

# Trastuzumab in the management of early and advanced stage breast cancer

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**Abstract:** Trastuzumab is a monoclonal humanized antibody that has revolutionized the treatment of patients with Her2-positive breast cancer. Already well established in advanced stage disease, the substance was recently introduced in the adjuvant setting, reducing disease recurrences by more than 50% and mortality by approximately one third. Trastuzumab is a rationally designed substance which binds to cancer cells expressing the targeted antigen, and, by different mechanisms, causes tumor cell degradation. However, only one third of patients have an initial response to trastuzumab therapy, and the majority of initial responders demonstrate disease progression within 1 year of treatment initiation. It is therefore necessary to gain further insight into mechanisms of resistance, and develop ways to overcome those. In this article, the role of trastuzumab in early and advanced stage breast cancer is reviewed. We discuss current understandings of the specific tumor biology of Her2-positive breast cancer, and review the mechanism of action of trastuzumab. Further, we try to highlight possible mechanisms of resistance.

**Keywords:** Her2-positive breast cancer, monoclonal antibodies, trastuzumab

## Background

Breast cancer is one of the most prevalent cancers in the world, and holds responsible for almost half a million deaths per year worldwide. While incidence is rising, screening and recent advances in adjuvant treatment caused reduction in mortality in early stage disease over the last decade (American Cancer Society Facts and Figures 2005; World Health Organization Facts and Figures 2007). Yet, even in stage I and II disease, approximately one third of patients are expected to experience disease recurrence (Faneyte et al 2004). With few exemptions, breast cancer in this situation is incurable, and patients will finally succumb to their disease. Today, a prolongation of overall survival due to a wide range of treatment options is possible, yet median life expectancy after recurrence remains low, between 24 and 30 months (American Cancer Society Facts and Figures 2005; World Health Organization Facts and Figures 2007).

Relatively early it was anticipated that major biologic differences exists between estrogen receptor- (ER) positive and ER-negative disease. More recently it was discovered that up to 25% of breast cancers have overexpression of Her2, defining a high-risk breast cancer subtype (Slamon et al 1987; Paik et al 1990; Kallioniemi et al 1991; Boss et al 2003). Progresses in microarray technology allowed the simultaneous evaluation of the expression of tens of thousands of genes, thereby increasing our understanding of cancer biology, and proving that breast cancer is a heterogeneous disease (Sørlie et al 2001; Clarke et al 2001; van de Vijver 2002; van't Veer 2002). At least four different subtypes are currently defined: Her2 positive, normal breast-like, basal-like (often also referred to as triple negative), and the two luminal subtypes, A and B (possibly endocrine responsive and Her2 negative) (Sørlie et al 2001; van de Vijver 2002; van't Veer 2002). Due to these insights, while yet far from perfect, patients are treated with

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regimens that are risk adapted, and, wherever possibly, tailored to the specific tumor biology of their individual disease.

Still, a number of patients are not adequately treated, as they will eventually develop metastatic disease. Other patients might not have needed adjuvant therapy at all, and are therefore considered overtreated. In the future, it is hoped for that differences in gene expression profiling might lead our decision to treat (or not to treat) a certain patient, or might even help in choosing the most appropriate substances.

## Breast cancer biology: the Her2 subtype

The Her2 subtype of breast cancer can be identified both by histopathologic methods and by gene expression profiling. Overexpression of Her2 in breast cancer cells is well known to be associated with high recurrence rate and poor outcome (Slamon et al 1987; Paik et al 1990; Kallioniemi et al 1991; Boss et al 2003). Her2 (Her2/neu, erbB2) is a transmembrane tyrosine kinase molecule of the Her family of human growth factor receptors, coded by the Her2/neu gene on chromosome p17 (Bargmann et al 1986; Akiyama et al 1988). The molecule forms homo- or heterodimers with other members of this family (EGFR, Her3, and Her4). It is of importance that heterodimers of EGFR and Her2 have higher stability and therefore cause prolonged receptor signaling compared with EGFR homodimers, a fact that serves as rationale for dual targeting of EGFR and Her2 (Konecny et al 2006; Xia et al 2006). Following receptor activation, phosphorylation of tyrosine residues on the cytoplasmatic domain activates downstream signaling pathways, namely the cell survival PI3K (phosphatidylinositol 3-kinase)/Akt pathway, and cell proliferation pathways mediated by mitogen-activated protein kinase (MAPK). Furthermore, increased signaling via those pathways leads to an increase in vascular endothelial growth factor (VEGF) expression (Yen L et al 2000; Izumi et al 2002), most probably via an increase in hypoxia inducible factor (HIF) 1 $\alpha$  (Laughner et al 2001). This is due to mTor and MAPK signaling, which in turn may be blocked by trastuzumab.

It was therefore suggested that the positive association between Her2 and VEGF expression implicates VEGF in the aggressive phenotype exhibited by Her2 overexpression, and this might support the use of combination therapies directed against both Her2 and VEGF (Pegram and Reese 2002; Konecny et al 2004).

In recent years, existence of a molecular crosstalk between the ER and signaling pathways downstream of

growth factor receptors was reported by different groups. MAPK and pAKT may cause a ligandless activation of the AF-1 domain of membrane bound and cytoplasmatic ER. As this domain is not blocked by tamoxifen, it is widely believed that this constitutes the main mechanism of resistance to tamoxifen in Her2-positive disease (Schiff et al 2004).

Interestingly, while other receptors of the Her family have specific ligands, Her2 appears to represent a ligandless receptor, as no specific Her2 ligand was yet identified. Characterization of the crystal structure of the extracellular domain showed that Her2 has a constitutively open conformation. This open conformation is needed for receptor dimerization and signal transduction. Indeed, the extracellular domain of Her2 is similar to that of ligand bound EGFR (Cho et al 2003). As mentioned above, stability of EGFR and Her2 heterodimers is higher compared with EGFR homodimers (Konecny et al 2006; Xia et al 2006). Therefore, Her2 is believed to act mainly as enhancer of signal transduction.

