzumab in combination with paclitaxel was not part of the initial trial design testing trastuzumab in combination with chemotherapy. It was only after difficulty arose in accruing patients to a study employing doxorubicin and cyclophosphamide as therapy for first-line metastatic disease that a provision for treatment of patients with prior anthracycline was added (Shak S, Personal communication). It is therefore intriguing to ask what other chemotherapeutic agents might show significantly increased efficacy when administered with trastuzumab. Along those lines, Pegram *et al*. [7] evaluated trastuzumab in combination with cisplatin in a phase II study and demonstrated an overall response rate of 24%. In the preclinical setting, trastuzumab appears to augment the activity of most anticancer drugs (Slamon D, Personal communication). Selection of the best combination is complicated by the fact that many of these drugs are not considered optimal therapy for patients with metastatic breast cancer.

Trastuzumab cardiotoxicity remains an unfortunate and poorly explained attribute of this antibody. Preclinical data did not suggest that this toxicity would be seen in humans, and in fact, serial cardiac evaluations were only included in the initial registration trial as the standard of care for those patients receiving anthracycline. Although a number of theories have been suggested about the cause of trastuzumab cardiotoxicity, no single theory currently explains this troublesome phenomenon, and no effective means to abrogate this toxicity has been found [8••].

Perhaps the greatest challenge will be to define the role of trastuzumab in the adjuvant setting. Currently, a number of clinical trials are either planned or in progress for this indication. Of particular concern is the issue of combining trastuzumab with anthracycline. Although no trial in the adjuvant setting is evaluating the use of trastuzumab and anthracycline simultaneously, whether administration of these two agents sequentially will result in an acceptably safe regimen remains to be seen. Other investigators have chosen to evaluate non-anthracycline–based chemotherapy in combination with trastuzumab in the hope that the addition of trastuzumab will more than offset the absence of anthracycline. In either case, concern has been raised that long-term side effects may result from use of trastuzumab—particularly delayed or accelerated cardiac dysfunction that was not apparent from the initial registration trials.

Edrecolomab

Edrecolomab is a murine monoclonal antibody (17-1A) directed against the epithelial adhesion molecule EpCAM. EpCAM is widely expressed in epithelial tumors, which led to its evaluation in the adjuvant setting of colorectal cancer. Edrecolomab received approval for this indication in Germany in December 1994. This antibody may gain approval in the United States pending the results of a US trial conducted for the same indication. As with HER2, which is expressed in many different tumors of epithelial origin, 17-1A is found in both colon and breast tumors. For this reason, Braun *et al*. [9••] evaluated the ability of this antibody to clear the bone marrow of microscopic residual disease in patients with metastatic breast cancer. They report that a single dose of edrecolomab (500 mg, intravenously) resulted in a one-log reduction in detectable tumor cells in the marrow of 10 patients with breast cancer.

Microscopic residual disease in the marrow may be a more appropriate target for adjuvant therapies, and the ability to clear such microscopic disease may serve as a surrogate marker for the effectiveness of adjuvant therapies in the future [10]. Use of such a surrogate could speed the development of adjuvant therapies, which currently depend on the completion of large clinical trials requiring long follow-up [11•]. The investigators also point out that tumor cells are far from uniform in their expression of surface antigens, and for this reason, it is likely that future therapies will include cocktails of antibodies or will consist of therapies that are capable of attacking poly-antigen targets (*ie*, vaccination).

Bispecific antibody (MDX-H210) with granulocyte-colony stimulating factor

Native antibodies have two antigen-binding sites per molecule, both of which share the same binding specificity and affinity. However, it is possible to bioengineer antibodies with two different binding sites per molecule. The result is a "bispecific" antibody with the ability to cross-link structures containing the two antigens of interest. Pullarkat *et al*. [12] evaluated the utility of a bispecific antibody (MDX-H210) with affinity for both HER2 and the FcGamma receptor (CD64) in patients with breast cancer. The ability of MDX-H210 to bind to both HER2 and CD64 was anticipated to result in coupling of immune effector cells, specifically T cells, to breast cancer cells expressing HER2. Such coupling was expected to lead to immune activation and destruction of HER2 positive tumor cells. As with many biologic therapies, no maximum tolerated dose was reached in this phase I/II trial. Changes were observed in the immune parameters of patients (expression of IL-6, G-CSF, and TNF-α and recruitment of monocytes into the periphery), consistent with the purported mechanism of action of this compound, but no tumor responses were seen. Rather, dose escalation was halted at the highest available dose (40 mg/m^2) in the absence of significant toxicity. Whereas the lack of tumor responses in this patient population was disappointing, the ability to control the trafficking of specific immune cells should prove useful in designing therapies in the future.

