Endocrine-Related Cancer (2002) **9** 75–85

The development and clinical use of trastuzumab (Herceptin)

M Harries and I Smith

Breast Unit, The Royal Marsden Hospital and Institute of Cancer Research, Fulham Rd, London SW3 6JJ, UK

(Requests for offprints should be addressed to M Harries; Email: mark.harries@rmh.nthames.nhs.uk)

Abstract

HER-2 is a member of the c-erbB family of receptor tyrosine kinases and is overexpressed by 20–30% of human breast cancers. HER-2 overexpression is an independent adverse prognostic factor and may also predict for response to both chemotherapy and endocrine agents. Trastuzumab is a humanised monoclonal antibody that binds with high affinity to the extracellular domain of HER-2. In HER-2-overexpressing preclinical models trastuzumab has been shown to have a marked antiproliferative effect and demonstrates synergy with a number of cytotoxic drugs. Several phase II and phase III clinical trials have now been performed in patients with advanced breast cancer that overexpress HER-2. Trastuzumab was initially shown to be active and well tolerated as a single agent in heavily pretreated women. Subsequently, studies of first-line treatment for metastatic breast cancer have demonstrated an improvement in survival for trastuzumab when used in combination with either paclitaxel or an anthracycline–cyclophosphamide regimen compared with chemotherapy alone. Unexpectedly, the combination of trastuzumab and the anthracycline-containing regimen was associated with a significant incidence of cardiac dysfunction. The benefit of trastuzumab is generally confined to patients whose tumours have gene amplification as detected by fluorescence *in situ* hybridisation (FISH) and this is tightly associated with immunohistochemical (IHC) staining at the highest (3+) level. A small number of patients have IHC 2+ tumours together with FISH evidence of gene amplification and may also derive benefit from treatment. Trastuzumab has also been shown to be effective when used as first-line monotherapy for advanced breast cancer. Trials to date have employed trastuzumab in a weekly schedule, but there is emerging evidence that a three-weekly regimen may be as effective. Trastuzumab has shown encouraging activity when used with other agents including docetaxel and vinorelbine. The combination of trastuzumab, docetaxel, and platinum salts also appears to be very active. The role of trastuzumab as adjuvant therapy for early breast cancer is being tested in a number of large randomised trials.

Endocrine-Related Cancer (2002) **9** 75–85

Introduction

In recent years a number of the molecular pathways responsible for tumorigenesis have been identified (Hanahan & Weinberg 2000). With this knowledge has come the possibility of effective anti-cancer treatments aimed at novel targets with few of the toxicities associated with conventional cytotoxic therapies. This review focuses on the development and use of trastuzumab (Herceptin), a humanised monoclonal antibody specific for HER-2, a growth factor receptor found to be overexpressed in 20–30% of human breast cancers (Slamon *et al*. 1987, 1989). The clinical data that have led to the recent licensing of trastuzumab as a treatment for metastatic breast cancer will be presented together with a discussion of the controversial areas that surround its use and an assessment of current clinical trials to define further the optimal usage of this agent. First, the background to the discovery of the HER-2 target and the preclinical validation of antibody therapies directed against it will be presented.

Background

HER-2 is a member of the erbB epidermal growth factor receptor tyrosine kinase family. In the early 1980s the erbB receptor tyrosine kinases became implicated in cancer when it was found that the avian erythroblastosis tumour virus encoded an oncogene that was highly homologous to the human epidermal growth factor receptor (HER-1, also known as ErbB1 and EGFR). Subsequently a gene called *neu* was

1351-0088/02/009–075 © 2002 Society for Endocrinology *Printed in Great Britain*

identified from a chemically induced rat neuroblastoma that was able to transform fibroblast cell lines in culture and was shown to be related to but distinct from the HER-1 gene (Shih *et al.* 1981, Schechter *et al.* 1984). At about the same time two other groups independently isolated human *erb*B-related proto-oncogenes and named them HER-2 (Coussens *et al.* 1985) and c-*erb*B2 (Semba *et al.* 1985). These genes were then shown to be the same as *neu*. King and colleagues also identified an EGFR-related gene that was overamplified in a human mammary carcinoma cell line; this gene was also found to be identical to the HER-2/*neu*/erbB2 gene (King *et al.* 1985).

HER-1 and HER-2 differ in a number of ways: the HER-2 gene is located on chromosome 17 whereas the HER-1 gene has been mapped to chromosome 7, and the HER-2 mRNA and protein are of different sizes from the HER-1 gene products. The erbB receptor tyrosine kinase family has two other members, HER-3 and HER-4 (erbB3 and erbB4), with the four receptors sharing an overall membrane spanning structure composed of extracellular and transmembrane components together with an intracellular region containing a kinase domain flanked by tyrosine autophosphorylation sites. There are a number of functional differences between the domains of the different family members. For example, HER-2 appears to have no direct ligand and HER-3 has no intrinsic kinase activity and therefore a number of complex interactions between the different family members involving dimerisation are required for signalling. The HER-2 receptor can signal by forming heterodimers with other members of the HER family that are bound to a ligand, or two HER-2 molecules can combine to form a homodimer which has intrinsic kinase activity. Overexpression of HER-2 favours the production of both activated homo- and hetero-dimers. erbB receptor kinase activation recruits a number of adaptor proteins to the cytoplasmic domains which in turn trigger a number of downstream signalling cascades. The end results of HER-2 activation are effects on cell growth, division, differentiation, migration and adhesion (reviewed in Yarden & Sliwkowski 2001).

Slamon and colleagues initially reported that the HER-2 receptor was overexpressed in 20–30% of human breast cancers (Slamon *et al.* 1987). In the vast majority of cases overexpression is caused by amplification of the HER-2 gene (Pauletti *et al.* 1996). HER-2 gene amplification results in increased levels of mRNA as detected by Northern blot and of the HER-2 receptor as detected by immunohistochemistry (IHC) or Western blot analysis. Over amplification of the gene is most strikingly seen using fluorescence *in situ* hybridisation (FISH), when multiple copies of the HER-2 gene can be seen in the nuclei of affected cells. This technique has become a useful method of detecting HER-2 gene amplification in clinical samples.