## Role of Her2 in healthy tissue

In healthy tissue, Her2 signaling regulates cell differentiation, survival, and repair mechanisms (Casalini et al 2004). Cardiac myocytes for instance, are dependent on Her2 activation in their response to cellular stress. In this case, downstream signaling induces repair mechanisms. Blocking this repair pathway in connection with anthracycline exposure is often held responsible for the well known cardiotoxicity of trastuzumab (Fukazawa et al 2003). Another possible cause of cardiac toxicity is the interaction of Her2 with RLIP76 (RALBP1), a multifunctional transporter involved in signaling and transmembrane movement of solute allocrites which includes several antineoplastic agents. In fact, RALBP1 appears to be the dominant anthracycline transporter in the heart (Yadav et al 2004). In embryogenesis, Her2 was found to assist in proper cell differentiation (Casalini et al 2004). In cases where the Her2 pathway is deregulated, carcinogenesis results.

## Assessment of Her2 status

Definition of Her2-positivity results from increased receptor expression or gene amplification. As Her2 overexpression and amplification have important consequences both for prognosis and treatment, accurate determination is necessary. Currently, two different methods are being used worldwide, immunohistochemistry (IHC) and fluorescence in situ hybridization (FISH). IHC is a semiquantitative method that identifies Her2 expression on the cell surface, with a grading system of 0 to 3+ positive. Tumors are classed as Her2-positive if they have a staining intensity of 3+ (when

**Table 1** Mechanism of action, trastuzumab

Molecular mechanism	Evidence
Inhibition of Her2 extracellular domain cleavage Immune effects	Inhibition of proteolytic cleavage of the extracellular domain in vitro (Molina et al 2001) Trastuzumab activated ADCC in many breast cancer cell lines. Level of ADCC correlated with response to therapy in vivo (Cooley et al 1999; Gennari et al 2004; Clynes et al 2006) Downregulation of Her2 protein in breast cancer cell lines (Cuello et al 2001)
Her2 receptor Internalization and degradation	
Decreased CDK2 (cyclin dependent kinase 2) activity	In vitro treatment of breast cancer cell lines increased p27, resulting in reduced CDK2 activity (Lane et al 2001)
Blockade of Her2 signaling	Blockade of the PI3-Kinase/Akt pathway, as evidenced by decrease of phosphorylated Akt levels (Yakes et al 2002)
Inhibition of DNA repair	Inhibition of DNA repair and blockade of unscheduled DNA synthesis after damage by cisplatin and radiation (Pietras et al 1994, 1998, 1999)
Reduced angiogenesis	Reduced VEGF levels on trastuzumab treatment in breast cancer xenograft models (Yen et al 2002)

receptor levels approach 2 million); if a score of ++ (receptor levels of approximately 500,000) is gained, the tumors should be reanalyzed using FISH; tumors with Her2 gene amplification again are deemed Her2 positive. This testing algorithm is accepted by many authors as a viable, cost effective approach (Ellis et al 2000; Larsimont et al 2002). More recently, alternative options for Her2 testing have been described: chromogenic in situ hybridization (CISH) (Denoux et al 2003), and real time polymerase chain reaction (RT-PCR) (Gjerdum et al 2004). The exact assessment of Her2 status is of utter importance, as only patients with strong overexpression or gene amplification may expect to derive benefit from trastuzumab treatment.

## Trastuzumab

For nearly 10 years, the use of trastuzumab has been firmly established in Her2-positive breast cancer. The drug has dramatically changed response rate and progression-free survival in metastatic disease. More importantly even, disease-free survival and overall survival in the adjuvant setting were improved.

## Mechanism of action

Trastuzumab is a recombinant, humanized monoclonal antibody (rhMab4D5), targeting the extracellular domain of Her2 (human EGFR related), also known as erbB2 or neu (Hynes and Stern 1994; Nahta et al 2006). It is currently the only Her2-targeted therapy approved by the FDA for metastatic breast cancer. The drug inhibits cleavage of the extra cellular domain (Molina et al 2001) and, via antibody-dependent cellular cytotoxicity (ADCC) and complement dependent cytotoxicity (CDC), leads to cell degradation (Cooley et al 1999; Gennari et al 2004; Clynes et al 2006). Natural killer (NK) cells are necessary for the ADCC response to

trastuzumab. They express Fc gamma receptors which bind the Fc domain of the IgG1 antibody, thereby activating NK cells to destroy cells expressing Her2 by lyses. This was validated in a mouse model where a significant reduction in trastuzumab efficacy was observed in mice lacking the Fc receptor (Clynes et al 2006). Other important mechanisms of action comprise the internalization and degradation of Her2 receptor protein (Cuello et al 2001), decrease of cyclin dependent kinase 2 (CDK2) activity via p27 induction (Lane et al 2001), blockade of downstream Her2 signaling pathways (Yakes et al 2002), and inhibition of DNA repair (Nahta et al 2006). The latter point is deemed to be especially important for the described synergistic interaction with cisplatin and irradiation (Pietras et al 1994, 1998, 1999). It is unclear, however, whether trastuzumab actually causes downregulation of Her2, as some studies have demonstrated that receptor levels remain basically unchanged on trastuzumab therapy (Lane et al 2001; Yakes et al 2002; Nahta et al 2002). A summary of potential mechanisms of action is provided in Table 1.

## Mechanisms of resistance

In general, while considerable knowledge has been gained during recent years, different questions remain unanswered still. This mainly concerns the mechanisms of resistance to trastuzumab. Obviously, de novo resistance exists, as only approximately one third of patients on trastuzumab monotherapy have objective response, thereby rendering response rate less than satisfactory (Vogel et al 2002). Also, secondary resistance eventually develops, as the majority of initial responders demonstrate disease progression within 1 year of treatment initiation (Nahta et al 2006).