Immuno-conjugates

Another permutation on the theme of using antibodies to treat cancer involves the development of antibody conjugates. Interestingly, with careful selection of the coupling site, most antibodies will tolerate linkage to large molecules with only minor changes in their binding affinity to antigen. Coupling a compound to an antibody was thus recognized early on as an ideal strategy for focusing the effect of a cytotoxic agent or radioisotope to areas of antigen distribution.

Coupling radioactive isotopes to antibodies offers the advantage of delivering high doses of radiation to areas of antigen concentration while sparing normal, non-antigen–bearing tissue. Wong *et al*. [13] evaluated an Yttrium-90–labeled antibody (T84.66) in seven patients with refractory breast cancer. T84.66 is a human/mouse chimeric antibody with specificity for carcinoembryonic antigen (CEA). Given the expectation that dose-limiting toxicity would be hematologic, patients received stem-cell support in order to allow further dose escalation. Evidence of an antitumor response was seen in two of seven patients, but no patient demonstrated a complete or partial response.

Antibodies can also be coupled to cytotoxic molecules in an attempt to increase the therapeutic index of the native cytotoxic. BMS-182248-1 is such a molecule and consists of doxorubicin linked to an antibody directed against the Lewis-Y antigen. Lewis-Y is expressed by many epithelial tumors, including the majority of breast cancers. Tolcher *et al*. [14] compared this antibody conjugate with standard doxorubicin in 23 women in a randomized phase II study. Unfortunately, responses were seen in only one of 14 patients receiving BMS-182248-1 and four of nine patients receiving standard doxorubicin. In addition, gastrointestinal toxicity in the group receiving BMS-182248-1 included significant gastritis, nausea, and vomiting and elevations in both amylase and lipase. The authors concluded that the profound gastric toxicity was likely related to binding of BMS-182248-1 to normal tissues expressing the Lewis-Y antigen. Further development of BMS-182248-1 was not recommended.

It bears repeating that the toxicity of any immunoconjugate or antibody is not always predictable. Trastuzumab demonstrated unexplained cardiotoxicity when it was first used in humans, and BMS-182248-1 demonstrated profound gastrointestinal toxicity, which was not commonly seen with either doxorubicin or the native antibody alone. Another example of unexpected toxicity comes from the experience of Pai-Scherf *et al*. [15], who evaluated a single-chain immunotoxin consisting of the antigen-binding portion of an anti-erbB2 antibody bound to a truncated portion of *Pseudomonas* exotoxin A. In their study, five patients with breast cancer treated with this immunotoxin developed hepatotoxicity believed to be due to the presence of erbB-2 on normal hepatocytes.

Use of Antibodies in the Future

Antibodies continue to offer the promise of less toxic therapy for patients with cancer. However, much work remains to be done. As new antibodies are introduced, each will require further evaluation to determine its optimal dose, schedule, and appropriate clinical setting. Initially, antibodies will be used in conjunction with currently accepted therapy such as radiation and chemotherapy. As more is learned about the immune system and cancer, and as we become better able to recruit the immune system to fight cancer, we will see immune therapies augment and possibly supplant chemotherapy and radiotherapy as the preferred method of treatment.

Vaccine Therapy

Vaccination has theoretic advantages over passive (antibody administration) immunization for the treatment of cancer. Although one could conceivably administer a cocktail of antibodies that would cover many different antigens present on a tumor cell, current antibody therapies (such as trastuzumab) are directed against specific monoclonal targets (such as HER2). Cancer cells lacking these targets or cells capable of reducing or eliminating these targets on their surface are therefore capable of surviving in the face of such monospecific antibody therapy.

Active immunization (*ie*, following successful vaccination) results in a more robust immune response than is possible following antibody administration. Generally, an antibody response following immunization is not limited to a single monospecific antibody. Rather, a polyclonal response is elicited, resulting in generation of antibodies that may cover a wide range of cellular targets. Active immunization may also generate a cellular immune response in addition to a humoral or antibody response. Such cellular responses can be important in eliciting an immune reaction against intracellular targets.

