

# Trastuzumab (Herceptin®): Monoclonal Antibody in the Treatment of HER2/neu-Overexpressing Breast Cancer in the Metastatic and (Neo)adjuvant Situation

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## Key Words

Herceptin® · Trastuzumab · Monoclonal antibody, humanized · HER2/neu

## Summary

Trastuzumab (Herceptin®) is a humanized monoclonal antibody that specifically targets HER2/neu (human epidermal growth factor receptor-2) breast cancer cells, which are overexpressed in about 25–30% of breast carcinomas. After phase I and II trials, several phase III studies of trastuzumab alone or in combination with various chemotherapies were conducted. Patients with HER2/neu overexpression levels of 3+ determined by immunohistochemical assay or gene amplification (fluorescence in situ hybridization) derive most clinical benefit from trastuzumab. Taking into consideration efficacy and side effect profile, the combination of trastuzumab and paclitaxel showed an improvement of all clinical parameters, including overall survival, for the first time in the history of palliative breast cancer therapy. The application of trastuzumab has meanwhile become an established part of systemic therapy of metastatic breast cancer, and excellent data of its application in the adjuvant setting now exist (NSABP-B31, NCCTG-N9831, HERA), with significantly better relapse-free survival in the treatment arms with trastuzumab. Ongoing trials investigate the role of trastuzumab in the neoadjuvant setting. Trastuzumab is generally well tolerated. Cardiotoxicity is the main concern, thus monitoring of cardiac function is recommended.

## Schlüsselwörter

Herceptin® · Trastuzumab · Monoklonaler Antikörper, humanisierter · HER2/neu

## Zusammenfassung

Trastuzumab (Herceptin®) ist der erste humanisierte monoklonale Antikörper zur Behandlung des Mammakarzinoms. Er ist gegen den humanen epidermalen Wachstumsfaktor-Rezeptor-2 (HER2/neu) gerichtet, der bei 25–30% aller humanen Mammakarzinome überexprimiert ist. Nach erfolgreichen Phase-I-/II-Studien folgten mehrere Phase-III-Studien mit Einsatz von Trastuzumab alleine oder in Kombination mit unterschiedlichen Chemotherapien. Trastuzumab ist für Patientinnen indiziert, deren Tumor in einem validierten immunhistochemischen Test eine HER2/neu-Überexpression (3+) und/oder Genamplifikation (Fluoreszenz-in-situ-Hybridisierung) aufweist. Unter Berücksichtigung von Wirksamkeit und Nebenwirkungsprofil zeigte die Kombinationstherapie von Trastuzumab und Paclitaxel erstmals in der Geschichte der palliativen Therapie des Mammakarzinoms eine Verbesserung aller klinischen Parameter, einschließlich des Gesamtüberlebens. Mittlerweile ist der Einsatz von Trastuzumab ein etablierter Bestandteil der Systemtherapie beim metastasierten Mammakarzinom und sollte seit der exzellenten Datenlage zur adjuvanten Situation (NSABP-B31, NCCTG-N9831, HERA) mit signifikant besserem rezidiv- und metastasenfreiem Überleben für die Therapiearme mit Trastuzumab hier zum Einsatz kommen. Derzeit laufende Studien untersuchen die Wirksamkeit einer Kombinationstherapie mit Trastuzumab in der neoadjuvanten Situation. Trastuzumab gilt nach aktueller Datenlage als gut verträgliche Substanz. Die wichtigste Nebenwirkung ist die Ausbildung einer Herzinsuffizienz, weshalb sich die Empfehlung einer basalen kardialen Diagnostik vor Therapiestart sowie unter und nach der Therapie ergibt.

## Introduction

HER2 (human epidermal growth factor receptor-2) is a transmembrane tyrosine kinase receptor belonging to the family of epidermal growth factor receptors. It is encoded by the HER2/neu proto-oncogene located on chromosome 17 q 21 [1]. HER2 is overexpressed in approximately 25–30% of all breast cancers [2]. HER2 overexpression stimulates cancer cell growth and correlates with a shorter relapse-free interval and overall survival time [3]. The unfavorable prognosis associated with HER2 overexpression has been confirmed by numerous studies [4]. HER2 overexpression is determined immunohistochemically. Currently, the HercepTest® (Dako, Glostrup, Denmark) using a polyclonal antibody against HER2/neu is the most commonly used test. A HER2 score of 0 and 1+ is classified as negative, a HER2 score of 3+ as clearly positive. In the case of a HER2 score of 2+ (marginally positive), fluorescence in situ hybridization (FISH) should follow. Accurate determination of HER2 status is an indispensable prerequisite to every trastuzumab treatment.