Possible mechanisms of resistance comprise activation of downstream signaling pathways via insulin-like growth factor-1 (IGF-1) (Lu et al 2001), and phosphatase and tensin homologue

loss (PTEN) (Nagata et al 2004), which enables activation of downstream signaling independent of receptor activity. Further, apparently decreased interaction between trastuzumab and its target receptor might occur, which in some cases is due to steric hindrance of Her2 by cell surface proteins such as mucin-4 (MUC4) (Nahta et al 2006). In other cases, resistance is conveyed by a truncated Her2 receptor without extracellular domain due to alternative splicing. This specific receptor is commonly known as truncated Her2. From that, a constitutively activated Her2 protein results, without possibility of trastuzumab binding (Esparis-Ogando et al 1999). Also, constitutive Akt signaling was shown to inhibit the cell-cycle arrest and apoptotic effect of trastuzumab (Clark et al 2002). As outlined above, trastuzumab activity is in part due to p27 induction. Consequently, the downregulation of p27 was reported to be associated with trastuzumab resistance in breast cancer cell lines (Nahta et al 2004). Possible mechanisms of resistance are summarized in Table 2.

## Advanced breast cancer

### Early trials

In parallel to two small phase II that established the activity of trastuzumab in humans (Baselga et al 1996; Pegram et al 1998), a large, randomized, single-agent study was initiated in April 1995. Response rate was found to be 15%, and median overall survival was 13 months. In the Her2 3+ subgroup, a considerably higher activity was observed, with an overall response rate of 18% and median survival of 16.4 months. Those results suggested that this group might derive the greatest benefit from trastuzumab (Cobleigh et al 1999).

### Trastuzumab, taxanes, and anthracyclines

Two randomized, combination trials of trastuzumab and chemotherapy led the path to establishing the antibody in the

treatment of Her2-positive metastatic breast cancer: H0648g and M77001.

A total of 469 patients without prior therapy for metastatic breast cancer were included in H0648g. Patients who were not eligible for anthracycline treatment or who already were exposed to an anthracycline in the adjuvant setting, were randomized to receive paclitaxel or paclitaxel plus trastuzumab. The reminders were randomized to doxorubicin plus cyclophosphamide with or without trastuzumab. Response rate, progression-free survival, and overall survival were superior in the combination group. A subset analysis revealed that patients with IHC 3+ positive disease gained the largest benefit, compared with the total population (IHC 2+ and 3+ positive). Further, it was observed that in the trastuzumab plus anthracycline group, 28% of patients experienced a cardiac event, compared with 13% in the population treated with paclitaxel plus trastuzumab (Slamon et al 2001). Due to these findings, the combination of anthracyclines and trastuzumab is currently not deemed standard of care, although cardiotoxicity might be less pronounced when liposomale doxorubicin is applied. This was evaluated in the M77035 trial. Within this trial, the triple combination of trastuzumab, paclitaxel, and TLC D-99 (Myocet®) was generally well tolerated. By formulating doxorubicin within a liposome, cardiac toxicity was minimized compared with conventional anthracyclines, while activity was maintained (Baselga et al 2004).

M77001 included patients with Her2-positive disease and randomized them to either receive docetaxel monotherapy or a combination of docetaxel plus weekly trastuzumab. Again, response rates, time to progression, and overall survival were superior in the trastuzumab containing arm, without adding significantly to the toxicity profile of docetaxel alone (Marty et al 2005).

Owing to these results, the combination of trastuzumab plus taxanes is deemed the golden standard in the first line treatment of Her2-positive metastatic breast cancer (Jakisch et al 2006). Yet, as taxanes are increasingly incorporated into the adjuvant setting, interest in further combination strategies evolved. Based on a preclinical trial conducted by Pegram et al cytotoxic agents and trastuzumab were classed either as synergistic, agonistic, or antagonistic (Pegram et al 1999). Data were gained both from cell culture and a tumor xenograft model. Based on the results of this trial, special emphasis was put on combinations with vinorelbine and platinum compounds (as for these agents a synergistic interaction was assumed), while due to a suggested antagonistic effect of trastuzumab with 5-FU, the oral 5-FU prodrug capecitabine was of less interest until recently.

**Table 2** Mechanism of resistance, trastuzumab

Proposed mechanism
Activation signaling pathways downstream insulin-like growth factor-I (IGF-I) (Lu et al 2001)
Loss of PTEN enables activation of downstream signaling independent of receptor activity (Nagata et al 2004)
Decreased interaction of trastuzumab and Her2 due to steric hindrance of Her2 by cell surface proteins such as mucin-4 (MUC4) (Nahta et al 2006)
Truncated Her2 receptor without extracellular domain, resulting in a constitutively activated Her2 protein, without the possibility of trastuzumab binding (Esparis et al 1999)
Constitutive Akt signaling activates downstream signaling independent of receptor activation (Clark et al 2002)
Downregulation of p27 is associated with trastuzumab resistance in breast cancer cell lines (Nahta et al 2006)

### Trastuzumab and vinorelbine

A number of phase II trials reported high activity and good tolerability of trastuzumab plus vinorelbine combinations in metastatic breast cancer. In these trials, different schedules of trastuzumab (weekly and every 3 weeks) and vinorelbine (both intravenous and oral vinorelbine) were used. In general, combination therapy was well tolerated, with reported response rates in the range of 50%–80% (Jahanzeb et al 2002; Burstein et al 2003a; Papaldo et al 2006). As expected, first-line therapy yielded higher objective response rates and longer time to disease progression, possibly indicating that first-line therapy with this combination may be more effective than later treatment (Jackisch et al 2006). A recently published phase II trial of oral vinorelbine plus trastuzumab suggested that intravenous vinorelbine could be substituted for by the oral formulation, without a decrease in efficacy (Bartsch et al 2007a). Burstein et al addressed the important question whether the combination of trastuzumab with vinorelbine or a weekly administered taxane would be the optimal first-line regimen for Her2-positive metastatic breast cancer (Burstein et al 2006). The trial was closed due to slow accrual in December 2003, after having accrued only 85 of a planned 250 patients. While therefore the results must be interpreted with caution, this trial suggested at least comparable clinical activity of the two combinations, with a trend towards superior time to progression in the vinorelbine group (8.5 months vs 6.0 months;  $p = 0.09$ ).

