REVIEW ARTICLE

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Biological Therapy of Breast Cancer

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Abstract

Breast cancer treatment has now entered a new era in which biological therapies, based on a rapidly expanding cellular and molecular understanding of breast cancer pathogenesis, have joined the standard armamentarium of surgery, radiation, chemotherapy, and hormone therapy. In 1998, the anti-HER2 humanised monoclonal antibody trastuzumab became the first biological therapy to receive US Food and Drug Administration (FDA) approval for the treatment of breast cancer, thus marking a milestone that almost certainly will be repeated with other new agents. HER2 (ErbB2) has been the focus of many therapeutic strategies because of its frequent gene amplification and overexpression in breast cancer, its role in

tumourigenesis and cancer progression, and its prognostic and predictive significance in clinical studies.

In preclinical studies, trastuzumab showed antiproliferative activity against *HER2*-overexpressing breast cancers *in vitro* and in tumour xenograft models. In a phase II clinical trial of 222 stage IV patients, trastuzumab was associated with an objective response rate of 15%. A randomised phase III clinical trial demonstrated that first-line chemotherapy for stage IV patients in combination with trastuzumab was significantly superior to chemotherapy alone. Chemotherapy plus trastuzumab was associated with a median time to progression of 7.2 months, versus 4.5 months for chemotherapy alone ($p < 0.001$), and a response rate of 45% versus 29% for chemotherapy alone ($p = 0.001$).

Other novel therapies involving antibody targeting of HER2 are under development, including bispecific antibodies, immunotoxins, and immunoliposomes. Vaccine approaches are also under active investigation, including those directed against HER2 and mucin antigens. Gene therapy strategies under development include gene transfer of immunomodulatory genes and of anti-oncogene constructs. Other biological therapies include agents designed to induce differentiation or inhibit invasion, angiogenesis and metastasis.

For many years, the only effective treatment for breast cancer was mastectomy, epitomised by the development of the Halsted procedure in 1894. The subsequent introduction of radiation therapy and the advent of improved surgical techniques, including breast conserving approaches, have greatly improved local-regional management of breast cancer. In a parallel development, systemic treatment of breast cancer has also undergone tremendous progress, and now includes an array of active cytotoxic agents for chemotherapy and hormone-directed agents for endocrine therapy. However, further progress is clearly required, as the treatment of breast cancer remains incomplete or ineffective for many patients, and treatment-related toxicities are considerable.

Breast cancer treatment now appears on the threshold of another major revolution, in which these established modalities can be complemented by novel biological therapies based on a rapidly expanding cellular and molecular understanding of breast cancer pathogenesis. In 1998, the anti-HER2 monoclonal antibody trastuzumab became the first biological therapy to receive US Food and Drug Administration (FDA) approval for the treatment of breast cancer, thus marking a milestone that almost certainly will be repeated with other new agents. These new biotherapeutic strategies include other monoclonal antibody (MAb)-based agents, vaccine-based therapies, prodifferentiation agents, antimetastatic agents, antiangiogenesis agents and gene therapies.

This review discusses representative examples of this new wave of biological therapies for breast cancer, with a focus on agents that have been recently approved or are currently in or near clinical trials.

1. Monoclonal Antibody-Based Therapies

1.1 Anti-HER2 Monoclonal Antibody-Based Therapies

1.1.1 HER2 as a Target for Immunotherapy

The rodent homologue of *HER2*, the *neu* oncogene product, was first identified in neuroblastomas that were generated by *in utero* treatment of rats with ethylnitrosourea.[1] Identification of the transforming gene, designated *neu* because of its association with neuroblastoma, revealed it to encode a 185kD membrane-bound glycoprotein closely related to the epidermal growth factor receptor (EGFR).[2] Subsequent studies demonstrated that this original sequence was a mutant allele that differed

from the wild type at a single position within the transmembrane domain, and which produced constitutive tyrosine kinase activity.[3] The human gene was identified by screening human genomic and cDNA libraries for receptors related to EGFR; using this strategy, several groups simultaneously isolated the human homologue of *neu*.[4-6] This gene was designated *c-erbB-2* (*ErbB2*) or *HER2* to reflect its relation to *EGFR* (*c-erbB-1*, *HER1*), and has also been referred to as *neu* or *HER2*/*neu*.

In contrast to the wild-type rat *neu* proto-oncogene, from which an activated tyrosine kinase can arise via a single mutation, analogous mutations of the *HER2* gene have not been observed in human cancer.[7] However, the *HER2* gene was observed to be amplified in certain human cancer cell lines,[4,5,8] suggesting that overexpression of wild-type HER2 might be an alternative oncogenic mechanism. The direct role of HER2 in breast tumourigenesis has since been demonstrated in multiple laboratory studies. For example, HER2 functions as a classical oncogene that transforms cells *in vitro* and confers tumourigenicity *in vivo*.[9,10] Transgenic mice overexpressing the mutant or wild-type rat *neu* gene or the mutant or wild-type human *HER2* gene developed various cancers, including mammary cancer.[11-15] Finally, certain MAbs directed against HER2 or rodent Neu are able to inhibit cancer cell growth *in vitro* and/or *in vivo*.