## Therapy of HER2-Overexpressing Breast Cancer with Trastuzumab

Beside surgery, radiation, chemotherapy and antihormonal therapy, immunotherapy is a promising new approach in breast cancer treatment. Trastuzumab (Herceptin®, Hoffman-La Roche, Grenzach-Whylen, Germany) was the first antibody approved for breast cancer therapy by the FDA (U.S. Food and Drug Administration). Trastuzumab is a murine monoclonal antibody that targets the extracellular domain of the HER2 protein. The exact mechanism of its anticancer activity is not yet fully understood, and several different ways of action have been suggested. The response to therapy correlates with HER2 overexpression. Cells with normal receptor levels are not efficiently inhibited. Trastuzumab is clearly indicated in patients with immunohistochemical scores of 3+ or, if the score is 2+, with amplification detected by FISH analysis. In the USA, trastuzumab was approved for the treatment of metastatic breast cancer in 1998, and European approval followed in 2000. Phase II and III studies demonstrated efficacy and tolerability of trastuzumab in metastatic breast cancer patients with HER2 overexpression. Trastuzumab has demonstrated antitumor effects when administered as a single agent and additive and synergistic effects when given in combination with other antineoplastic agents or hormonal therapy. Studies examining the effects of a combination of trastuzumab and chemotherapeutic agents on tumor growth in tissue culture and xenograft models demonstrated synergy between trastuzumab and cisplatin/carboplatin, vinorelbine, thiotepa, gemcitabine at low concentrations and etoposide, and additivity between trastuzumab and doxorubicin, epirubicin, paclitaxel, docetaxel, vinblastin and methotrexate. In vitro fluo-

rouacil was found to have antagonistic interaction with trastuzumab [5]. At standard doses of 4 mg/kg loading dose followed by 2 mg/kg weekly, the mean half-life is approximately 28.5 days (95% confidence interval (CI) 25.5–32.8 days) with a washout period of up to 20 weeks (95% CI 18–24 weeks). The steady-state concentration is reached at approximately 20 weeks (95% CI 18–24 weeks). Weekly intravenous infusions of trastuzumab show a volume of distribution which approximates the serum volume (2.95 l). The recommended initial loading dose of trastuzumab is 4 mg/kg administered via intravenous infusion over 90 min, followed by a weekly dose of 2 mg/kg given over 30 min. Three-weekly intervals with 8 mg/kg loading dose followed by 6 mg/kg treatment dose are also being used. In 2 phase II trials (BO15935, WO16229), patients were treated with a loading dose of 8 mg/kg trastuzumab administered as a 90-min intravenous infusion followed by doses of 6 mg/kg every 3 weeks (q3w). The q3w regimen was well tolerated, with safety profiles similar to that of standard weekly regimen [6]. No formal drug interaction studies were performed. According to the population pharmacokinetic analysis by Genentech (San Francisco, CA, USA), administration of concomitant drugs (anthracycline/cyclophosphamide or paclitaxel) does not appear to influence the pharmacokinetics.

Serum levels of the circulating HER2/neu shed antigen can be measured by enzyme-linked immunosorbent assays (ELISA). At the moment the serum level testing has to be seen as a complementary method rather than an alternative to HER2/neu tissue analysis. Due to only minimal penetration into cerebrospinal fluid, trastuzumab is not suitable for treatment of cerebral metastases [7].

## Trastuzumab in the Metastatic Situation

Trastuzumab is applied without interruption at least until appearance of relevant cardiac toxicity or disease progression. Clinical data of the retrospective admission study show a benefit for patients with progressive disease under trastuzumab treated further on with trastuzumab. The encouraging results of preliminary clinical trials [8] were followed by initiation of major pivotal trials using chemotherapy +/- trastuzumab and trastuzumab as monotherapy in metastatic breast cancer. Efficacy and safety data of these trials led to the FDA approval of trastuzumab in metastatic HER2/neu-overexpressing breast cancer.