### Trastuzumab and platinum compounds

In one of the earliest clinical trials, Pegram and colleagues reported on 37 patients with metastatic breast cancer who had received extensive prior chemotherapy. The subjects were treated with a 250 mg loading dose of trastuzumab followed by weekly doses of 100 mg until disease progression, in combination with cisplatin. Nine patients (24%) had a partial response (Pegram et al 1998).

In the last decade, a number of trials have reported results of trastuzumab in combination with platinum compounds, either as single agent or in combination with taxanes. These trials reported response rates ranging from 48% (single agent cisplatin) to approximately 80% in trials using the combination of platinum salts and taxanes (for a review see Tokunaga et al 2006; Demonty et al 2007). A recently published study compared the triple combination of trastuzumab, paclitaxel and carboplatin with trastuzumab plus paclitaxel alone (Robert et al 2006). The addition of carboplatin improved both overall response rate (52% vs 36%;  $p = 0.03$ ) and progression-free survival (13.8 months vs 7.6 months;  $p = 0.005$ ). A second trial (BCIRG 007), evaluating

the efficacy of docetaxel, carboplatin plus trastuzumab, however, brought different results. The authors concluded that the already effective docetaxel plus trastuzumab regimen does benefit from the addition of carboplatin in women with Her2 overexpressing metastatic breast cancer (Forbes et al 2006).

In general, bearing in mind the relative high toxicity of such regimens, the combination of platinum compounds and trastuzumab appears to be a reasonable treatment option in selected patients with metastatic breast cancer.

### Combinations with other cytotoxic agents

Data for the combination of trastuzumab with gemcitabine are available from two phase II trials. In both of these studies, patients with prior exposure to at least an anthracycline and/or taxane were included. Response rates were 36% and 38% respectively, and treatment in general was well tolerated. Notably, in a total of 89 patients included, no case of congestive heart failure was observed (Christodoulou et al 2003; O'Shaughnessy et al 2004).

As outlined above, *in vitro* studies suggested an antagonistic effect of combining 5-FU and trastuzumab; in effect, the combination was considered less effective than either drug alone (Pegram et al 1999). However, further trials with human breast cancer models showed at least an additive effect of this combination (Fujimoto-Ouchi et al 2002). This conclusion was strengthened by available clinical data. A small trial incorporated heavily pretreated patients with Her2-positive breast cancer. At the ASCO 2005 meeting an encouraging response rate of 60% was reported (Schaller et al 2005). These results were mirrored by data presented by Xu et al who conducted a phase II trial of capecitabine and trastuzumab in the first line setting (Xu et al 2006). A recent single-center trial of trastuzumab plus capecitabine beyond progression reported a time to progression (TTP) of 8 months and a clinical benefit rate of 70% (Bartsch et al 2007b). Still, while there is growing evidence that the combination of capecitabine with trastuzumab is active, this regimen remains largely experimental.

In the MO16419 (CHAT) trial, patients were randomized to receive docetaxel plus trastuzumab, either with or without capecitabine, as first-line therapy for Her2-positive metastatic breast cancer. Overall response rate, which was defined as primary endpoint, was high in both treatment arms, with no significant difference (71% in the triple combination arm, compared with 73% in patients on docetaxel plus trastuzumab). In terms of TTP, there was a significant benefit for the docetaxel, trastuzumab plus capecitabine (18.2 months vs 13.8 months,  $p = 0.045$ ) combination. Differences in pro-

gression-free survival and overall survival, however, did not reach statistical significance. It must therefore be assumed that the addition of capecitabine does not add to the efficacy of docetaxel plus trastuzumab.

Independently of cytotoxic combination partners, current results clearly recommend early use of trastuzumab in the metastatic setting (ie, from first line) (Slamon et al 2001; Harris and Smith 2002).

### Trastuzumab and endocrine therapy

New data are available concerning the combination of trastuzumab and endocrine substances in the palliative setting (Kaufmann et al 2006). In the Tandem trial, combination of trastuzumab and anastrozole was superior to anastrozole alone in Her2- and ER- and/or PgR-positive breast cancer in terms of response rate and progression-free survival. A non-significant trend towards prolonged overall survival was observed. As outlined above, the assumed biological basis for this study is a crosstalk between the estrogen receptor and growth factor pathways, making a ligandless activation of the ER via Her2 possible, thereby causing endocrine resistance (Schiff et al 2004).

### Scheduling trastuzumab

In the pivotal trials and most of the following studies evaluating the role of trastuzumab either alone or in combination with chemotherapy, the standard weekly schedule of administering the antibody was used. Based on newer data suggesting a longer half-life of trastuzumab than originally believed, Leyland-Jones et al initiated a trial evaluating trastuzumab and paclitaxel with a more convenient regimen with a 3-weekly administration of both drugs. A pharmacokinetics sub-protocol revealed no major differences between the three-weekly schedule and data from trials utilizing the weekly regimen (Leyland-Jones et al 2003). The same results were reported by a European group evaluating a 3-weekly schedule (Baselga et al 2005). While unfortunately no formal, direct, randomized, comparison of these two regimens was performed, in the light of available data it appears reasonable to offer patients the possibility of the 3-weekly regimen.

### Treatment beyond progression

A major unresolved question about trastuzumab treatment in advanced breast cancer concerns treatment beyond progression. After progressing on a combination of trastuzumab and chemotherapy, should trastuzumab be discontinued, or only the cytotoxic partner switched to another substance, while antibody treatment continues? As of today, no results from randomized clinical trials are available. For

daily clinical practice, however, some preclinical and clinical evidence supports ongoing trastuzumab therapy beyond disease progression.