HER2 amplification was first shown to be clinically relevant in breast cancer by Slamon and coworkers,[16] who observed *HER2* amplification in approximately 25% of primary breast cancers from axillary lymph node–positive patients, which correlated with poor prognosis. A subsequent study demonstrated that HER2 overexpression detected at the RNA and protein levels was similarly prognostic for poor outcome.[17] It has since been clearly confirmed that HER2 overexpression is associated with poor prognosis in node-positive and probably also node-negative patients.[18] In addition to its prognostic significance, HER2 overexpression has also been found to have predictive significance with respect to specific therapies. In experimental models, HER2 overexpression has induced resistance to tamoxifen therapy.[19,20] In clinical studies involving adjuvant chemotherapy of early breast cancer, HER2 overexpression correlated with increased benefit with anthracycline chemotherapy,[21-23] and has also been correlated with resistance to other chemotherapies.[24]

Among the earliest breast cancer-associated antigens to be targeted by MAb therapy, HER2 has since become a paradigm for immunotherapy of solid tumours in general. Indeed, HER2 provides an attractive target for MAb-based therapy: it is accessible as a cell surface receptor, and when overexpressed is present at up to 10^6 receptors/cancer cell, or 100-fold higher than in normal cells. In normal tissues, its expression is detected only in certain predominantly epithelial cell types.[25] As an oncogene, its continued expression appears to remain important throughout malignant progression, including metastasis.[26]

1.1.2 Trastuzumab

As mentioned, certain MAbs against HER2 can inhibit cancer cell growth.[27-31] Murine MAb 4D5 displays cytostatic antiproliferative activity against breast cancer cells overexpressing HER2 in both *in vitro*[32] and *in vivo* models.[33,34] A recombinant humanised version of 4D5 (trastuzumab) retains these binding and biological activities, but contains consensus sequences of human IgG_1 in place of the parental murine MAb sequences.[35] This minimises immunogenicity while enhancing the potential to recruit human immune effector cells via antibody-dependent cellular cytotoxicity, although this has not been clearly demonstrated in patients. The mechanism(s) of the growth inhibitory activity of trastuzumab are not precisely known. Whereas the physiological activator heregulin causes activation of the HER2 receptor by heterodimerisation with related receptors EGFR, HER3, and HER4, trastuzumab appears to induce altered receptor interactions, and possibly generates a different cellular signal.[36]

Phase I studies of trastuzumab showed that this therapy was generally well tolerated, was not associated with significant antibody induction (human antihuman antibodies or HAHA), and could be

combined with cisplatin chemotherapy. Phase II trials showed that trastuzumab has anticancer activity as a single agent[37] as well as in combination with cisplatin in patients with advanced and refractory metastatic breast cancers that overexpress HER2.[38] Based on these early results, 2 expanded multicentre studies were initiated to assess the efficacy and safety of trastuzumab.[39,40] Results of these phase II trials are summarised in table I. In a phase II clinical trial of 222 stage IV patients, trastuzumab was associated with an objective response rate of 14% (table I).

In these studies, the likelihood of responding to trastuzumab appeared to be higher in patients who had not previously received chemotherapy for metastatic breast cancer. Similar tumour response rates were seen in patients with visceral metastases and in patients who had previously undergone high dose chemotherapy with stem cell or bone marrow transplant. In the study that included patients previously untreated for metastatic disease, no difference in response rates was seen between the 2 trastuzumab doses. Toxicities included an infusion reaction consisting of self-limited fevers and chills, mostly after the first infusion only, mild upper airway congestion, and diarrhoea. Cardiac dysfunction, defined as symptoms of congestive cardiomyopathy or subclinical declines in cardiac ejection fraction, was seen in 3 to 5% of patients. The mechanisms of cardiotoxicity remain unclear, since myocytes do not express HER2 at appreciable levels, although the HER2 pathway may be important in prenatal cardiac development and possibly in myocardial remodelling.

A phase III study was performed to directly compare chemotherapy plus trastuzumab versus chemotherapy alone in patients with HER2-overexpressing breast cancers as first-line therapy for metastatic (stage IV) breast cancer.[41] Chemotherapy plus trastuzumab was associated with a median time to progression of 7.2 months, versus 4.5 months for chemotherapy alone ($p < 0.001$), and a response rate of 45% versus 29% for chemotherapy alone ($p = 0.001$) [table II]. Chemotherapy was given for 6 cycles or longer (at the investigator's discretion) in conjunction with weekly trastuzumab. Trastuzumab therapy was given until disease progression, which was the primary end-point of the study.

Patients who had previously received an anthracycline-based chemotherapy regimen in the adjuvant setting received paclitaxel 175 mg/m^2; the others received doxorubicin 60 mg/m^2 (or epirubicin 75 mg/m^2) plus cyclophosphamide 600 mg/m^2 (AC), with all chemotherapy given every 3 weeks. Improvements in time to disease progression, response rate and 1-year survival were seen with the addition of trastuzumab to chemotherapy, particularly in the paclitaxel stratum (table II). Updated information continues to demonstrate an improvement in survival due to trastuzumab therapy, with a median survival of 20.3 months with chemotherapy alone versus 25.4 months with chemotherapy plus trastuzumab ($p = 0.025$).[42] This result is particularly noteworthy, since not all patients responded, and also since 65% of patients progressing on chemotherapy alone crossed over to receive trastuzumab following progression, as allowed by protocol.