HER-2 gene amplification is seen early in the development of invasive breast carcinoma and can also be seen in ductal carcinoma *in situ* (Barnes *et al.* 1992). Overexpression of HER-2 in breast cancer cells correlates with a number of well recognised adverse histological prognostic features including tumour size, high grade, a high percentage of S-phase cells, aneuploidy and lack of steroid receptors (Slamon *et al.* 1987, Ross & Fletcher 1999). Overexpression of HER-2 correlates with poor breast cancer prognosis. In 1987, Slamon and colleagues examined tumour samples from 86 node-positive patients and found that overamplification of the HER-2 gene was a strong independent adverse prognostic factor (Slamon *et al.* 1987). In 1989, Slamon and co-workers collected a total of 668 human breast cancer specimens, 526 of which had sufficient clinical information to examine a link between HER-2 expression and outcome. Of 345 node-positive patients, 27% had HER-2 gene amplification. This was found to be a significant predictor of disease-free and overall survival in multivariate analysis ($P = 0.006$ and 0.045 respectively) and was superior to all other prognostic factors except nodal status. In this initial study there was no association found between gene amplification and disease outcome in the 181 node-negative patients (Slamon *et al.* 1989). In a subsequent retrospective series of 1506 patients from the Ludwig International Breast Cancer Study Group, HER-2 overexpression was again found to be prognostically significant in node-positive but not in node-negative patients with breast cancer (Gusterson *et al.* 1992). However, more recent studies have demonstrated that HER-2 gene amplification is an independent prognostic factor even in node-negative patients (Seshadri *et al.* 1993, Press *et al.* 1997 and reviewed in Ross & Fletcher 1998).

Current evidence suggests that there may be an association between the overexpression of HER-2 and response to therapy. For example, in endocrine therapy a recent neoadjuvant trial has suggested a significantly higher response rate to letrozole than to tamoxifen in a small subset of patients with both HER-1- and/or HER-2-positive and oestrogen receptor (ER)-positive cancers (15/17 88% compared with 4/19 21%, odds ratio 28, $P = 0.004$) (Ellis *et al.* 2001). Likewise, in chemotherapy circumstantial data suggest that anthracyclines may be more effective than cyclophosphamide, methotrexate and 5-fluorouracil (CMF) schedules for patients with HER-2-overexpressing breast cancer. This complex area has recently been reviewed by Piccart *et al.* (2000).

Although the evidence is intriguing, there are currently insufficient data to suggest that HER-2 status be used to select therapy for patients other than their suitability for HER-2-directed therapies. In 2000, the American Society of Clinical Oncology (ASCO) committee on the update of recommendations for the use of tumour markers in breast and colorectal cancer stated that 'levels of c-erbB2 expression should not be used to exclude patients from anthracycline treatment' and 'the use of c-erbB2 data to decide whether to prescribe endocrine therapy either in the adjuvant or metastatic setting is not recommended' (Bast *et al.* 2001).

Development of trastuzumab

The discovery of HER-2 overexpression in a significant minority of human breast cancers and its adverse prognostic significance prompted investigators to develop agents using HER-2 as a target for treatments. Several groups including workers at Genentech Inc. raised murine monoclonal antibodies to the extracellular domain of HER-2 and showed that some of these antibodies were capable of inhibiting the growth of cell lines that overexpressed the receptor (Hudziak *et al*. 1989, Fendly *et al*. 1990). This effect was also seen in HER-2-overexpressing human breast cancer xenografts where the effects of the antibody were found to be synergistic to anti-neoplastic agents such as cisplatin (Pietras *et al*. 1994).

The Genentech researchers developed a panel of murine monoclonal antibodies capable of inhibiting HER-2 + cell lines; the most potent of these was muMAb 4D5. This antibody was found markedly to inhibit proliferation of cell lines that overexpressed HER-2 but had little or no effect on cells without elevated levels of HER-2 (Sarup *et al*. 1991). 4D5 was found to be a potent inhibitor of growth of human breast cancer xenografts (Baselga & Mendelsohn 1994) and was therefore selected for further clinical development.

In order to reduce the potential for generating a human anti-mouse immune response the 4D5 murine monoclonal antibody was humanised. Carter and colleagues subcloned the hypervariable region of the antibody into plasmids encoding a human κ light chain and the IgG1 constant region to generate a vector encoding a chimeric antibody which was then further humanised by site-directed mutagenesis (Carter *et al*. 1992). The vector was transduced into Chinese hamster ovary (CHO) cells that then secrete the antibody into the culture medium from which it is purified. The chimeric antibody called trastuzumab is 95% human and 5% murine and retains the high affinity for the HER-2 epitope of the parental antibody.

Preclinical pharmacology

Trastuzumab has a binding affinity for HER-2 that is three times greater than that of its parent murine antibody 4D5. Like 4D5, it has been shown to have a marked anti-proliferative effect on HER-2-overexpressing cell lines and very little effect on cells not expressing HER-2 (Carter *et al*. 1992). This anti-proliferative effect has also been demonstrated *in vivo* in breast cancer xenograft experiments by Baselga and colleagues in which established BT-474 tumour xenografts were inhibited from growing by trastuzumab. In doses of less than 1 mg/kg growth was inhibited in a dose-dependent fashion and no growth at all was seen at higher doses (Baselga *et al*. 1998). In the same study, the researchers explored the addition of trastuzumab to either paclitaxel or doxorubicin. Chemotherapy alone was shown to have only modest anti-tumour activity, whereas combined treatment with trastuzumab resulted in a marked enhancement of the effect of chemotherapy with the greatest growth inhibition being seen with paclitaxel and trastuzumab (Baselga *et al*. 1998).

Pegram and colleagues examined the effect of trastuzumab on a number of other chemotherapeutic agents in a HER-2 transfected MCF7 xenograft model. Synergistic interactions were seen with cisplatin, docetaxel, thiotepa, cyclophosphamide, vinorelbine and etoposide. Additive effects were seen with doxorubicin, paclitaxel, vinblastine and methotrexate and the combination of trastuzumab with 5-fluorouracil (5-FU) was found to be antagonistic (Pegram *et al*. 1999, Konecny *et al*. 2001 and reviewed in Pegram *et al*. 2000). The synergy seen in these *in vivo* models has led to the exploration in clinical trials of trastuzumab in combination with chemotherapy.