In a Her2-positive human xenograft model, a decrease of trastuzumab levels caused tumor regrowth (Pietras et al 1998). This might implicate a rebound effect after cessation of antibody therapy. Recently presented preclinical data showed the following results: Combination of trastuzumab with a taxane after failure of trastuzumab monotherapy was more effective than taxanes alone in this setting (Fujimoto-Ouchi et al 2005). Clinical data are also available: In an extension study of the pivotal phase III trial, patients were given the opportunity to continue trastuzumab when on disease progression. Continuation of antibody therapy was safe and well tolerated; response rate was 11%, and response duration 6.7 months (Tripathy et al 2004). Other different groups also reported encouraging results (Fountzilias et al 2003; Bartsch et al 2006a). In one study, response rates and time to progression were maintained from first to beyond second line treatment. Response rates were 42.6% in first line trastuzumab therapy, 25.9% in second line, and 30% in beyond second line respectively. Median time to progression was 6 months in all treatment lines (Bartsch et al 2006a).

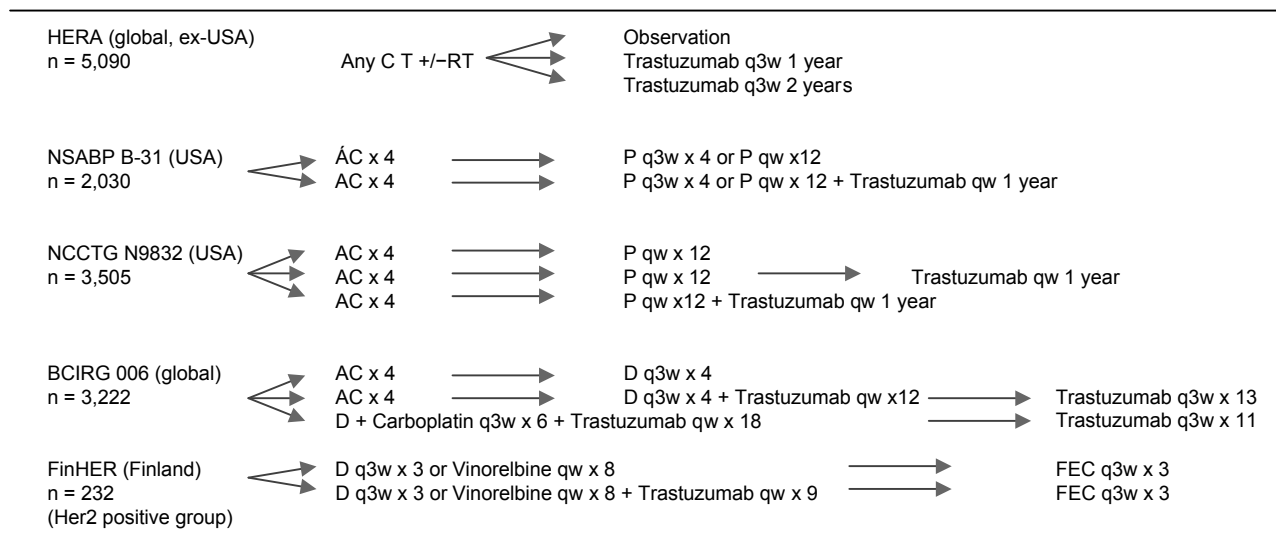
### Early breast cancer

As outlined, up to 25% of women with early stage breast cancer have Her2-positive disease, which is well known to correlate with aggressive disease and a higher likelihood of recurrence after adjuvant therapy (Slamon et al 1989; Boss et al 2003). Due to the benefits of trastuzumab in the palliative setting, a number of adjuvant trials were initiated, to evaluate the possible role of the antibody in the prevention of cancer recurrence in this high risk population.

More than 13,000 women were included in a total of 5 prospective adjuvant phase III trials that have ultimately led to the definition of trastuzumab as golden standard in the adjuvant therapy of Her2-positive early breast cancer. Importantly, chemotherapy regimens and the timing of trastuzumab administration varied among the different studies. Therefore a number of unanswered questions concerning the optimal way to administer trastuzumab adjuvant remain. The designs of the adjuvant breast cancer trials are summarized in Figure 1.

### HERA

This international, non-US, adjuvant multicenter trial randomized patients after standard adjuvant therapy (minimal of 4 cycles of adjuvant/neoadjuvant chemotherapy with or



**Figure 1** Trials evaluating the role of trastuzumab in early stage breast cancer.

**Abbreviations:** A, doxorubicin; C, cyclophosphamide; D, docetaxel; E, epidoxorubicin; F, 5-FU; P, paclitaxel; q3w, every 3 weeks; qw, weekly; RT, radiotherapy.

without radiotherapy) to 3 arms: a control arm, 1 year of trastuzumab, and 2 years of therapy. Disease-free survival was defined as primary endpoint. Initial data have been published in 2005. At 1 year of median follow up, a relative risk reduction of recurrence of 46% was observed in the treatment group as compared with the observation group (hazard ratio [HR], 0.54; 95% confidence interval [CI], 0.43–0.67;  $p < 0.0001$ ). Further, a trend towards a prolonged overall survival was seen in the treatment group (Piccart-Gebhart et al 2005). This trend reached statistical significance at the second planned interim analysis at a median follow up of 24 months (HR, 0.64; 95% CI, 0.54–0.76;  $p = 0.005$ ) (Smith et al 2006). Differing from the US trials NSABP-B31 and NCCTG N9831, a large number of node negative patients were included (approximately 32% in each arm).