Table I. Phase II studies of trastuzumab alone or with chemotherapy

Therapy	No. of patients	Prior chemotherapy for advanced disease	Response rate (%)	Median response duration (mo)	Median time to disease progression (mo)	Reference
Trastuzumab	46	Any	12	6.6	5.1	37
Trastuzumab plus cisplatin	39	1 or 2 prior regimens	24	5.3	Not reported	38
Trastuzumab[a]	222	1 or 2 prior regimens	15	9.1	3.0	39
Trastuzumab[b]	113	None	26	9 (estim)	3.5	40

a Trastuzumab was given as a loading dose of 4 mg/kg followed by 2 mg/kg intravenously every week.

b Patients were randomised to 4 mg/kg followed by 2 mg/kg intravenously every week *vs* 8 mg/kg followed by 4 mg/kg every week.

Table II. Phase III randomised trial of chemotherapy versus chemotherapy plus trastuzumab[41]

Treatment	No. of patients	Median time to disease progression (mo) [p value]	1-year survival (%) [p value]	Response rate (%) [p value]	Median response duration (mo) [p value]
Chemotherapy	234	4.5 [<0.001]	68 [0.01]	29 [0.001]	5.8 [0.0001]
Chemotherapy + trastuzumab	235	7.2	79	45	8.3
AC	138	5.7 [0.001]	73 [0.04]	38 [0.1]	6.4 [0.0025]
AC + trastuzumab	143	7.6	83	50	8.4
Paclitaxel	96	2.5 [0.0001]	61 [0.08]	15 [0.001]	4.3 [0.0001]
Paclitaxel + trastuzumab	92	6.7	73	38	8.3

AC = anthracycline (doxorubicin or epirubicin) plus cyclophosphamide.

Toxicities in the phase III trial attributable to trastuzumab were generally similar to those seen in the single agent studies. Cardiotoxicity was higher in the trastuzumab group, especially in the substratum of patients receiving AC chemotherapy.[43]

FDA-approved indications for the use of trastuzumab currently include treatment of patients with advanced metastatic breast cancer whose tumours overexpress HER2. For those who have not received chemotherapy for advanced disease, trastuzumab is indicated in combination with paclitaxel. For previously treated patients, trastuzumab alone is indicated. Patients should receive a loading dose of 4 mg/kg intravenously over 90 minutes, and premedication with paracetamol (acetaminophen) and diphenhydramine may lessen the potential for infusion reactions. Subsequent doses of 2 mg/kg are given weekly; the drug can be administered over 30 minutes if there are no infusion-related symptoms. Baseline evaluation of cardiac function and extreme caution in patients with cardiac problems are recommended.

The FDA has recently approved an immunohistochemical kit to determine candidates for trastuzumab therapy (HercepTest™ **1**). The performance of this kit is somewhat concordant with the method used in the trastuzumab clinical trials, but there is more discordance in the intermediate (1-2+) expression level. A gene-based assay for *HER2* amplification, fluorescence *in situ* hybridisation (FISH), has also been approved to stratify risk and aid in the choice of adjuvant chemotherapy for patients with early stage breast cancer. Since the likelihood of response may depend on the actual level of overexpression, further studies will be required to clarify the optimal method and cutoffs for choosing patients most likely to benefit from trastuzumab. As with other biological therapies, many mechanisms for resistance to therapy are likely to exist or develop with time. Furthermore, many HER2-overexpressing tumours may use alternative oncogenic pathways that are not modulated by trastuzumab.

Trials are ongoing or planned to study the efficacy of trastuzumab with other chemotherapeutic agents, such as docetaxel, carboplatin, vinorelbine, gemcitabine and capecitabine. Preliminary results from a phase II trial using weekly paclitaxel at 90 mg/m^2 with weekly trastuzumab showed a response rate of 62% in patients with HER2-overexpressing tumours, compared with 44% in patients with non-overexpressing tumours who were also enrolled.[44] Likewise, early results of a study using vinorelbine plus trastuzumab as first-line therapy for metastatic breast cancer show a response rate of 71%.[45] Combinations with hormonal therapies will also be studied; as mentioned, HER2-overexpression may mediate resistance to tamoxifen, and this potentially may be reversible with trastuzumab. The use of trastuzumab as part of neoadjuvant therapy for locally advanced breast cancer and adjuvant therapy for early stage breast cancer will be tested in large cooperative group trials.

1.1.3 Anti-HER2 Bispecific Antibodies

Bispecific antibodies (BsAbs) are hybrid constructs in which two MAb fragments recognising distinct antigenic targets are linked together. This

1 Use of a trade names is for product identification purposes only, and does not imply endorsement.

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