### *Trastuzumab Monotherapy*

Cobleigh et al. [9] treated 222 metastatic breast cancer patients with weekly trastuzumab as second- or third-line therapy. 68% of them had previously received chemotherapy (H0649g). The overall response rate was 15% with a median

time to progression of 9.1 months and a median survival of 13 months. There were 8 complete and 26 partial remissions. Of all patients, 40% suffered mild toxicity. Cardiac side effects were observed in 4.7% (10 patients) of which 9 were pretreated with doxorubicin. Vogel et al. [10] treated 114 metastatic breast cancer patients with 2 different dosages of first-line trastuzumab (H0650g). One group received a loading dose of 8 mg/kg and 4 mg/kg maintenance dose, the other group 4 mg/kg followed by 2 mg/kg. Treatment was well tolerated in both groups. Toxicity was more pronounced in the dose-intensified group but generally mild. The overall response rate was 26% (95% CI 18–34%) without a significant difference between the groups (standard dose 24%, dose intensified 28%). Subgroup analysis demonstrated an elevated response rate in immunohistochemistry (IHC) 3+ (35%) and FISH-positive patients (41%). The median survival was 24.4 months, approximating the survival rate in the pilot phase III combination study (25 months).

#### *Trastuzumab in Combination Therapies*

In Germany, trastuzumab is approved in combination with paclitaxel or docetaxel as a first-line regimen. First results of a treatment combining paclitaxel (90 mg/m<sup>2</sup> q1w) and trastuzumab were published in 1999. The response rate was 61% [11]. Slamon et al. [12] compared the treatment effects of chemotherapy alone versus chemotherapy and weekly trastuzumab combined in a cohort of 469 patients with HER2-overexpressing tumors and without previous treatment for metastatic breast cancer (H0648g). Patients were randomized to one of 4 treatment regimens based on the presence or absence of prior anthracycline adjuvant therapy: trastuzumab plus anthracycline (doxorubicin 60 mg/m<sup>2</sup> or epirubicin 75 mg/m<sup>2</sup>) and cyclophosphamide (6 × 60/600 mg/m<sup>2</sup> q3w), versus anthracycline and cyclophosphamide alone (in the case of no prior anthracyclines), or trastuzumab plus paclitaxel (6 × 175 mg/m<sup>2</sup> q3w) versus paclitaxel alone. The trial demonstrated favorable effects of trastuzumab on response rate, median time to disease progression, median duration of response and survival. Overall response rates of chemotherapy plus trastuzumab versus chemotherapy alone were 50% and 32% (p < 0.001). The combination therapy with anthracycline chemotherapy plus trastuzumab showed an increased response rate of 56% versus 42% for the same chemotherapy regimen without trastuzumab (p = 0.02). Response rates to trastuzumab plus paclitaxel versus paclitaxel alone were 41% and 17%, respectively (p < 0.001). Both trastuzumab groups showed an improved time to disease progression compared to groups receiving chemotherapy alone. Time to disease progression was 7.8 months for anthracycline/cyclophosphamide plus trastuzumab versus 6.1 months for anthracycline/cyclophosphamide alone (p < 0.001) and 6.9 months for paclitaxel plus trastuzumab versus 3.0 months for paclitaxel alone

(p < 0.001). The median duration of response in all 469 patients was 9.1 months with trastuzumab versus 6.1 months without trastuzumab (p < 0.001). Trastuzumab improved the median survival time by 4.8 months compared to chemotherapy alone (p = 0.046). Symptomatic and asymptomatic cardiac dysfunction was observed in 63 patients. NYHA (New York Heart Association) class III or IV dysfunction was developed by 16% of patients treated with anthracycline/cyclophosphamide and trastuzumab, 3% of patients treated with anthracycline/cyclophosphamide alone, 2% of patients treated with paclitaxel and trastuzumab and 1% of patients treated with trastuzumab alone.