### Combined analysis of NSABP B-31 and NCCTG N9831

NSABP B-31 is a 2-arm, randomized phase III trial of 4 cycles of doxorubicin and cyclophosphamide (AC) followed by 4 cycles of paclitaxel every 3 weeks (or 12 cycles of paclitaxel weekly) with or without trastuzumab. Treatment was initiated after completion of AC therapy. In contrast, NCCTG N9831 had a 3-arm design. Two arms were identical to the corresponding arms of B-31, a third arm initiated trastuzumab after the end of chemotherapy (ie, similar the HERA trial). Treatment also consisted of the US standard AC  $\times$  4 followed by 12 cycles of weekly paclitaxel. Therefore, after approval by the FDA was gained, a combined analysis of the two control arms and concurrent arms of those trials

was possible. After 2 years of median follow up, patients treated with trastuzumab had a significantly longer disease-free survival as compared with the control group (HR, 0.48; 95% CI, 0.39–0.59;  $p < 0.001$ ). Also, a significant benefit in terms of overall survival was observed (HR, 0.67; 95% CI, 0.48–0.93;  $p = 0.015$ ) (Romond et al 2005).

### BCIRG 006

A third, international trial, was initiated by the BCIRG. This 3-arm study, conducted at centers in the US, Europe, South Africa, Asia, and Venezuela, had a very interesting design: a standard arm of AC  $\times$  4 followed by 4 cycles of docetaxel every 3 weeks was compared with the same regimen plus trastuzumab (initiated after AC) and a third (anthracycline-free) arm consisting of carboplatin, docetaxel, and trastuzumab (TCH). The special value of this trial, therefore, is the evaluation of an anthracycline-free regimen. In the light of well known cardiac safety concerns, this might be an effective regime in patients with known pre-existing cardiac conditions. Initial results were presented at the 28th San Antonio breast cancer symposium (SABCS) in 2005. Both trastuzumab containing treatment arms had a significantly improved disease free survival (which was defined as primary endpoint) as compared to the control arm. Still, there seemed to be a special benefit for the anthracycline-containing arm, with a 51% lower risk of recurrence compared with a 39% risk reduction in the TCH arm (Table 3). Due to the fact that up to one third of tumors are co-amplified for Her2 and topoisomerase II $\alpha$ , it was assumed that this difference may become statistically significant over time, and

**Table 3** Results BCIRG 006

<b>BCIRG 006</b>		
	Disease-free survival	Overall survival
AC→TH vs AC→T	HR, 0.49; 95% CI, 0.37–0.65; $p < 0.0001$	HR, 0.59; 95% CI, 0.42–0.85; $p = 0.004$
TCH vs AC→TH	HR, 0.61; 95% CI, 0.47–0.83; $p = 0.0003$	HR, 0.66; 95% CI, 0.47–0.93; $p = 0.017$

**Abbreviations:** A, doxorubicin; C, cyclophosphamide; T, docetaxel; H, trastuzumab; TCH, docetaxel, carboplatin, trastuzumab.

the anthracycline plus trastuzumab arm would eventually prove to be the superior regimen (Slamon et al 2005). In 2006, however, updated information became available. The trend observed before then had all but vanished. It must therefore be assumed that targeting a single, amplified gene of the Her2 amplicon is sufficient, and double targeting by trastuzumab plus an anthracycline is of no additional value (Slamon et al 2006). While these data are interesting, final results and possibly further studies will have to be awaited before anthracycline-free regimens could be deemed standard in Her2 overexpressing breast cancer.

### FinHER

The smallest of the five adjuvant trials included only 232 Her2-positive patients. The design was radically different from the others: before having 3 cycles of CEF every 3 weeks, patients either received 3 cycles of docetaxel every 3 weeks or 8 cycles of weekly vinorelbine. Further, Her2-positive patients were randomized either to a group of 9 cycles of weekly trastuzumab, or control (Figure 1). Again, recurrence-free survival was defined as primary endpoint. While no difference in overall survival has been observed up to now, a significant advantage in recurrence-free survival was found in patients receiving trastuzumab (HR, 0.42; 95% CI, 0.21–0.83;  $p = 0.01$ ) (Joensuu et al 2006).

Due to the different designs of the adjuvant trials, a number of questions concerning the optimal use of trastuzumab in the adjuvant setting remain unanswered. For example, it is not known whether the optimal use of chemotherapy and trastuzumab is sequential or concurrent. Also, in the light of BCIRG 006 data, it must be asked which chemotherapy plus trastuzumab combination is the most effective and safe.

The question concerning the optimal duration of trastuzumab therapy is also still open, although it was indirectly approached in a study presented by Sledge et al at the SABCS 2006 meeting. E2198 was a pilot trial examining the cardiac effects of paclitaxel plus trastuzumab prior to doxorubicin plus cyclophosphamide (AC) in Her2-positive patients. Patients received paclitaxel plus weekly trastuzumab for 10 weeks followed by 4 cycles of AC. After completion of chemotherapy, one arm received trastuzumab for a further

52 weeks. Disease-free survival and overall survival showed no difference between the two groups. The authors concluded that although the trial was not designed or powered to test the question of trastuzumab duration, a significant advantage for prolonged trastuzumab administration was not observed.

Therefore, although trastuzumab is now well established in the adjuvant therapy of Her2-positive breast cancer, further trials addressing the mentioned questions are awaited.

### Role of trastuzumab in the neoadjuvant setting

Neoadjuvant chemotherapy (systemic chemotherapy before surgery) for breast cancer is standard of care in patients with locally advanced disease. While there is little doubt concerning the efficacy of trastuzumab in the adjuvant setting, the exact role of the antibody in the preoperative setting awaits further clarification. A number of phase II data, with pathologic complete remission (pCR) rates ranging from 18% to 39%, are currently available, and definitely support evaluation in larger trials (Wenzel et al 2002; Burstein et al 2003b; Jahanzeb et al 2005; Hurley et al 2006). Trials were utilizing highly different substances and regimens, and there is limited evidence from large phase III studies.

