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Review

Perspectives of Immunotherapy in Metastatic Breast Cancer

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Abstract. *Further improvements in the treatment of breast cancer can be expected with a better understanding of its pathophysiology and through biologically-oriented therapeutic interventions, as well as better identification of patient populations likely to benefit from specific therapies. Trastuzumab (Herceptin®) is the first biological modifier, showing significant activity in patients with advanced breast cancer who exhibit HER-2/neu gene amplification and/or protein overexpression. Trastuzumab is approved for use in combination with paclitaxel or docetaxel as first-line chemotherapy. Combinations of a taxane, a platinum salt and trastuzumab are feasible and active and have proven an increased survival advantage. This is in addition to the benefit that has been shown for Herceptin in combination with mono-chemotherapy alone. Several groups have demonstated the ratio of serum HER-2/neu levels prior to initiation of Herceptin treatment to levels at the time of re-staging examination to be significantly higher in patients with a significant benefit from therapy as compared to patients with progressive disease. As a result of the survival improvements in the metastatic setting, Herceptin was quickly entered into development trials for adjuvant treatment. The significant cardiac toxicity that has been observed with trastuzumab/anthracycline combinations has led to two main strategies for integrating trastuzumab in the adjuvant setting: either the addition of trastuzumab to mostly anthracycline-based programs in a sequential approach, or the biologically-oriented strategy based on synergism between trastuzumab and chemotherapy agents including platinum compounds. Last but not least, the most important prerequisite for the optimal efficacy of Herceptin-based therapy remains a very strict selection of those patients with tumours that have HER-2/neu over-expression.*

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Pathophysiology of the EGF receptor family

Signal transduction refers to essential cell processes of growth, differentiation and survival, and acts through complex biochemically-related networks (1, 2). Aberrations in signal transduction elements can lead to increased proliferative potential, sustained angiogenesis, tissue invasion and metastasis, and apoptosis inhibition (3, 4). The *HER-2/neu* (for human epidermal growth factor receptor) gene, which encodes the growth factor receptor HER-2/neu, is amplified and/or overexpressed in 25 to 30 per cent of breast cancers, increasing the aggressiveness of the tumour by initiating various signal transduction pathways, such as the ras/raf, PKC, AKT and JNK systems (5-8). While HER-2/neu shows an increased density in the cell membrane, the growth-stimulatory effect of HER-1 (or EGFR) is more related to mutated receptors and the persistent activation of autoregulatory loops by paracrine activation through different growth factors (9, 10).

HER-2/neu positivity is defined as those tumours with >10% of cells with moderate/strong, complete membrane staining, by IHC (11-13). Confirmatory HER-2/neu testing using FISH is recommended for indeterminate cases. The comparison of IHC and FISH demonstrated an excellent correlation of high-level HER-2/neu overexpression (3+) with gene amplification, which implies that FISH does not provide further information about these tumours. However, weakly-positive IHC results (2+) obtained with the DAKO HercepTest® share only a minor association with gene amplification. FISH positivity is generally defined as a gene copy number > 4 per nucleus.

Herceptin, a monoclonal antibody against the cell-surface receptor HER-2/neu, was shown to induce normalisation and regression of the vasculature in an experimental mouse xenograft of a HER-2/neu over-expressing human breast tumour. Its mechanism of action was determined to be the modulation of the effects of different pro- and anti-angiogenic factors (14). In addition, trastuzumab treatment results in down-regulation of the HER-2/neu receptor. *In vivo*, trastuzumab induces antibody-dependent cellular cytotoxicity. Trastuzumab also inhibits constitutive HER-2/neu cleavage/shedding mediated by metalloproteases. The ability of trastuzumab to inhibit HER-2/neu cleavage may correlate with the clinical anticancer activity of the multifunctional HER-2/neu-targeting antibody (15, 16).