Trastuzumab was shown to be safe when administered chronically to a range of animals including primates (reviewed in Roche 2001).

Mechanism of action

The mechanism by which trastuzumab inhibits the growth of HER-2-expressing breast cancer cells and interacts with cytotoxic drugs is uncertain. It has been shown that trastuzumab downregulates the HER-2 receptor by accelerating endocytic degradation (De Santes *et al*. 1992). The reduction in available HER-2 receptor results in fewer homo- and heterodimers capable of signalling. Either as a result of reduced numbers of receptors or by a direct inhibitory effect, trastuzumab antagonises growth and cell division signals associated with HER-2 signalling (reviewed in Sliwkowski *et al*. 1999). In addition to cell cycle effects, trastuzumab may also antagonise the induction of angiogenic factors such as vascular endothelial growth factor by HER-2 (Petit *et al*. 1997). The ability of cells to repair DNA damage after radiation or chemotherapy damage may also be impeded by trastuzumab (Pietras *et al*. 1999). In addition to direct effects on HER-2 signalling, trastuzumab may induce a host tumour response via antibody-dependent cell cytotoxicity (ADCC) mechanisms and complement activation (Hotaling *et al*. 1996).

Early clinical trials

The first single agent phase I studies of trastuzumab began in 1992. In the first study, 16 patients with HER-2-positive metastatic breast cancer were treated with fixed doses of 10–500 mg as a single dose. Subsequently, two phase I studies evaluated a weekly schedule of trastuzumab either alone or in combination with cisplatin. In neither study was dose-limiting toxicity reached with trastuzumab doses of 10–500 mg, and 100 mg was taken forward as the recommended phase II dose. Pharmacokinetic data showed a half-life of 8.3

days, thus suggesting a weekly schedule (summarised in Roche 2001).

Baselga and colleagues reported on 46 patients with metastatic breast cancer who had received extensive prior chemotherapy treated with a 250 mg loading dose of trastuzumab followed by weekly doses of 100 mg until disease progression. Five out of 43 assessable patients had clinical responses (12%) and 16 additional patients also had minor responses or stable disease with a median time to progression of 5.1 months (Baselga *et al*. 1996). A separate study evaluated the same dose of trastuzumab in combination with cisplatin in a similar group of heavily pretreated patients. Of 37 patients evaluable for response, 9 patients had a partial response (24%). No excessive toxicity was seen above that expected for cisplatin alone (Pegram *et al*. 1998).

Pivotal trials

In parallel with these early trials a large multinational phase II study was initiated to confirm the efficacy and safety of trastuzumab. Two hundred and twenty-two women were enrolled into the study between April 1995 and September 1996. All women had previously received one or two chemotherapy regimens for metastatic breast cancer and had tumours that overexpressed HER-2 as determined by IHC in a central reference laboratory by weak to strong membrane staining in >10% of cells (IHC 2+ or 3+). Patients received trastuzumab 4 mg/kg as a loading dose and then 2 mg/kg weekly thereafter until disease progression. The primary endpoint was response rate and secondary endpoints were duration of response, time to disease progression, time to treatment failure, and survival. In an intent to treat analysis the overall response rate was found to be 15% (95%, confidence interval (CI) 11–21%) as determined by an independent response evaluation committee. The median duration of response was 9.1 months (95%, CI 6.5–10.5 months). Amongst all treated patients median survival was 13 months, median time to progression was 3.1 months and median time to treatment failure was 11 months. In the subgroup of patients whose tumours had IHC staining that was 3+, the response rate was 18% and median survival 16.4 months, suggesting that this group of patients derived greatest benefit from trastuzumab. A retrospective analysis of gene expression by FISH showed that the response rate was 19% in patients with gene amplification (FISH+) and was 0% in the FISH-negative group. The median number of infusions received was 12 (range 1–96) and treatment was generally found to be well tolerated, with the most common adverse event being mild to moderate infusion reactions that usually only occurred with the first treatment and were managed successfully with paracetamol and/or antihistamines. The incidence of cardiac dysfunction, defined as congestive heart failure, cardiomyopathy and/or a decrease in ejection fraction of >10% was examined by retrospective analysis and was identified in 4.7% of patients. This trial confirmed that trastuzumab was active and generally well tolerated as a single agent in heavily pretreated patients (Cobleigh *et al*. 1999).

The synergy seen in the preclinical studies between trastuzumab and cytotoxic agents encouraged investigators to evaluate combination therapy in the clinic. A large multinational phase III study was performed to compare chemotherapy in combination with trastuzumab to chemotherapy alone as first-line therapy in patients with metastatic breast cancer whose tumours were found to overexpress HER-2 (again as determined to be IHC 2+ or 3+ in a central reference laboratory). Four hundred and sixty-nine patients were randomised to receive chemotherapy alone or chemotherapy plus trastuzumab. Patients who had received an anthracycline in an adjuvant setting received paclitaxel 175 mg/m^2 three-weekly and for other patients chemotherapy was an anthracycline (the majority receiving doxorubicin 60 mg/m^2) and cyclophoshamide 600 mg/m^2 three-weekly. Chemotherapy was given every three weeks for 6 cycles and then further chemotherapy was allowed to be given at the discretion of the investigator. Trastuzumab was given as a loading dose of 4 mg/kg and then 2 mg/kg weekly thereafter until disease progression. Patients had measurable disease and were of good performance status. The primary study endpoint was time to progression, with secondary endpoints of objective response rate, duration of response, time to treatment failure and 1-year survival. At a median of 30 months of follow-up the time to progression for patients receiving both trastuzumab and chemotherapy was 7.4 months compared with 4.6 months for patients who received chemotherapy alone. The overall response rate and response duration were also improved in patients receiving the combination treatment. The addition of trastuzumab to paclitaxel increased the response rate from 17% to 41% and median response duration from 4.5 to 10.5 months. The addition of trastuzumab to the anthracycline regimens increased response rates from 42 to 56% and median duration of response from 6.7 to 9.1 months compared with chemotherapy alone.