The efficacy and safety of the combination of trastuzumab and taxanes was confirmed by another current phase III trial. First-line trastuzumab plus docetaxel compared with docetaxel alone was investigated in 188 patients who had IHC 3+ and/or FISH+ disease (M77001). Weekly trastuzumab was administered until progression. Docetaxel was given at a dose of 6 × 100 mg/m<sup>2</sup> q3w. The addition of trastuzumab to docetaxel improved all clinical outcomes, compared with docetaxel alone: overall response rate (61 vs. 34%, p = 0.0002), median duration of response (11.4 vs. 5.1 months, p = 0.0011), median time to disease progression (10.6 vs. 5.7 months, p = 0.0001) and median survival (30.5 vs. 22.1 months, p = 0.0062). Median survival was prolonged in patients who received trastuzumab concomitantly with docetaxel (30.5 months) compared with those who received trastuzumab after progressing on docetaxel (24.5 months; 48% of patients crossed over) and those who received docetaxel alone (19.1 months) [13].

In the pivotal trial, the combination of trastuzumab with anthracyclines was associated with a higher incidence of cardiotoxicity which is why this regimen was not approved by the FDA. In the first part of phase II, 26 HER2-positive patients were treated with epirubicin and cyclophosphamide (EC) 4 × 60/600 mg/m<sup>2</sup> q3w (EC60) plus trastuzumab every week until week 103. The overall response rate was 71%. In the second part of phase II, 25 HER2-positive patients were treated with an elevated EC dose of 4 × 90/600 mg/m<sup>2</sup> q3w (EC90) and weekly trastuzumab for 103 weeks. 25 HER2-negative patients were treated with EC90. Cardiac function was closely monitored. 2 EC90 plus trastuzumab-treated patients experienced symptomatic congestive heart failure, and 1 EC60 plus trastuzumab-treated patient experienced an asymptomatic decrease in left ventricular ejection fraction (LVEF) to < 50%. Objective response rates with EC60 plus trastuzumab and EC90 plus trastuzumab were 62% and 64%, respectively and 26% for EC90 alone [14]. In the following phase III study (M77003), 100 HER2-positive patients were treated with EC 90/600 mg/m<sup>2</sup> q3w followed by trastuzumab. As control group, 100 HER2/neu-negative patients were treated with EC 90/600 mg/m<sup>2</sup> q3w without trastuzumab. Liposomal doxorubicin formulations provided an advantage over conventional doxorubicin in terms of cardiac safety [15]. One trial reported the use of liposomal doxorubicin in combination with trastu-

zumab. The overall response rate in 33 evaluable patients was 58%.

In combination therapies with trastuzumab and navelbine, 40 HER2-positive patients with metastatic disease (82% with prior chemotherapy) showed an overall response rate of 75%. The combination therapy was well tolerated. The most common hematological side effect was neutropenia. 3 patients developed grade II cardiotoxicity. Two confirmatory multicenter trials using the same treatment regimen as first-line therapy demonstrated a similar degree of activity [16]. Further combination therapies of trastuzumab and capecitabine, gemcitabine and platinum salts presently demonstrate few impressive results because of small patient cohorts and short observation periods [17–19]. Table 1 shows recommendations for trastuzumab therapy in combination with chemotherapy. Future and ongoing studies will provide further data on the therapeutic efficacy.

### Trastuzumab and Anti-Hormonal Therapy

There is evidence for interaction or ‘cross talk’ between HER2/neu and estrogen receptor (ER) signaling pathways in breast cancer. According to the results of several studies, HER2/neu overexpression is associated with resistance to anti-hormonal therapy [20]. The International Breast Cancer Study Group analyzed the data of 1,506 patients and found a correlation of HER2 overexpression and negative hormone receptor (HR) status. Borg et al. [21] and Ravdin et al. [22] reported a decreased effect of tamoxifen in HR-positive and HER2-overexpressing patients. To investigate the relationship of HER2 overexpression and HR expression, HER2, ER and progesterone receptor (PR) were examined as continuous variables in 1,359 breast cancer specimens in 14 cell lines some of which had been transfected with the HER2/neu gene. Transfected cell lines expressed significantly lower levels of ER and PR than parental lines. In 2 clinical cohorts, primary tumors with HER2 overexpression or pathologic amplification in the nucleus had significantly lower levels of ER and PR than patients without HER2 overexpression or amplification. Therefore, pathologic HER2 amplification and overexpression seems to downregulate expression of ER and PR, thus impairing the efficacy of hormone therapy. Results of a neoadjuvant study comparing letrozole versus tamoxifen in ER-positive and/or PR-positive postmenopausal primary breast cancer patients – having shown a good response in ER-positive, HER2/neu-positive cancers and infrequent responses to tamoxifen – indicate that the response in HER2/neu-positive tumors to aromatase inhibitors, such as letrozole, may differ from that seen with tamoxifen [23]. A decreased effect of tamoxifen in HR-positive and HER2/neu-overexpressing patients was demonstrated [21]. A potential mechanism is the observed downregulation of ER and PR in HER2/neu-overexpressing tumor cells [24]. Thus, it has been suggested that