Trastuzumab in combination with taxanes only

Trastuzumab has received regulatory approval for the treatment of patients with metastatic breast cancer, based on the results of a phase III trial showing that the compound increases the clinical benefit of first-line chemotherapy in cancers that overexpress HER-2/neu (17). A total of 234 patients were assigned to receive standard chemotherapy alone and 235 patients to receive standard chemotherapy plus trastuzumab. Patients who had not previously received postoperative therapy with an anthracycline were treated with doxorubicin (or epirubicin in the case of 36 women) and cyclophosphamide alone (138 women) or with trastuzumab (143 women). Patients who had previously received adjuvant anthracycline were treated with paclitaxel alone (96 women) or paclitaxel with trastuzumab (92 women). The addition of trastuzumab to the chemotherapy was associated with 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%; $p<0.001$) and 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; $p=0.01$) with a 20% reduction in the risk of death. Similar results were obtained by a confirmatory phase II trial replacing paclitaxel by docetaxel as the combination partner of Herceptin (18). Overall survival was increased from 18.3 months for docetaxel alone to 27.7 months for the combination with the antibody. Notably, this trial provided the first high-level evidence that the up-front use of Herceptin as compared to a later-line therapy resulted in an additional benefit to the patient. The patients who had been randomised to the combination arm from the very beginning showed an estimated survival time of 27.7 months. In contrast, those patients who had been treated in the monotherapy arm and later crossed to Herceptin therapy after the study was concluded (*i.e.*, those with progressive disease), in a form of compassionate use design, presented only an estimated survival of 21.9 months.

The most important adverse event in the pivotal trial was cardiac dysfunction of NYHA class III or IV. This occurred in 27% of the group given an anthracycline, cyclophosphamide and trastuzumab; in 8% of the group given an anthracycline and cyclophosphamide alone; in 13% of the group given paclitaxel and trastuzumab; and in 1% of the group given paclitaxel alone. Although the cardiotoxicity was potentially severe and, in some cases, life-threatening, the symptoms generally improved with standard medical management. To investigate the pathophysiology of HER-2/neu signalling in the adult heart, a mouse model was generated with a ventricular-restricted deletion of HER-2/neu (19). The analysis revealed the onset of multiple independent parameters of dilated cardiomyopathy, including chamber dilation, wall thinning and decreased contractility. Additionally, cardiomyocytes isolated from these conditional mutants were more susceptible to anthracycline toxicity. HER-2/neu signalling in cardiomyocytes must, therefore, be considered essential for the prevention of dilated cardiomyopathy.

Trastuzumab in combination with platinum-based chemotherapy

Metastatic breast cancer is a partially chemotherapy-sensitive neoplasm. An emerging literature suggests that taxane-platinum combinations have substantial activity in HER-2/neu-overexpressing disease. Preclinical data suggest a powerful synergistic interaction between trastuzumab and both platinum and the taxanes. In early trials, platinum-taxane-trastuzumab combinations exhibited promising clinical activity (20). These results led to a phase III trial in which paclitaxel (175 mg/m^2) was combined with Herceptin and carboplatin (AUC 6) (trastuzumab, paclitaxel and carboplatin =TPC) and compared to the new standard therapy in HER-2/neu-overexpressing disease, (*i.e.*, the combination of paclitaxel plus Herceptin alone (trastuzumab and paclitaxel= TP)) (21). In this study the response rate was 57% with TPC *vs.* 38% with TP ($p<0.01$), and time to progression was 13 months with TPC *vs.* 7 months with TP ($p=0.002$). Grade 3 and 4 hematological toxicities were more frequent for TPC, but did not lead to an increased frequency of neutropenic fever. There were also no toxicity-related deaths.

In order to reduce the toxicities associated with the TPC schedule as mentioned above, the North Central Cancer Treatment Group study compared the efficacy and tolerability of paclitaxel, carboplatin and trastuzumab q3w *versus* weekly as first-line therapy for HER-2/neu-positive metastatic breast cancer, defined as HER-2/neu 3+ by immunohistochemistry or HER-2/neu gene amplified by

fluorescence-*in situ*-hybridisation (22). The q3w regimen was paclitaxel 200 mg/m^2, carboplatin AUC 6 and trastuzumab (8 load then 6 mg/kg) administered on day 1 q3w for 8 cycles (6 months), followed by weekly trastuzumab until progression. The weekly regimen was paclitaxel 80 mg/m^2, carboplatin AUC 2 and trastuzumab administered on day 1 weekly for 3 out of 4 weeks for 6 cycles (6 months), followed by weekly trastuzumab until progression. Preliminary data indicate neutropenia was the most common toxicity, with grade 4 toxicity in 69% of patients on the q3w arm and 12% of patients on the weekly arm. Thus, the trial demonstrated that the combination of paclitaxel, carboplatin and trastuzumab is better tolerated when administered by the weekly regimen than the every 3-week regimen.