Taking both groups of patients together, overall survival was significantly improved with the addition of trastuzumab to chemotherapy from 20.3 to 25.1 months ($P = 0.046$). Seventy-two percent of patients who had been randomised to receive chemotherapy alone subsequently received trastuzumab and therefore the magnitude of the survival advantage may be underrepresented due to this crossover effect. As with the multicentre phase II trial, the benefit for the addition of trastuzumab was particularly marked for patients whose tumours were 3+ on IHC staining. In addition, the benefit of trastuzumab was only seen in patients whose tumours had amplification of the HER-2 gene as determined by FISH. In patients without gene amplification, response rates were not significantly improved in the trastuzumab-containing regimens. In contrast, for patients with HER-2 amplification the response rate to chemotherapy alone was 31% and this

increased to 54% in the trastuzumab-containing regimens. In this group median survival was increased from 20 to 26.2 months ($P = 0.007$) (Mass *et al*. 2001*a*, Slamon *et al*. 2001).

Trastuzumab as first-line monotherapy

Subsequent to the two pivotal studies discussed above, Vogel and colleagues (2002) have reported the results of an important phase II study of single agent trastuzumab as first-line treatment for 114 patients with metastatic breast cancer. In this study HER-2-overexpressing patients were randomised to receive either standard dose (4 mg/kg loading then 2 mg/kg weekly) or a higher dose regimen (8 mg/kg loading dose and 4 mg/kg weekly thereafter) until disease progression. The overall response rate was 24% for the standard trastuzumab dose arm, 28% for those receiving the higher dose (non-significantly different) and was 26% for all patients in the study. A further 13 patients had stable disease that persisted for greater than 6 months. As in the previous studies, higher response rates were seen in IHC 3+ patients and FISH+ patients (35% and 41% respectively). In FISH-negative patients, the response rate was only 7%. Median survival for all patients was 24.4 months but follow-up is short and at the time of censor 49 patients were alive or lost to follow-up. In this group of patients single agent trastuzumab was extremely well tolerated with only 2 (2%) patients discontinuing therapy due to adverse events, both of whom had a history of cardiac disease and were withdrawn due to cardiac dysfunction. Furthermore, there was an overall improvement in global quality of life and fatigue scores (Vogel *et al*. 2002).

Measuring HER-2 expression

These clinical studies have shown a strong correlation between the efficacy of trastuzumab and the strength of IHC staining, with patients with IHC 3+ tumours responding better than those with 2+ tumours. Trastuzumab efficacy was also highly correlated with gene amplification with almost no benefit seen in tumours that had normal copy numbers of the gene. FISH has been shown to select almost all IHC 3+ HER-2-expressing tumours. In addition, in these studies and others about 24% of patients whose tumours stained 2+ by IHC were found to be FISH+ (Mass *et al*. 2001*b*). It is therefore becoming the practice of many clinicians to select patients for trastuzumab on the basis of IHC 3+ reactivity and if staining is 2+ then treatment is only given if gene amplification is present on FISH analysis. Unfortunately FISH is not so far available in all centres.

Clinical safety of trastuzumab

The large studies of trastuzumab have shown it to be generally a well tolerated treatment. The commonest side effects were mild to moderate infusion reactions that were easily manageable and usually only associated with the first treatment.

Unexpectedly, cardiac dysfunction that had not been seen or predicted from the preclinical studies occurred in a number of patients. In the single agent phase II study for pretreated patients, the incidence of symptomatic heart failure was 6–8.8% and all but one of these patients had received prior anthracyclines (Cobleigh *et al*. 1999). In the first-line phase II study of single agent trastuzumab only 2% of patients had to stop treatment due to cardiac dysfunction (Vogel *et al*. 2002). In the trastuzumab–chemotherapy combination study there was a marked increase in the incidence of heart failure for patients receiving trastuzumab plus an anthracycline, with an incidence of cardiac dysfunction (all grades) of 26–28% compared with 6–9.6% for patients who had received the anthracycline regimen alone. The incidence of marked symptomatic dysfunction (New York Heart Association (NYHA) grade III–IV) was 16% and 3% respectively. Only 1–4% of patients treated with paclitaxel alone developed cardiac dysfunction (1% at NYHA grade III/IV) compared with 8.8–11% (2% at NYHA grade III/IV) in patients treated with the trastuzumab and paclitaxel combination. In the small numbers of patients who had not received prior or concurrent anthracycline the incidence of cardiac dysfunction was 4%, all of whom were found to have had risk factors for cardiac disease. Most of the patients with symptomatic heart failure continued to receive trastuzumab and 75% improved with standard medical management. Only in the anthracycline/trastuzumab-treated patients were a small minority of patients (6%) left with NYHA grade III/IV toxicity at the end of treatment. Deaths related to cardiac dysfunction were very rare with no significant difference in incidence between the subgroups (Slamon *et al*. 2001, Tripathy *et al*. 2001). It should be noted that these data were collected retrospectively but prospective monitoring of cardiac function is an integral part of all current trastuzumab studies.

Thus experience with trastuzumab to date suggests that cardiac toxicity is particularly related to the concurrent use of anthracyclines. In addition, the incidence of cardiac dysfunction seems greatest in patients with pre-existing risk factors for heart failure.

The mechanism of cardiotoxicity is unclear. It appears unlikely that trastuzumab-associated heart damage occurs directly as a result of HER-2 expression on heart muscle. In 60 patients with evidence of cardiac dysfunction, including 25 patients with previous anthracycline exposure, Fuchs and colleagues found only faint membrane staining in less than 10% of cardiac biopsies and no evidence of gene amplification by FISH (Fuchs *et al*. 2001). It also seems unlikely that trastuzumab alters the pharmacokinetic properties of anthracyclines and one study has failed to demonstrate any difference in the pharmacokinetics of doxorubicin when it was administered before or after trastuzumab (Gianni *et al*. 2001).

One possible hypothesis, partly born out by preclinical data, is that trastuzumab impairs growth factor-mediated repair of anthracycline-induced cardiac damage (reviewed in Chien 2000).

Therefore, it is current policy not to recommend concurrent treatment with trastuzumab and doxorubicin. In patients with previous exposure to anthracyclines or with a predisposition to heart disease careful monitoring of cardiac function either with echocardiography or multigated radionucleotide angiography (MUGA) should be performed to pick up early evidence of cardiac dysfunction that may necessitate withdrawal of trastuzumab. If symptomatic cardiac dysfunction develops whilst on trastuzumab then standard medical therapy should be initiated and is generally effective.

