Targeting HER2 as a therapeutic strategy for breast cancer: a paradigmatic shift of drug development in oncology

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Targeted therapies are causing a dramatic change in cancer drug development. Trastuzumab, a humanised recombinant monoclonal antibody that recognizes the extracellular domain of HER2 trans-membrane protein, is among the first target-specific drugs that have been licensed for clinical use and its development represents a model of integration of new agents with classical treatment strategies. In preclinical models, trastuzumab has demonstrated a marked antiproliferative effect and a synergistic action with several chemotherapeutic agents. Monotherapy trials indicate that trastuzumab is active as a single agent in HER2 positive patients, is well tolerated, and is associated with preservation of quality of life (QoL). Furthermore, as first line therapy for metastatic breast cancer overexpressing HER2 receptor, the addition of trastuzumab to taxane-based chemotherapy significantly increased rate of objective response, time to disease progression and survival when compared with chemotherapy alone. Trastuzumab has shown important activity when used with many chemotherapeutic agents such as platinum salts, gemcitabine, vinorelbine and capecitabine and liposomal anthracyclines. Various trials are now ongoing to optimize the use of trastuzumab and to investigate its role in the adjuvant and in the neo-adjuvant setting.

Key words: HER2, breast cancer, targeted therapy, trastuzumab

Introduction

Current treatment strategies have impacted significantly on survival in patients with breast cancer. The use of chemotherapeutic agents confers about a 30% improvement in survival in the adjuvant treatment of early stage breast cancer. This improvement is mostly the result of the widespread use of anthracyclines. Taxanes and dose-dense chemotherapy, where drugs are given at shorter intervals, were lately shown to improve adjuvant treatment benefits further.

Recently, the design of drugs directed to specific molecular targets is dramatically changing and, possibly, accelerating the therapeutic development for this disease. Breast cancer has been an ideal model for targeted therapy. The observation that hormonal deprivation causes regressions of primary and metastatic breast cancers (MBC) led to the development of agents that block hormone receptors. Furthermore, in the late 1980s the discovery of HER2/neu directed researchers to investigate treatments targeting this receptor.

HER2 is a protein that belongs to the Epidermal Growth Factor Receptor (EGFR) family, a group of trans-membrane receptors with intracellular tyrosine–kinase activity and extracellular binding domain. HER2-receptor does not appear to have a specific ligand [1], but it can signal by forming heterodimers with other members of the EGFR family that are bound to a ligand. The amplification of the gene yields overexpression of the cell membrane receptor protein, thus causing homodimerization and activation of the downstream signal transduction pathway. The end results of HER2 activation are: effects on cell growth, division, differentiation, migration, and adhesion. HER2 is overexpressed in 20–30% of invasive breast cancer, leading to an inferior prognosis [2] and to a relative resistance to some cytotoxic and hormonal therapy [3–6]. Because of this, HER2 receptor has been considered as an important therapeutic target, so that drugs to limit the abnormal function of over-expressed HER2 receptors have been developed. Trastuzumab (Herceptin®; Genentech, Inc, South San Francisco, CA), a humanized monoclonal antibody that recognizes the extracellular domain of HER2, has been the first of such drugs to gain the clinical setting: its availability impacts deeply on the treatment and clinical research of breast cancer.
In this paper, we will review the pre-clinical and clinical development of trastuzumab as a model of the evolving scenario in the cancer therapeutics research.

Preclinical studies
Several studies have been conducted to test the antiproliferative activity of trastuzumab in vitro and in animal models.

Studies conducted in vitro showed that trastuzumab effectively prevented cell proliferation of the breast cancer cell line SK-BR-3, which overexpressed the HER2 receptor protein. Anti-HER2 antibodies have also proved to enhance the antiproliferative activity of conventional chemotherapeutic agents [7]. In these in vitro experiments synergistic and additive interactions were observed. Of particular interest for the potential therapeutic implications, trastuzumab significantly enhances the antiproliferative activity of anti-estrogens on human breast cancer cell lines which over-express HER2 [8].