In the phase III trial conducted by Buzdar et al 42 patients were treated with 4 cycles of paclitaxel followed by another 4 cycles of 5-FU, epirubicin and cyclophosphamide (FEC) with or without weekly trastuzumab for 24 weeks. The trial was terminated prematurely after the first 34 patients completed therapy, because a significant difference in terms of pCR rate favoring the trastuzumab arm was observed (66.7% vs 25%;  $p = 0.02$ ) (Buzdar et al 2005). Interestingly, a study conducted by the Austrian breast and colorectal study group (ABCBSG) showed a significantly higher pCR rate after 6 cycles of epirubicin and docetaxel (ED) in patients with Her2-positive tumors in a multivariate analysis as compared with the Her2 negative subgroup. This possibly indicates the assumed higher responsiveness of Her2-positive tumors to anthracyclines exposure due to coamplification of topoisomerase II $\alpha$  and Her2 in one third of cases (Steger et al 1999; Slamon et al 2006; Steger et al 2007). Therefore, while results of preoperative targeting of Her2 and its amplicon are definitely encouraging, the



neoadjuvant approach with trastuzumab still remains largely experimental. To further evaluate this question, ABSCG initiated another randomized neoadjuvant protocol (ABSCG-24), incorporating preoperative chemotherapy with or without trastuzumab (ClinicalTrials.gov2007). Another important trial that will help clarifying the role of trastuzumab in the neoadjuvant setting will be conducted by the American college of surgeons oncology group (ACOSOG). In the ACOSOG Z1041 study, patients will be randomized to receive either paclitaxel and trastuzumab weekly for 12 weeks followed by 4 cycles of FEC with weekly trastuzumab before surgery, or 4 cycles of FEC followed by 12 cycles of weekly paclitaxel and trastuzumab (www.acosoc.org).

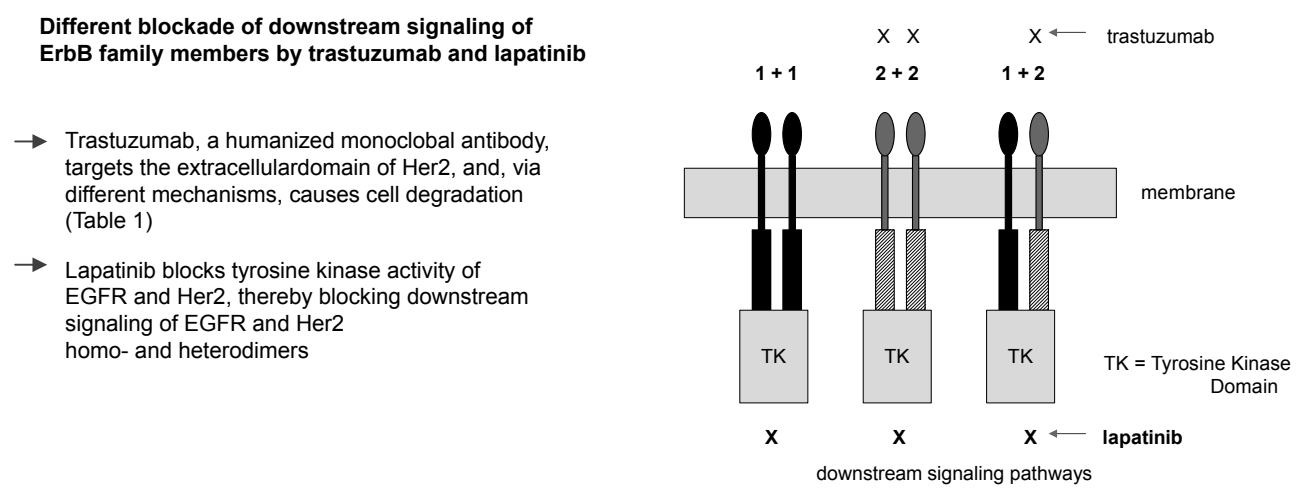
Trastuzumab retreatment after recurrence following adjuvant trastuzumab With the establishment of antibody therapy in early stage Her2-positive breast cancer, recurrence rate was reduced by approximately 50%. Still, some patients will experience disease recurrence on, or after antibody therapy. Therefore, there is an urgent need to answer the question, whether trastuzumab should be re-induced in these individuals. Data from the RHEA (retreatment after herceptin adjuvant) phase II trial, which is currently ongoing, will hopefully provide information concerning this problem (ClinicalTrials.gov2007).

## Drawbacks and side-effects

A problem arises from the fact that monoclonal antibodies cannot pass an intact blood–brain barrier. With trastuzumab, an increased incidence of breast cancer brain metastases was observed (Bendell et al 2003; Clayton et al 2004; Shmueli et al 2004). This is explained by two interacting

mechanisms. First, as outlined above, large molecules cannot pass into the brain and the cerebral liquor, and thus cannot prevent the development of cerebral disease. Secondly, survival in this high risk population is prolonged due to the antibody. As result, patients who might have died from systemic disease progression, survive to develop cerebral disease. Although there is some evidence concerning possible activity of systemic treatment in brain metastases due to an impairment of the blood-brain-barrier around metastatic sites and due to irradiation (Rosner et al 1986; Boogerd et al 1992; Bartsch et al 2006b), no truly and long-term active treatment of this debilitating complication exists, and brain metastases remain a major problem. Therefore, other solutions must be sought. A possible answer lies in the use of lapatinib in this situation. Due to their molecular weight, tyrosine kinase inhibitors are in theory able to pass the blood brain barrier. This was strengthened by data presented at the ASCO 2006 meeting, where a stabilization of cerebral lesions in patients treated with lapatinib was reported (Lin et al 2006). The question was also approached in a trial comparing the combination of capecitabine and lapatinib versus capecitabine monotherapy after trastuzumab failure (Geyer et al 2006). Fewer patients in the combination group developed cerebral disease.