Post-marketing safety analysis has indicated that there is also a rare incidence of severe pulmonary events associated with trastuzumab that may or may not follow severe infusion reactions. The clinical picture can include dyspnoea, pulmonary infiltrates, pleural effusions, non-cardiogenic pulmonary oedema, and adult respiratory distress syndrome. Patients with symptomatic intrinsic lung disease or with extensive tumour involvement of the lungs resulting in dyspnoea at rest may be at greater risk of such events (Roche 2001).

Summary of the pivotal trials and licensing of trastuzumab

The two large pivotal trials discussed above demonstrated that trastuzumab was active as a single agent in patients with metastatic breast cancer who had previously received chemotherapy and when used in combination with paclitaxel or anthracycline–cyclophosphamide regimens was associated with improved survival in first-line therapy of metastatic breast cancer compared with chemotherapy alone. The benefit of trastuzumab was greatest for patients whose tumours expressed HER-2 at the 3+ level by IHC and was generally only seen in patients who had evidence of gene amplification as detected by FISH. Although trastuzumab was generally well tolerated, cardiac dysfunction was seen in some patients who received an anthracycline–cyclophosphamide regimen together with trastuzumab.

As a result of these trials trastuzumab is now licensed in many countries for use as first-line therapy in combination with paclitaxel for patients whose tumours overexpress HER-2 and as a single agent in paclitaxel pretreated patients. It is our view that overexpression of HER-2 in this context should be defined as IHC 3+ or IHC 2+ and FISH+.

Timing of trastuzumab treatment

An important area of debate concerns the optimal timing of trastuzumab treatment. Options for a patient with HER-2-overexpressing metastatic breast cancer might include trastuzumab as first-line monotherapy followed by chemotherapy at progression, or in combination with chemotherapy, or as single agent therapy after failure of first-line chemotherapy. As discussed above the pivotal phase III trial demonstrated a survival advantage for the trastuzumab and chemotherapy combination compared with chemotherapy alone despite a large number of the patients on the chemotherapy alone arms receiving trastuzumab on disease progression. These data and the synergy seen in preclinical xenografts would suggest that combination therapy should be superior to sequential therapy with chemotherapy followed by trastuzumab.

On the other hand, for patients whose tumours were FISH+ the phase II study of first-line single agent trastuzumab reported by Vogel and colleagues reported an overall survival on a par with the combination of chemotherapy and trastuzumab seen in the large phase III studies (23 months compared with 26.8 months). This suggests that there may be no survival disadvantage for sequential therapy compared with combination treatment for this group (Slamon *et al*. 2001, Vogel *et al*. 2002). Furthermore, some patients relapsing after adjuvant chemotherapy might prefer the improved quality of life associated with trastuzumab alone compared with trastuzumab in combination with more chemotherapy. Trials addressing this issue are ongoing: for example, a European study is comparing first-line trastuzumab followed at disease progression by combination trastuzumab and paclitaxel compared with a first line combination of trastuzumab and paclitaxel.

Duration of therapy for metastatic disease

The trials discussed above have all continued the use of trastuzumab until disease progression based on the preclinical tumour xenograft models that demonstrated tumour regrowth could occur after trastuzumab withdrawal (Pietras *et al*. 1998). Nevertheless, clinical trials to determine the optimum duration of therapy are necessary on both clinical and cost grounds.

For patients being treated with chemotherapy and trastuzumab conventional practice in cancer therapy would suggest that both agents should be stopped at disease progression. But it could be argued that there might be benefit in the continued use of trastuzumab either alone or in combination with an alternative chemotherapy agent based on trastuzumab having a residual effect in slowing tumour growth or in promoting synergy with other cytotoxic agents. Trials examining this issue are warranted.

Newer initiatives

Trastuzumab and docetaxel

Preclinical studies have demonstrated the synergy between docetaxel and trastuzumab to be greater than that seen with

paclitaxel (Pegram *et al*. 1999, Konecny *et al*. 2001). Two phase II studies of this combination have been reported. Kuzur and colleagues evaluated the combination of trastuzumab in standard dose (4 mg/kg loading then 2 mg/kg weekly thereafter) together with docetaxel 75 mg/m^2 three-weekly as first- or second-line treatment for patients with HER-2-overexpressing metastatic breast cancer. At the time of reporting, 21 patients had been treated with a median of 6 cycles of chemotherapy. Of the 21 patients enrolled, 16 patients were evaluable and 6 responses had been seen (response rate 45%) (Kuzur *et al*. 2000, Burris 2001). The Eastern Cooperative Oncology Group (ECOG) is conducting a pilot study of weekly docetaxel (35 mg/m^2) in conjunction with trastuzumab. Preliminary data have been reported in abstract form for 22 evaluable patients and a response rate of 63% has been seen, rising to 73% in patients with tumours that stain 3 + by IHC (Uber *et al*. 2001). This weekly regimen is also being evaluated in another ongoing study in which responses have been seen in 9 out of 22 patients (41%) in a preliminary report (Malik *et al*. 2000). This is clearly an active combination that requires further evaluation.

Trastuzumab, platinum salts and taxanes

Synergy was seen in preclinical models with both platinum salts and taxanes and trastuzumab. Concern with the incidence of cardiac dysfunction seen in patients in the phase III pivotal trial who received concurrent anthracyclines and trastuzumab has led investigators to explore trastuzumab–chemotherapy combinations with classes of drug less likely to cause cardiac toxicity. This issue is felt to be particularly important in designing adjuvant studies where long-term cardiac toxicity due to treatment is clearly unacceptable.