Trastuzumab and different anthracyclines in sequence or combination

As described above, trastuzumab in combination with chemotherapy results in higher response rates, longer control of disease and superior survival in patients with metastatic breast cancer. In a recently presented phase II study, the hypothesis was tested whether the addition of Herceptin to anthracycline-containing chemotherapy in the neoadjuvant setting could increase the pathological complete response rates (23). A total of 42 patients with HER-2/neu-positive disease (IHC 3+ or FISH +) with operable breast cancer were randomised to 4 cycles of paclitaxel followed by 4 cycles of FEC, or the same chemotherapy with simultaneous weekly Herceptin at 2 mg/kg for 24 weeks. The primary objective was to demonstrate a 20% improvement in pathological complete remission (assumed 21% to 41%) with the addition of Herceptin to the chemotherapy. The planned sample size of this investigation was 164 patients, however, after 34 patients had completed therapy, the trial's Data Monitoring Committee stopped the study because of the superiority of chemotherapy plus Herceptin. Pathological complete remission rates were 25% and 67%, respectively, for chemotherapy alone (n=16) and chemotherapy plus Herceptin (n=18) (p=0.02). A decrease in the cardiac ejection fraction (>10%) was observed in 6 and 4 patients, respectively, in the chemotherapy arm as compared to the chemotherapy plus Herceptin arm. No patient had clinical congestive heart failure or other clinically relevant cardiac toxicity. Based on these results, the trial was stopped with a Bayesian predictive probability of 0.95 that the trial would conclude a significant benefit for chemotherapy plus Herceptin if completed to the originally planned 164 patients.

These results indicate that the combination of an anthracycline, a taxane plus Herceptin is extraordinarily active in HER-2/neu-positive breast cancer. On the other hand, the potential for cardiac toxicity when trastuzumab is combined with anthracyclines suggests a further rationale for the integration of liposomal anthracyclines into combined chemo-immunotherapy (24). The liposome-encapsulated form of doxorubicin, Myocet®, is as effective as doxorubicin in metastatic breast cancer, with significantly reduced cardiotoxicity. A regimen of Myocet 50 mg/m^2, paclitaxel 80 mg/m^2 and Herceptin 4 mg/kg followed by 2 mg/kg was selected for a phase II efficacy study for which previously untreated patients with HER-2/neu-positive (IHC 3+/FISH+) locally advanced or metastatic breast cancer were eligible. Cardiac function was assessed every 3 weeks and tumor response every 6 weeks, with the primary end-point being response rate. In a preliminary analysis, the overall response rate was 87.5% (CR 15, PR 13). An additional 4 patients had stable disease. The most common events were febrile neutropenia (n=8) and infections (n=7). One case of cardiac insufficiency was reported, but was judged to be not related to the therapy. Thus, Myocet in combination with Herceptin and paclitaxel resulted as a very active regimen with acceptable toxicity. No treatment-related symptomatic cardiotoxicity had occurred.

Pathophysiological background for adjuvant trastuzumab therapy

In the original publication by Slamon *et al.*, the amplification of the *HER-2/neu* gene was a significant predictor of both overall survival and time to relapse in patients with breast cancer (25). It retained its significance even when adjustments were made for other known prognostic factors. Moreover, HER-2/neu amplification had greater prognostic value than other traditional prognostic factors, including hormonal receptor status, in lymph node-positive disease. One major reason for the prognostic value of HER-2/neu may be the high incidence of HER-2/neu expression on micrometastatic breast cancer cells in the bone marrow, which suggests that these cells might have been positively selected during the early stages of metastasis (26, 27). The majority of these cells appear to be in a dormant state of cell growth, making them a biologically relevant target for adjuvant Herceptin therapy. This is even more true as the cytotoxic agents currently used for chemotherapy in high-risk breast cancer patients do not completely eliminate cytokeratin-positive tumour cells in the bone marrow (28).

For the reasons described above, the adjuvant use of trastuzumab may become paramount for HER-2/neu-overexpressing breast cancer patients. Therefore, it must be evaluated in randomised controlled trials (29). There is disagreement regarding the design of such a trial, largely because of the ubiquitous use of anthracyclines in the adjuvant setting and the opposing necessity of avoiding