Simple administration of tumor antigens to a patient is unlikely to elicit a meaningful immune response. Rather, most vaccination strategies include the use of an adjuvant in the vaccine preparation such as granulocyte-macrophage stimulating factor (GM-CSF) or keyhole-limpet hemocyanin (KLH). Several vaccines also make use of a priming dose of cyclophosphamide, which has been demonstrated to increase antibody titers following vaccination in humans. Other strategies include alteration of the native antigen in the hope that slight changes in the tertiary structure or sequence will render the vaccine more likely to be viewed as foreign by the immune system. Interestingly, small peptides derived from larger proteins are often capable of eliciting an immune response where the intact protein is not. This is presumably due to the differences in folding and orientation between a native protein and a small fragment of that protein presented to the immune system "out of context." Such "peptide" vaccines are a promising approach to cancer vaccination.

Sandmaier *et al*. [16] reported on the use of such a vaccine against carbohydrate antigens in patients following cytoreduction with a stem-cell transplant. Concern has been raised about the ability of such patients to mount a meaningful immune response following transplantation. These authors demonstrate that the majority of such patients can mount an immune response to the specific carbohydrate used in the vaccine, sialyl-Tn. Seventeen of 27 patients receiving at least three vaccinations demonstrated evidence of a T cell–based response that was specific for sialyl-Tn. Furthermore, in those patients with elevated tumor markers at the time of vaccination, five of seven demonstrated decreasing markers over time. This promising vaccination strategy is currently being evaluated in a phase III trial in patients with metastatic breast cancer following cytoreductive chemotherapy.

Disis *et al*. [17••] evaluated a peptide-based vaccine using GM-CSF as an adjuvant treatment in patients with breast and ovarian cancer. Peptide segments were chosen from the larger HER2 protein using a computer program that selected peptide fragments likely to elicit a cellular immune response. Peptide sequences were chosen from both the intracellular and extracellular domain of the HER2 protein in anticipation that intracellular peptides might be more likely to be immunogenic. All patients developed T-cell responses to the peptides administered in the vaccine, and most patients also developed reactions against other peptides in HER2 that were not included in their vaccine. The induction of an immune response to portions of a protein not included in a vaccine is referred to as epitope spreading and is considered to be important when eliciting a cellular immune response to a tumor. Tumor cells have more difficulty escaping an immune reaction directed at multiple targets in the cell as opposed to an immune reaction focused on a single epitope or antigen. Interestingly, the extracellular sequences were as good at eliciting an immune response as were the intracellular sequences.

Vaccines require time to be effective and for that reason are unlikely to benefit patients with advanced cancer. Consequently, most vaccine strategies represent an attempt to treat patients in a situation of minimal residual disease such as that seen following successful treatment with chemotherapy, radiotherapy, or surgery. Given the myriad of tumor types and the uncertainty of developing tumors even in high-risk individuals, it is unlikely that cancer vaccines will be used in patients who are only "at risk" for tumor development.

Conclusions

Cancer therapy has progressed dramatically in the past decade and will continue to improve in the future. Cancer therapies will be less toxic, more patient specific, and almost certainly designed to recruit the patients' immune system into the battle against their tumor. The development of these new therapies will require a change in the manner in which we conduct clinical trials. We will no longer be able to make use of a drug's toxicity to guide its dosing; rather, we will be required to develop new endpoints such as the clearance of tumor cells from bone marrow, or the resolution of specific genetic markers from the blood. Similarly, response rates may be less important in decisions regarding whether a drug warrants further development.

The development of new biologic therapies will be greatly facilitated in those situations where the mechanism of action is clearly defined (as was the case with trastuzumab). Such an understanding allows for the targeting of patient populations with the highest likelihood of benefit.

In addition, new biologic therapies are likely to be used and developed in combination with existing therapies, particularly chemotherapy. Given the favorable toxicity profiles of newer biologic therapies, such combinations will be practical. The identification of effective combinations will provide insight into the mechanism of action of both the biologic therapy and existing chemotherapy. As with most drugs currently in clinical practice, the optimal combinations and schedules will be determined empirically in the setting of clinical trials performed long after drug approval by the FDA.

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An excellent review of the issues surrounding trastuzumab cardiotoxicity.

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An interesting use of edrecolomab as treatment for microscopic residual disease in the bone marrow is reported. This may represent a new method of evaluating adjuvant therapies in the future.

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A good example of peptide-based vaccines is presented, along with a helpful discussion of the issues surrounding vaccine development.