The Breast Cancer International Research Group (BCIRG) has conducted two multicentre phase II clinical studies of the combination of either carboplatin or cisplatin together with docetaxel and trastuzumab. The BCIRG study 101 has evaluated docetaxel 75 mg/m^2 in combination with cisplatin 75 mg/m^2 (both three-weekly) and trastuzumab (4 mg/kg loading then 2 mg/kg weekly). The BCIRG study 102 has evaluated docetaxel 75 mg/m^2 in combination with carboplatin AUC 6 (both three-weekly) and trastuzumab (4 mg/kg loading then 2 mg/kg weekly). Sixty-two patients were entered into each study and primary endpoints were safety and response rate with secondary endpoints of duration of response, time to progression and survival. Treatment was well tolerated with no increase in toxicity seen above that expected for the combination of chemotherapy agents, and importantly cardiac dysfunction was found to be minimal with National Cancer Institute (NCI) grade III or IV cardiac toxicity seen in 2 patients in the carboplatin trial (3.2%) and in 1 patient in the cisplatin trial (1.6%). Six cycles of treatment were deliverable in most patients. In a recent update to these studies the overall response rate to the cisplatin-containing regimen was 79% (CI 66–88%) and median time to progression was 9.9 months (CI 8.3–13.1 months) in all patients and 12.7 months in the FISH + subgroup. For the carboplatin-containing combination response rates were 56% (CI 40–69%) and the median time to progression was 12.0 months (CI 7.4–16.3 months) in all patients and 17 months in the FISH + subgroup (Slamon 2001). The BCIRG is now conducting a study evaluating the combination of platinum salts, docetaxel and trastuzumab in the adjuvant setting (BCIRG study 006 discussed below).

Trastuzumab and vinorelbine

Preclinical data also suggested a synergy between vinorelbine and trastuzumab (Konecny *et al*. 2001). Burstein and colleagues have published the results of a phase II study of standard dose trastuzumab in combination with vinorelbine 25 mg/m^2 weekly as first-, second- or third-line treatment for HER-2-overexpressing metastatic breast cancer. Forty patients were enrolled with an overall response rate of 75% (CI 57–89%) and 84% in patients who had received no prior treatment for metastatic disease (Burstein *et al*. 2001*b*). Myelosuppression was as expected for weekly single agent vinorelbine, and non-haematological toxicities were mild. A multicentre phase II study of this combination appears to confirm the efficacy and safety of this combination in a first-line setting with response rates of 60% in 20 evaluable patients (Jahanzeb *et al*. 2001). Further investigation of this regimen is warranted.

Trastuzumab with other anthracyclines

The problems seen with the combination of doxorubicin and trastuzumab has led investigators to explore alternative anthracyclines with potentially less cardiotoxicity such as epirubicin and liposomal doxorubicin. A large multicentre study is ongoing to evaluate first-line therapy for metastatic breast cancer in which patients are randomised to receive epirubicin and cyclophosphamide or epirubicin, cyclophosphamide and trastuzumab. The study has a dose escalation design with the first 25 patients receiving epirubicin 60 mg/m^2 and only if the incidence of cardiotoxicity is judged to be acceptable is the epirubicin dose to be escalated to 90 mg/m^2. The liposomal doxorubicin preparation TLC D-99 and trastuzumab have been delivered to 29 patients in a phase 1 study without significant cardiotoxicity and with responses seen in 8 of 14 evaluable patients (57%) (Winer *et al*. 2000).

Trastuzumab and capecitabine

5-FU and trastuzumab were found to be antagonistic in xenografts (Pegram *et al*. 1999). This antagonism may not be seen with capecitabine and some activity has been reported using this combination in a small study, with 8 out of 18 patients

achieving a response (Bangemann *et al*. 2000). Further larger studies of this combination are ongoing.

Endocrine agents and trastuzumab

A neoadjuvant endocrine trial has suggested that tumours that overexpress both ER and HER-2 might respond better to the removal of the oestrogen ligand by aromatase inhibition rather than to the competitive antagonism of the receptor by the partial agonist tamoxifen. In this study (discussed above) the response rates for letrozole and tamoxifen for tumours that were positive for HER-1 and/or HER-2 and ER + were 88% and 21% respectively ($P = 0.0004$) (Ellis *et al*. 2001). How this translates into the use of endocrine therapy in conjunction with trastuzumab is being examined in a series of clinical trials. One phase II trial will randomise patients whose tumours are ER + and/or progesterone receptor-positive (PGR +) together with HER-2-positive to receive either anastrozole alone or anastrozole plus trastuzumab. Other studies are looking at the combination of tamoxifen and trastuzumab and also the ability of trastuzumab to reverse tamoxifen resistance.

Newer schedules for trastuzumab

The weekly schedule of trastuzumab has been widely adopted following the initial pharmacokinetic studies that suggested that its half-life was in the order of 6–8 days. However, it has now been shown that with chronic administration the elimination half-life is much longer and that a three-weekly schedule should be feasible. Indeed pharmacokinetic data from a three-weekly trastuzumab trial have shown that plasma levels with an 8 mg/kg loading dose followed by a three-weekly dose of 6 mg/kg are similar to the conventional 4 mg/kg loading dose followed by 2 mg/kg weekly (Gelmon *et al*. 2001). The three-weekly schedule is being further evaluated in both metastatic and adjuvant settings.

Adjuvant trastuzumab trials

The improvements in survival seen with trastuzumab in the metastatic setting have prompted trials of trastuzumab as part of adjuvant therapy for patients with high-risk HER-2-overexpressing early breast cancer. Eligibility in all adjuvant trials is confined to patients whose tumours overexpress HER-2 at the 3 + level by IHC or that are FISH +.

The National Surgical Adjuvant Breast and Bowel Project (NSABP) is running a large multicentre trial (B-31) for patients with HER-2-positive and node-positive breast cancer. All patients will receive standard dose doxorubicin/cyclophosphamide three-weekly for four courses followed by either paclitaxel 175 mg/m^2 three-weekly alone for four courses or paclitaxel three-weekly for four courses in combination with weekly trastuzumab. In this arm, trastuzumab is then continued for a total of 52 weeks.

The Intergroup N9831 trial has a similar design to the NSABP trial but paclitaxel is delivered on a weekly basis at a dose of 80 mg/m^2 for 12 weeks after four courses of standard doxorubicin/cyclophosphamide. Patients are randomised to receive paclitaxel alone, paclitaxel in conjunction with trastuzumab followed by 40 weeks of weekly trastuzumab (total 52 weeks) or paclitaxel alone for 12 weeks followed by trastuzumab for 52 weeks.