**Table 1.** Recommendations for trastuzumab therapy in metastatic breast cancer

Combination	Oxford		AGO
	LOE	GR	
Paclitaxel	1b	A	++
Docetaxel	1b	A	++
Paclitaxel/carboplatin	1b	A ++	
Vinorelbine	2b	C	+
Docetaxel/platinum complex	2b	C	+/-
Epirubicin/cyclophosphamide	2b	C	+/-
PEG-liposomal doxorubicin	2b	C	+/-
Capecitabine	2b	C	+/-
Gemcitabine	2b	C	+/-

LOE = Levels of evidence, GR = grade of recommendation, AGO = Arbeitsgemeinschaft Gynäkologische Onkologie.

combining hormonal agents with trastuzumab may be a rational approach for clinical trials. A phase III randomized, controlled, open label trial of anastrozol with or without trastuzumab is being conducted in 202 HR-positive, HER2/neu-positive metastatic breast cancer patients. Moreover, there are several ongoing trials with letrozole +/- trastuzumab. The largest is enrolling 300 patients and will investigate letrozole with or without trastuzumab as first-line therapy in metastatic breast cancer.

### Trastuzumab in the Adjuvant and Neoadjuvant Situation

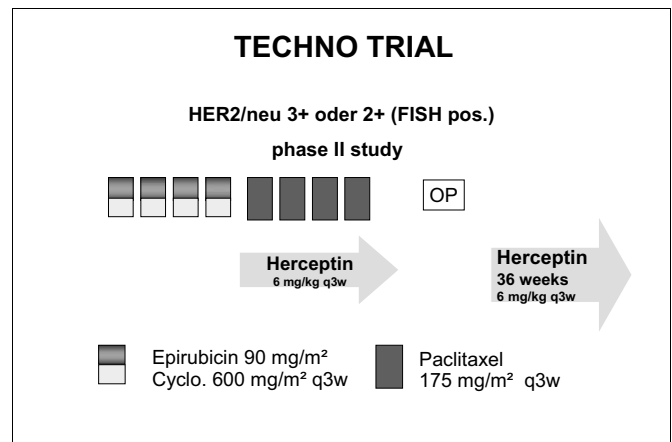
The efficacy of trastuzumab plus chemotherapy in patients with HER2/neu-positive metastatic breast cancer generated interest in the use of trastuzumab in the adjuvant situation. After a pilot study of 40 stage II and III breast cancer patients, several multicenter, randomized, adjuvant trials were initiated, each with a cardiac monitoring plan:

1. The NSABP (National Surgical Adjuvant Breast and Bowel Project) trial B-31 uses anthracycline/cyclophosphamide q3w (4 cycles), followed by paclitaxel plus trastuzumab q3w (4 cycles) versus the same chemotherapy regimen without trastuzumab in node-positive, HER2-positive breast cancer patients.
2. The NCCTG (North Central Cancer Treatment Group) Intergroup trial N9831 is a randomized 3-arm trial. Treatment with anthracycline/cyclophosphamide q3w (4 cycles) in all 3 arms is followed by weekly paclitaxel (12 cycles) versus weekly paclitaxel (12 cycles) plus trastuzumab for 1 year versus weekly paclitaxel and trastuzumab (12 cycles) followed by trastuzumab monotherapy (40 cycles). Recruited patients have node-positive or high-risk node-negative breast cancer overexpressing HER2.