While trastuzumab in general is well tolerated, the best known side-effect of course is the increased incidence of congestive heart failure (CHF). This was first observed in the pivotal trial in the metastatic setting, with notably the highest occurrence rates in patients treated with a combination of doxorubicin plus trastuzumab (Slamon et al 2001). Therefore this combination was avoided in following studies. In the



**Figure 2** Mechanism of action, trastuzumab, and lapatinib.

adjuvant setting, approximately 5% of patients are expected to develop some form of cardiac function impairment, and 1% may develop symptomatic CHF (Perez and Rodeheffer 2004; Piccart-Gebhart et al 2005; Romond et al 2005). In most cases, the systolic dysfunction appears to be reversible (Guarneri et al 2006); still, close monitoring of patients on trastuzumab treatment is mandatory. Especially at risk for developing a drop in left ventricular ejection fraction (LVEF) are patients of old age and with known hypertension; further risk factors may include pre-existing diabetes, coronary artery disease, and valvular dysfunction (Romond et al 2005; Guarneri et al 2006). An algorithm for controlling the cardiac function of patients while on trastuzumab was proposed by Keefe (2002). The author suggests close surveillance with echocardiography or MUGA scan every 4 weeks if a drop in LVEF occurs. In asymptomatic cases, antibody treatment should be held when a drop of >20 points to <40% is observed, or when LVEF falls below 30%. In symptomatic CHF, treatment should be stopped in case LVEF drops by more than 30 points.

Other possible side-effects consist mainly of anaphylactic reactions; the HERA trial reported a minimally increased risk of infections (22 patients [1.3%] vs 7 patients [0.4%] in the control group) (Piccart-Gebhart et al 2005), and the combined analysis of NSABP B-31 and NCCTG N9831 trials reported rare cases of interstitial pneumonitis that in some cases appeared to be related to trastuzumab therapy (4 cases in B-31 and 5 in N9831). In BCIRG 006, there were no significant differences in grade 3 or 4 hematologic or nonhematologic adverse events among the 3 treatment arms (Slamon et al 2005). Also, in the FinHER trial, no differences in adverse events were observed in the respective trastuzumab and non-trastuzumab groups (Joensuu et al 2006).

It may therefore be stated that while the cardiac toxicity is evident, trastuzumab in general is well tolerated, and the benefits by far outweigh the potential risks and side-effects. Still, close cardiac monitoring is required.

## **An outlook: combinations of trastuzumab and other targeted agents**

Lapatinib is an orally available tyrosinkinase kinase inhibitor blocking the tyrosine kinase domains of EGFR and Her2 (Figure 2). There is little doubt that this compound is of value in Her2-positive breast cancer, especially in the metastatic setting. Lapatinib is active in combination with capecitabine after trastuzumab exposure (Geyer et al 2006), and might be active also in cerebral lesions (Lin et al 2006). A phase I study presented at the ASCO 2005 meeting reported

an impressive, while short lived (2–8 months), response rate with a trastuzumab lapatinib combination (Storniolo et al 2005). The EGF 104900 trial is currently further investigating this combination. After exposure to capecitabine, a taxane, an anthracycline, and trastuzumab, patients are randomized to receive either lapatinib 1500 mg or a combination of lapatinib 1000 mg in combination with weekly trastuzumab (ClinicalTrials.gov2007). Further, a number of trials evaluating the combination of lapatinib, trastuzumab, and chemotherapy (docetaxel, paclitaxel, carboplatin, doxorubicin) are currently recruiting (www.ClinicalTrials.gov2007). The substance will be also evaluated in the adjuvant setting in the ALTTO trial (ClinicalTrials.gov2007). In this 4-arm trial following standard chemotherapy, an adjuvant 1 year trastuzumab arm will be compared with a 1 year lapatinib arm and 2 combination arms, one in which the two drugs are administered sequentially (12 weeks of lapatinib followed by 6 months of trastuzumab) and 1 combination arm in which the two drugs are administered simultaneously for 1 year.

Another approach is the combination of 2 monoclonal antibodies targeting different receptors of the Her family. At our center, a trial combining trastuzumab and cetuximab is being conducted in patients with metastatic breast cancer. Cetuximab is a chimeric monoclonal antibody, targeting the extracellular domain of EGFR (Lenz 2006). Although the drug is well established in metastatic colorectal cancer, and also in head and neck tumors (Cunningham et al 2004; Bonner et al 2006), it has not yet found its way into breast cancer therapy. As described, a powerful interaction between Her2 and EGFR exists. It is hypothesized that by blocking both growth factor receptors, the resulting downstream signaling can be blocked in a more efficient manner; further an increased immune response is anticipated. This combination might also be of value in Her2 1+ or 2+, FISH negative disease.

Bevacizumab is a humanized monoclonal antibody targeting vascular endothelial growth factor (VEGF), which is of great importance for tumor vasculature and therefore tumor growth. This antibody might find a role in Her2-positive disease. The rationale is that through increased signaling via the ras/raf/MAPKinase signaling pathway, HIF-1 $\alpha$  levels increase (Laughner et al 2001), and, consecutively, so does VEGF expression (Yen et al 2000; Izumi et al 2002). This mechanism may be blocked by trastuzumab by downregulating the MAPKinase pathway. Therefore, combining these two antibodies might target tumor vessel growth more effectively than either compound alone (Pegram et al 2002). A phase II trial recently

presented at the 2006 SABCS reported this combination to be clinically feasible and highly active in Her2 amplified recurrent or metastatic breast cancer (Pegram et al 2006). Based on this rationale, a randomized multicenter study is currently underway, evaluating docetaxel plus trastuzumab with or without bevacizumab in the first line metastatic setting (ClinicalTrials.gov2007).

## Conclusion

Molecular targeted treatment is of great promise in cancer therapy. Targeting Her2 with the monoclonal antibody trastuzumab has dramatically changed the prognosis of patients with Her2-positive breast cancer, both in advanced and early stage disease. While this drug therefore evolved as the gold standard, a number of questions remain unanswered.

In advanced breast cancer, among other uncertainties, there is no consensus regarding the optimal trastuzumab schedule (weekly vs every 3 weeks), the optimal chemotherapy combination partners, and treatment beyond progression. In early-stage disease, questions remain concerning anthracycline-free chemotherapy regimens, the time point of trastuzumab initiation, and treatment duration. Yet the largest challenge remaining is to identify mechanisms of resistance to trastuzumab, and possible ways to overcome these.

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