The potential antitumor activity of trastuzumab has also been confirmed in vivo. Trastuzumab was able to determine a strong dose-dependent growth suppression of HER2-over-expressing BT-474 breast cancer xenografts in athymic mice with eradication of tumors in a significant proportion of animals [9]. Furthermore, trastuzumab, in combination with either methotrexate, etoposide, cyclophosphamide, doxorubicin, vinblastine and paclitaxel significantly reduced tumor volume when administered to athymic mice bearing HER2-transfected MCF7 human breast cancer xenografts compared with single-agent therapy [10, 11].

Clinical Studies
Trastuzumab as a single agent
After pharmacokinetics studies conducted in a small number of patients with metastatic breast cancer (MBC), the activity and safety of trastuzumab were evaluated in two phase II trials of patients who had been already exposed to one or more chemotherapy regimens [12, 13]. The objective response rate (ORR) ranged between 11.6% and 15% in unselected patients. Retrospective FISH testing of patients’ tissues from the pivotal phase II trial showed a 19% response in FISH positive patients and a 0% ORR in FISH negative. Clinical benefit for IHC 3+ patients was similar to that of FISH positive patients [14].

In a randomized phase II trial of first line treatment [15], patients (n = 114) were treated with a weekly high-dose 8 mg/kg initially followed by 4 mg/kg weekly or with the standard dose 4 mg/kg initially followed by 2 mg/kg weekly. However, the study was not designed to carry out a formal comparison between the two dose levels. HER2 level of overexpression was defined as 3+ in 76% of patients. In the group receiving a weekly dose of 2 mg/kg the overall response was 24%; 3 patients experienced a complete response and 11 had a partial response. For patients receiving 4 mg/kg/week the overall response rate was 28% with 4 complete and 11 partial responses. The median duration of survival for all enrolled patients was similar for the standard and high dose trastuzumab regimens (22.9 vs 25.8 months, respectively).

Overall clinical studies have shown that trastuzumab administered as a single agent is well tolerated and is associated with a low incidence of side effects. In particular, trastuzumab is not associated with the toxic effects typically produced by chemotherapy such as nausea, vomiting, hair loss and bone marrow toxicity [12–15].

Trastuzumab in combination with chemotherapy
The favorable safety profile exhibited by trastuzumab as a single agent and the preclinical evidence of synergistic/additive effect with many cytotoxic drugs has prompted investigators to evaluate the addition of trastuzumab to chemotherapy regimens.

In a pivotal randomised phase III trial (H0648 g) [16], 469 women were enrolled to receive chemotherapy alone or chemotherapy plus trastuzumab as first line chemotherapy for MBC with a moderate or more than moderate HER2-over-expression, determined by IHC (score of 2+ or 3+). Chemotherapy consisted of an antracycline (intravenous doxorubicin 60 mg/m² or epirubicin 75 mg/m²) plus cyclophosphamide 600 mg/m² every 3 weeks for patients who had not received antracycline-based regimen for adjuvant setting, with or without trastuzumab; while patients who had received antracycline in adjuvant setting, received three-weekly paclitaxel at a dose of 175 mg/m² with or without trastuzumab. Trastuzumab was administered in the first week at a loading dose of 4 mg/m², then weekly at a dose of 2 mg/m². Patients receiving chemotherapy in combination with trastuzumab, showed a longer time to disease progression (median, 7.4 vs. 4.6 months; P <0.001), a higher rate of objective response (50% vs. 32%, P <0.001), a longer duration of response (median, 9.1 vs. 6.1 months; P <0.001) and a longer survival (median survival, 25.1 vs. 20.3 months; P = 0.046). Trastuzumab administration also correlated to an improvement of health-related quality of life (QoL) [17]. Trastuzumab given in combination with chemotherapy was associated with an increase of incidence of anemia, leukopenia, diarrhea and infections, but these events were not severe and in general were fairly manageable [16]. Cardiac toxicity was the most important side-effect involving a decrease in left ventricular ejection fraction at rest or congestive heart failure. Cardiotoxicity was more frequent in patients with pre-existing cardiac disease and, particularly, in patients receiving concomitant doxorubicin the incidence of marked symptomatic dysfunction (New York Heart Association (NYHA) grade III-IV) was as high as 16% [16]. Based on the results of this pivotal phase-III trial, trastuzumab has been approved for use in HER2 overexpressing metastatic breast cancer in combination with paclitaxel, while the combination of trastuzumab and anthracycline-containing regimens has been deemed inappropriate because of excessive cardiotoxicity.