The Intergroup NCCTG N9831 and the NSABP B-31 trastuzumab adjuvant trials have presented combined data of a first interim analysis based on 395 events of 3151 treated patients with a median follow-up of 2.4 years on B-31 and 1.5 years on N9831. Trastuzumab administered simultaneously with paclitaxel following doxorubicin/cyclophosphamide reduced the risk of recurrence by 52% compared to controls (stratified log rank p-value =  $3 \times 10^{-12}$ , stratified HR (95% CI) 0.48 (0.39–0.60)). The absolute difference in disease-free survival was 12% after 3 years and 18% after 4 years. The relative risk reduction benefit was present in all analyzed subgroups (age, HR status, tumor size, number of positive nodes). There was no statistical power to establish efficacy in the node-negative subset, as NSABP B-31 only included node-positive patients, and NCCTG N9831 only enrolled 13% of high-risk node-negative patients in the control and 11% in the investigational arm. As for the secondary efficacy endpoint, overall survival, the addition of trastuzumab showed a significant survival advantage with a relative risk reduction of 33% at a median follow-up of 2 years (stratified log rank p-value = 0.015, stratified HR (95% CI) 0.67 (0.48–0.93)). The absolute difference in time to first distant recurrence was 9% after 3 years and 16% after 4 years [25]. The NCCTG N9831 trial further evaluates the trastuzumab schedule given sequential to or concurrent with paclitaxel, each showing a decreased recurrence compared with controls. At the interim comparison, the concurrent versus sequential treatment led to a 36% decrease in recurrence (stratified log rank p-value = 0.0114, stratified HR (95% CI) 0.64 (0.46–0.91)) [26]. In the interim analysis of the NSABP B-31 trial, the incidence of cardiac events was 4.0% in the trastuzumab-containing regime versus 0.6% without trastuzumab. In the investigational group (n = 846), 30 cases of congestive heart failure (CHF) and no cardiac deaths were observed, compared with the control group (n = 811) where 3 CHF and 1 cardiac death occurred [25]. In the NCCTG N9831, there was 0% incidence of cardiotoxicity in the control, 2.2% in the sequential and 3.3% in the concurrent arm [25].

3. In the BCIRG (Breast Cancer International Research Group) phase III randomized trial 006, patients with node-positive or high-risk node-negative, HER-2-overexpressing primary breast cancer are randomized to one of 3 treatment arms: After 4 cycles of doxorubicin/cyclophosphamide (A/C) q3w, patients receive either 4 cycles of docetaxel q3w, or 4 cycles of docetaxel q3w plus trastuzumab for 1 year, or docetaxel plus carboplatin (6 cycles) followed by trastuzumab for 1 year. A total of 3,222 patients were recruited. After a median follow-up of 23 months, the 2 trastuzumab-containing arms reached the disease-free survival endpoint: HR of 0.49 with AC followed by docetaxel and trastuzumab and 0.61 with docetaxel, carboplatin and trastuzumab compared to AC followed by docetaxel [26].

4. The HERA (Herceptin Adjuvant Trial) study is a worldwide multicenter study in which patients with verified HER2



followed by postoperative therapy with trastuzumab in patients with HER2/neu-overexpressing primary breast cancer (fig. 1). So far, histopathological reports of surgery after chemotherapy and trastuzumab are available from 174 patients. According to the definition of the NSABP, 72 patients (41.4%) have a histopathological complete remission. In 127/174 (73%) of the patients, axillary nodes are histologically free of metastases at surgery.

The trastuzumab clinical trial program and the post-marketing surveillance demonstrated that trastuzumab is well tolerated, both when used as a single agent and in combination with chemotherapy. The most common side effects observed in clinical trials were mild to moderate infusion-related fever and chills that occurred mostly in the first few hours of the first infusion (25–40% incidence) [10, 13], with a trend toward increased incidence at higher doses [10]. The clinically

most significant trastuzumab-associated adverse events were serious infusion-related reactions and cardiotoxicity (especially in combination therapy with anthracyclines plus cyclophosphamide). The observed cardiac dysfunction has influenced the design of subsequent trials. Most studies request formal baseline measurements of LVEF (LVEF  $\geq$  50% is usually required) before initiating treatment and have adopted cardiac monitoring criteria [14]. The risk of cardiac failure associated with the use of trastuzumab can be justified by a significant improvement of treatment outcome, time to progression and a longer median survival. The already overwhelming results of the adjuvant trastuzumab trials mentioned above indicate that we have an incredibly potent drug at hand. However, it remains to be seen whether the long-term analysis as well as analysis of all randomized patients will support these promises.

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