anthracycline plus trastuzumab combinations. The HERA Trial, involving countries outside the US, has examined q3-weekly Herceptin monotherapy given for 1 and 2 years after the completion of adjuvant chemo-/radiation therapy (30). It is the only trial worldwide focussing on a 2-year adjuvant therapy with Herceptin, which will have important clinical implications for the after-care of those patients, since the first data on the risk profile of adjuvant Herceptin is now available from the NSABP-B 31 study. This adjuvant trial uses a control arm of doxorubicin/cyclophosphamide for 4 cycles followed by paclitaxel for 4 cycles, and the second arm contains the addition of trastuzumab to the taxane sequence (31). This study was the first to present 9-month safety data in terms of cardiac events. It could be shown that the frequency of patients with an ejection fraction <50% increased statistically significantly first during anthracycline therapy with doxorubicin, and then again under Herceptin sequential treatment. The overall cardiac event rate was 4.5%, while the per protocol acceptable rate was 5%. Thus, the trial was continued, but physicians must be aware of the increased cardiac risk, especially after 2 years of Herceptin therapy, as in the HERA trial, since 2 years of treatment should further increase the cardiac risk.

Monitoring of Herceptin-based therapies

From a clinical perspective, there is a great value for serum HER-2/neu monitoring of Herceptin-based forms of chemotherapy. Pegram *et al*. evaluated serum HER-2/neu changes in metastatic breast cancer patients undergoing treatment with cisplatin in combination with the monoclonal antibody, 4D5, the murine monoclonal antibody precursor to trastuzumab (32). The authors reported that the difference in circulating serum HER-2/neu levels between the pre-treatment sample and the day 70 post-treatment sample was significantly associated with clinical outcomes of disease progression, stable disease or response to therapy. The authors concluded that serial measurements of serum HER-2/neu levels are useful in determining treatment failure in patients who continually show increases of the circulating serum HER-2/neu concentration.

In the study by Esteva *et al.*, 30 patients with advanced breast cancer were monitored for serial changes of serum HER-2/neu while being treated with docetaxel and trastuzumab (33). The circulating HER-2/neu levels were measured at baseline and at the time of response evaluation (after cycles 2 and 4 and every 3 cycles thereafter). In 14 of the 16 patients who responded to therapy, there was a decrease of the serum HER-2/neu concentration. In 12 of these patients, the serum HER-2/neu levels fell below the upper limit of normal, 15 ng/mL. In another study, Köstler *et al.* monitored 75 metastatic breast cancer patients, with serum HER-2/neu levels being assessed immediately before each administration of trastuzumab-based therapies. These baseline levels were then compared with the serum HER-2/neu levels as determined at the time of re-staging examinations (34). Investigators observed that the baseline serum HER-2/neu levels prior to the initiation of therapy did not predict the patient objective response to therapy. However, the ratio of serum HER-2/neu levels prior to initiation of treatment and at the time of re-staging examinations was significantly ($p<0.01$) higher in those patients who had a significant benefit from the therapy.

Our own group evaluated serum HER-2/neu levels weekly in 54 advanced breast cancer patients being treated with trastuzumab (35). A receiver operating characteristics (ROC) curve for progressive disease *versus* clinical benefit (CR + PR + SD) was generated (AUC=0.78, 95% CI: 0.6-0.94) which indicated that the serum HER-2/neu levels at 3-5 weeks relative to the baseline were a significant discriminator of response. As an example, if a patient did not show a minimum of at least a 65% decrease in comparison to the baseline at week 4 after initiation of therapy, there was a 90% probability that the patient would not benefit from the therapy. Such information would prompt an earlier re-staging for that patient with, possibly, a change in the chemotherapeutic component of the regimen. In addition, the probability of response to trastuzumab-based therapies was greater with increasing baseline levels of serum HER-2/neu. It was determined that there was a 23.1% chance of response if the serum HER-2/neu level was less than 15 ng/mL, a 35.5% chance of response if the serum HER-2/neu level was between 15-50 ng/mL and a 46.7% chance of response if the serum HER-2/neu levels were greater than 50 ng/mL.

Currently, there are only a few published reports on the longitudinal assessment of metastatic breast cancer patients using the combination of CA 15-3 and serum HER-2/neu. In a study by Ali *et al.*, CA 15-3 was measured along with serum HER-2/neu in 566 ER-positive or PR-positive metastatic breast cancer patients (36). Overall, 30% of the patients had an increase in serum HER-2/neu levels and 60% had increases in CA 15-3 levels. This study found that serum HER-2/neu was a significant independent predictive and prognostic factor in hormone-receptor-positive metastatic breast cancer patients, even when adjusted for tumour burden as measured by CA 15-3. However, the combination of increased HER-2/neu and CA 15-3 predicted a worse prognosis than did increased CA 15-3 alone.

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