In a randomised phase II trial (M77001) 188 untreated MBC patients received docetaxel at a dose of 100 mg/m²
every 3 weeks for at least 6 cycles with or without trastuzumab administered in 2 mg/m² weekly doses until disease progression. Overall survival was 30.5 months in the combination arm, significantly better than 22.1 months of docetaxel monotherapy \( (P = 0.0062) \); TTP was 10.6 months in the docetaxel–trastuzumab group versus 5.7 months in the docetaxel arm \( (P = 0.0001) \); ORR was 61% in the combination arm, 34% for docetaxel alone \( (P = 0.0002) \) [18]. Based on this trial, the European Medicine Agency (EMEA) approved the combination of trastuzumab and docetaxel as first-line therapy in HER2-positive metastatic breast cancer. Trastuzumab–taxanes combinations are further supported by the results of phase II trials (Table 1).

Lately, in a randomized phase III trial a triple combination of trastuzumab, paclitaxel and carboplatin has shown to improve ORR \( (P = 0.04) \) and time to progression \( (P = 0.02) \) with trend to improved survival \( (P = 0.27) \) as compared with trastuzumab plus paclitaxel [19].

Furthermore, based on preclinical synergistic/additive interaction between trastuzumab and various cytotoxic agents, several other combinations have been investigated in phase II setting (Table 1). Interestingly, these combinations exhibit promising activity, favorable toxicity profiles and seem free of important cardiotoxicity.

## Current investigations with trastuzumab

### Trastuzumab in combination with anthracyclines

Several studies are examining the cardiac safety of trastuzumab combined with anthracyclines less cardiotoxic than doxorubicin, such as epirubicin or liposomal formulations of doxorubicin.

<table>
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<tr>
<th>Table 1. Response rate of the principal combinations of trastuzumab with various chemotherapeutic agents</th>
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<td><strong>Trastuzumab + Taxanes:</strong></td>
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<td>Meden et al. [33]</td>
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<td>Montemurro et al. [38]</td>
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<td><strong>Trastuzumab + Platinum salts:</strong></td>
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<td>Pegram et al. [40]</td>
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<td>Nabholtz et al. 1 [41]</td>
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<td>Slamon et al. 1 [42]</td>
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<td><strong>Trastuzumab + Capecitabine:</strong></td>
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<td>Bangemann et al. [43]</td>
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<td><strong>Trastuzumab + Vinorelbine:</strong></td>
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<td>Yardley et al. [49]</td>
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<td><strong>Trastuzumab + Gemcitabine:</strong></td>
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<td>O’Shaughnessy et al. [50]</td>
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<td>Polyzos et al. [51]</td>
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OR = Objective response; CR = Complete response; PR = Partial response; SD = Stable disease.

T = trastuzumab was administered as a 4 mg/kg initial dose followed by 2 mg/kg.

D = docetaxel; P = paclitaxel; CDDP = cisplatin; C = capecitabine; V = vinorelbine; G = gemcitabine; CBDCA = carboplatin.
A multicentre phase I–II, comparative trial (M77003; HER-CULES) is under way to compare the cardiac safety of trastuzumab plus epirubicin and cyclophosphamide (EC) versus EC alone as first-line therapy for MBC. A preliminary analysis did not show an excess of cardiac events in the trastuzumab-containing arm. The trial continues patient accrual to further investigate the efficacy and safety of trastuzumab plus EC [20].