The HERceptin Adjuvant trial (HERA) trial is being run in Europe and other parts of the world excluding the USA; this differs from the two American trials in three respects. First, it will recruit patients with HER-2-positive tumours that have completed any recognised adjuvant chemotherapy regimen; secondly, it will address the question of duration of trastuzumab, as patients will be randomised to receive either observation alone or trastuzumab for 1 year or 2 years; thirdly, it will use a three-week schedule in both experimental arms with trastuzumab given as an 8 mg/kg loading dose followed by 6 mg/kg three-weekly.

The BCIRG 006 trial adopts a different approach to adjuvant chemotherapy and is building on the encouraging results seen for platinum/docetaxel/trastuzumab combinations in its phase II trials in the metastatic setting. This is a three-arm trial in which the control arm will receive doxorubicin/cyclophosphamide for four courses followed by four courses of docetaxel. The first experimental arm will receive doxorubicin/cyclophosphamide for four courses followed by four courses of docetaxel in conjunction with weekly trastuzumab followed by weekly trastuzumab for 40 weeks. The third arm will receive docetaxel plus cisplatin or carboplatin for six courses with concurrent trastuzumab administered weekly during chemotherapy and continued at a dose of 6 mg/kg every three weeks for a total of 52 weeks of trastuzumab.

The Programmes d'Actions Concertées (PACS) 004 trial is being conducted in France and Belgium and will recruit patients with node-positive disease to six cycles of three-weekly FEC 100 (5-FU 500 mg/m^2, epirubicin 100 mg/m^2 and cyclophosphamide 500 mg/m^2) or six cycles of three-weekly docetaxel 75 mg/m^2 in combination with epirubicin 75 mg/m^2. Following completion of adjuvant therapy, patients who are HER-2-positive are randomised to receive either three-weekly trastuzumab for 1 year or observation alone.

These five trials will recruit more than 12 000 patients over the next five years and will hopefully define the role of trastuzumab as adjuvant therapy. Outside the context of such a trial, the use of adjuvant trastuzumab is neither recommended nor indeed licensed.

Neoadjuvant therapy

Preoperative chemotherapy of large breast cancers is now widely employed based on the ability to monitor tumour

response *in vivo*, and the potential to downstage and reduce the need for mastectomy. Several studies have been conducted to evaluate neoadjuvant trastuzumab. Hurley and colleagues have reported the results of treatment of locally advanced or inflammatory breast cancer with docetaxel, cisplatin and trastuzumab. Sixteen patients have been treated with a clinical response rate of 100% and a pathological complete response rate of 25% (Hurley *et al*. 2001). Burstein and colleagues have evaluated trastuzumab and paclitaxel as neoadjuvant therapy followed by surgery and postoperative doxorubicin/cyclophosphamide. The trastuzumab/paclitaxel combination produced responses in 73% of patients and a pathological complete remission was reported in 18% of patients (Burstein *et al*. 2001*a*).

Conclusion

All women with breast cancer should now be tested for overexpression of HER-2. Patients with early breast cancer whose tumours show high overexpression (IHC 3+ or FISH+) should be considered for entry into an adjuvant trastuzumab trial. Patients with advanced disease should be offered trastuzumab in combination with a taxane or trastuzumab alone if previously treated with a taxane. Single agent trastuzumab should also be available as first-line treatment for metastatic disease in the patients who prefer not to have immediate chemotherapy or in the context of trials addressing the issue of timing of treatment. The role of trastuzumab in combination with endocrine therapy is undefined and should only be given in the context of a clinical trial.

References

Bangemann N, Kuhle A, Ebert A, Buhler H & Schaller G 2000 Capecitabine combined with trastuzumab in the therapy of intensively pretreated HER2-overexpressing metastatic breast cancer (MBC). *Annals of Oncology* **11** (**S4**) 143 (abstract 653).

Barnes DM, Bartkova J, Camplejohn RS, Gullick WJ, Smith PJ & Millis RR 1992 Overexpression of the c-erbB-2 oncoprotein: why does this occur more frequently in ductal carcinoma *in situ* than in invasive mammary carcinoma and is this of prognostic significance? *European Journal of Cancer* **28** 644–648.

Baselga J & Mendelsohn J 1994 Receptor blockade with monoclonal antibodies as anti-cancer therapy. *Pharmacology Therapy* **64** 127–154.

Baselga J, Tripathy D, Mendelsohn J, Baughman S, Benz CC, Dantis L, Sklarin NT, Seidman AD, Hudis CA, Moore J, Rosen PP, Twaddell T, Henderson IC & Norton L 1996 Phase II study of weekly intravenous recombinant humanized anti-p185HER2 monoclonal antibody in patients with HER2/neu-overexpressing metastatic breast cancer. *Journal of Clinical Oncology* **14** 737–744.

Baselga J, Norton L, Albanell J, Kim YM & Mendelsohn J 1998 Recombinant humanized anti-HER2 antibody (Herceptin) enhances the antitumor activity of paclitaxel and doxorubicin against HER2/neu-overexpressing human breast cancer xenografts. *Cancer Research* **58** 2825–2831.

Bast RC Jr, Ravdin P, Hayes DF, Bates S, Fritsche H Jr, Jessup JM, Kemeny N, Locker GY, Mennel RG & Somerfield MR 2001 2000 update of recommendations for the use of tumor markers in breast and colorectal cancer: clinical practice guidelines of the American Society of Clinical Oncology. *Journal of Clinical Oncology* **19** 1865–1878.

Burris HA 3rd 2001 Docetaxel (Taxotere) plus trastuzumab (Herceptin) in breast cancer. *Seminars in Oncology* **28** 38–44.

Burstein HJ, Harris LN, Kaelin CM, Christian RL, Parker LM, Gelman R, Ellisen LW, Kuter I, Gadd MA, Kennedy PR *et al*. 2001*a* Preoperative Herceptin and paclitaxel (Taxol) for HER2 overexpressing (HER2+) stage II/III breast cancer. *Proceedings of the American Society of Clinical Oncology* **20** 26a (abstract 100).

Burstein HJ, Kuter I, Campos SM, Gelman RS, Tribou L, Parker LM, Manola J, Younger J, Matulonis U, Bunnell CA, Partridge AH, Richardson PG, Clarke K, Shulman LN & Winer EP 2001*b* Clinical activity of trastuzumab and vinorelbine in women with HER2-overexpressing metastatic breast cancer. *Journal of Clinical Oncology* **19** 2722–2730.