Liposomal formulations of doxorubicin show similar efficacy to doxorubicin but with fewer cardiac events. The liposomal doxorubicin, TLC D-99 (Myocet®) has been combined with trastuzumab [21], or with paclitaxel and trastuzumab [22]. Both regimens have shown interesting ORR (59% and 93%, respectively) along with an acceptable rate of cardiac events.

A pegylated liposomal formulation of doxorubicin, Caelyx®, has been examined in combination with trastuzumab [23] and produced an ORR of 52%. No symptomatic heart failure was reported; three patients (10%) experienced a protocol-defined cardiac event (all decreases in LVEF of >15%). The triple combination of trastuzumab with docetaxel and pegylated liposomal doxorubicin has also been evaluated [24]. There were no cases of CHF and all LVEF changes were asymptomatic.

**Trastuzumab in combination with endocrine therapy**

HER2 positivity predicts a relative resistance to endocrine therapy, particularly tamoxifen. [3, 5, 6]. There is evidence that this is caused by ‘crosstalk’ between the HER2 and the estrogen receptor (ER) intracellular signaling pathways [25], and that HER2 signaling stimulates the estrogenic, proliferative (agonist) effect of tamoxifen. In ER-positive breast cancer, this results in hormone-independent proliferation, which is insensitive to, and may be enhanced by, tamoxifen. Trastuzumab has been shown to reverse the agonistic effect of tamoxifen in HER2-positive cell lines [5]. Aromatase inhibitors such as letrozole have a different mechanism of action from tamoxifen and preliminary evidence suggests that their therapeutic action may be less affected by HER2-overexpression [26]. In view of this randomized clinical trials are now investigating the combination of trastuzumab with aromatase inhibitors.

A study of trastuzumab combined with letrozole (2.5 mg daily) in 26 patients with IHC 2+/3+ or FISH+ MBC has recently been reported [27] in a preliminary fashion showing encouraging activity (ORR 27% and clinical benefit rate 64%) and safety (1 G3-4 cardiac toxicity). A large (n = 300), international, randomized first-line trial of trastuzumab plus letrozole (MO16722; eLecTRA) with tighter HER2 inclusion criteria (IHC 3+ or FISH+) is ongoing. A similar trial (BO16216) of anastrozole (1 mg daily) with and without trastuzumab has completed recruitment (n = 202). Results of this trial are expected in early 2005. Also, a multicentre phase II trial of 3-weekly trastuzumab plus daily exemestane in post-menopausal patients with metastatic or locally advanced breast cancer has begun patient enrolment.

**Trastuzumab beyond disease progression**

Preclinical studies have shown a synergistic effect of trastuzumab with many chemotherapeutic agents and it has been hypothesized that it may act by interfering with the mechanism by which the tumor cell attempts to repair the chemotherapy-induced damage. In view of this, trastuzumab administration could theoretically be continued despite disease progression to take advantage from a potential interaction with second-line chemotherapy. However, there are no data that confirm this hypothesis. Nonetheless, there are enough data on the safety of continuing trastuzumab administration beyond progression. Patients in the pivotal phase III trial H0648g were given the opportunity to receive trastuzumab in a companion treatment-extension trial (H0659g) at the time of disease progression [28]. Extended trastuzumab administration up to 40 months was well tolerated in this study and no cumulative toxicities, including cardiac toxicity, emerged over this time frame. Furthermore, two retrospective studies have reported encouraging ORR of subsequent lines of trastuzumab-chemotherapy combinations [29, 30].

In all the above mentioned trials, however, it is not possible to discern whether the observed benefits are derived from chemotherapy alone or whether the continuation of trastuzumab have significantly contributed to the therapeutic results. Phase-III randomized trials are now ongoing to properly investigate this issue.

**Trastuzumab in the adjuvant setting**

The improvements obtained with trastuzumab in the metastatic setting have prompted trials of trastuzumab as part of adjuvant therapy for patients with high risk HER2 over expressing early breast cancer. There are at least five major ongoing trials (Figure 1).

Two are North American trials, the National Surgical Adjuvant Breast and Bowel Project B-31 and the North Central Cancer Treatment Group Intergroup Trial N9831. These will examine the use of trastuzumab with paclitaxel as part of the conventional US adjuvant treatment regimen of doxorubicin/cyclophosphamide (AC) followed by a taxane. The worldwide Breast Cancer International Research Group (BCIRG) trial 006, the Herceptin Adjuvant (HERA) trial and the Programmes d’Actions Concertées (PACS) 004 trial, which are conducted in countries other than North America, are evaluating different adjuvant regimens. These trials represent a major effort at analyzing the potential benefits of trastuzumab given concomitantly or sequentially with a variety of chemotherapy regimens in the adjuvant setting, and are also addressing the question of duration of trastuzumab therapy. Recruitment to these trials is expected to be complete by early 2005.
Primary systemic therapy (PST) with trastuzumab

PST with trastuzumab has been studied in phase II clinical trials in combination with taxanes and other cytotoxic agents [31]. These combinations have achieved pCR rates of 12% to 42% and clinical overall response rates of 70–100%, including 24–67% clinical complete response rates. Common adverse were manageable and no unexpected toxicities were reported. In the majority of the reported phase II PST studies, trastuzumab was administered for 12 weeks before surgery, continuing trastuzumab post surgery to complete 1 year for responding patients.

In a randomized trial paclitaxel followed by 5-fluorouracil, epirubicin and cyclophosphamide (FEC) has been compared with the same regimen plus concomitant trastuzumab. The trial was planned to enroll 164 patients, but was stopped at the interim analysis of first 34 patients showing a significant increase in pCR from 25% to 66.7% (P = 0.02) by the addition of trastuzumab to chemotherapy [32].

A large randomized international trial (MO16432; NOAH) in patients with either HER2+ (n = 230) or HER2- (n = 40) locally advanced breast cancer is currently ongoing. The HER2+ patients receive anthracycline plus paclitaxel given every 3 weeks for three cycles followed by paclitaxel alone given every 3 weeks for four cycles, and then CMF (cyclophosphamide/methotrexate/5-fluorouracil) on days 1 and 8, every 4 weeks for three cycles before surgery. Patients with HER2+ disease are randomized to receive the same regimen with or without the addition of trastuzumab. A further relatively large (n = 120) PST trastuzumab trial (M77041; TECHNO) is being conducted by the Arbeitsgemeinschaft Gynakologische Onkologie study group in Germany. Patients with HER2-positive, operable or locally advanced breast cancer will receive four cycles of epirubicin and cyclophosphamide every 3 weeks, followed by four cycles of paclitaxel along with trastuzumab for 12 weeks before surgery, followed by 3-weekly trastuzumab monotherapy for 36 weeks.
Conclusions

Targeted therapy in breast cancer presents a big challenge, but is an exciting area of research. Many new agents are currently being tested that block oncopgenic pathways. Although there was initial enthusiasm, it is becoming clear that single agent therapy will not significantly affect outcome and clinical research is now shifting towards the more successful integration of these new agents into traditional regimens. Trastuzumab is among the first target-specific drugs that have been licensed for clinical use and it has proved to yield substantial clinical benefits. The possibility of introducing this agent into the adjuvant setting and the introduction of new combinations, doses, and schedules remain exciting options. Trastuzumab represent a paradigmatic model of how the integration of targeted agents into therapeutic regimens may produce a significant prolongation of survival and improve QoL of cancer patients.

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