Carter P, Presta L, Gorman CM, Ridgway JB, Henner D, Wong WL, Rowland AM, Kotts C, Carver ME & Shepard HM 1992 Humanization of an anti-p185HER2 antibody for human cancer therapy. *PNAS* **89** 4285–4289.

Chien KR 2000 Myocyte survival pathways and cardiomyopathy: implications for trastuzumab cardiotoxicity. *Seminars in Oncology* **27** 9–14; discussion 92–100.

Cobleigh MA, Vogel CL, Tripathy D, Robert NJ, Scholl S, Fehrenbacher L, Wolter JM, Paton V, Shak S, Lieberman G & Slamon DJ 1999 Multinational study of the efficacy and safety of humanized anti-HER2 monoclonal antibody in women who have HER2-overexpressing metastatic breast cancer that has progressed after chemotherapy for metastatic disease. *Journal of Clinical Oncology* **17** 2639–2648.

Cook-Bruns N 2001 Retrospective analysis of the safety of Herceptin immunotherapy in metastatic breast cancer. *Oncology* **61** 58–66.

Coussens L, Yang-Feng TL, Liao YC, Chen E, Gray A, McGrath J, Seeburg PH, Libermann TA, Schlessinger J, Francke U, *et al*. 1985 Tyrosine kinase receptor with extensive homology to EGF receptor shares chromosomal location with neu oncogene. *Science* **230** 1132–1139.

De Santes K, Slamon D, Anderson SK, Shepard M, Fendly B, Maneval D & Press O 1992 Radiolabeled antibody targeting of the HER-2/neu oncoprotein. *Cancer Research* **52** 1916–1923.

Ellis MJ, Coop A, Singh B, Mauriac L, Llombert-Cussac A, Janicke F, Miller WR, Evans DB, Dugan M, Brady C, Quebe-Fehling E & Borgs M 2001 Letrozole is more effective neoadjuvant endocrine therapy than tamoxifen for ErbB-1- and/or ErbB-2-positive, estrogen receptor-positive primary breast cancer: evidence from a phase III randomized trial. *Journal of Clinical Oncology* **19** 3808–3816.

Fendly BM, Winget M, Hudziak RM, Lipari MT, Napier MA & Ullrich A 1990 Characterization of murine monoclonal antibodies reactive to either the human epidermal growth factor receptor or HER2/neu gene product. *Cancer Research* **50** 1550–1558.

Fuchs IB, Landt S, Buehler H, Evers K, Kleine-Tebbe A, Lichtenegger W & Schaller G 2001 Analysis of HER1 and HER2 in the heart to clarify the cardiotoxicity of Herceptin. *Proceedings of the American Society of Clinical Oncology* **20** 45a (abstract 176).

Gelmon K, Arnold A, Verma S, Ayoub J-P, Hemmings F & Leyland-Jones B 2001 Pharmacokinetics (PK) and safety of Herceptin when administered every three weeks to women with metastatic breast cancer. *Proceedings of the American Society of Clinical Oncology* **20** 69a (abstract 174).

Gianni L, Albanell J, Eirmann W, Bianchi G, Bourquez D, Viganò L, Molina R, Raab G, Locatelli A, Vanhauwere B & Baselga J 2001 Feasibility, pharmacology and antitumour activity of Herceptin (H) with doxorubicin and Taxol followed by weekly Taxol (AT&T) in women with HER2-positivie advanced breast cancer (ABC). *Proceedings of the American Society of Clinical Oncology* **20** 44a (abstract 174).

Gusterson BA, Gelber RD, Goldhirsch A, Price KN, Save-Soderborgh J, Anbazhagan R, Styles J, Rudenstam CM, Golouh R, Reed R *et al*. 1992 Prognostic importance of c-erbB-2 expression in breast cancer. International (Ludwig) Breast Cancer Study Group. *Journal of Clinical Oncology* **10** 1049–1056.

Hanahan D & Weinberg RA 2000 The hallmarks of cancer. *Cell* **100** 57–70.

Hotaling TE, Reitz B & Wolfgang-Kimball D 1996 The humanized anti-HER2 antibody rhuMAb HER2 mediates antibody dependent cell-mediated cytotoxicity via FcgRIII. *Proeedings of the American Association of Cancer Research* **37** 471.

Hudziak RM, Lewis GD, Winget M, Fendly BM, Shepard HM & Ullrich A 1989 p185HER2 monoclonal antibody has antiproliferative effects *in vitro* and sensitizes human breast tumor cells to tumor necrosis factor. *Molecular Cell Biology* **9** 1165–1172.

Hurley J, Franco S, Velez P, Doliny P, Gomez-Fernandez C, Powell J & Lee Y 2001 Primary therapy with Herceptin, Taxotere and cisplatin in locally advanced and inflammatory breast cancer. *Proceedings of the American Society of Clinical Oncology* **20** 31b (abstract 1871).

Jahanzeb M, Mortimer J, Yunus F, Irwin D, Speyer J, Koletsky A, Klein P, Lori Kronish L & Sabir T 2001 Multicentre phase II trial of weekly navelbine plus Herceptin in chemonaive patients with HER2 positive metastatic breast cancer. *Proceedings of the American Society of Clinical Oncology* **20** 59b (abstract 1986).

King CR, Kraus MH & Aaronson SA 1985 Amplification of a novel v-erbB-related gene in a human mammary carcinoma. *Science* **229** 974–976.

Konecny G, Fritz M, Untch M, Lebeau A, Felber M, Lude S, Beryt M, Hepp H, Slamon D & Pegram M 2001 HER-2/neu overexpression and *in vitro* chemosensitivity to CMF and FEC in primary breast cancer. *Breast Cancer Research and Treatment* **69** 53–63.

Kuzur ME, Albain KS, Huntington MO, Jones SF, Hainsworth JD, Greco FA, Erland JB, Vogel CL & Burris HA 2000 A phase II trial of docetaxel and herceptin in metastatic breast cancer overexpressing HER-2. *Proceedings of the American Society of Clinical Oncology* **19** 131a (abstract 1699).

Malik U, Sparano JA, Manalo J, Rajdev L, Sarta C, Hopkins U & Fineberg S 2000 Phase II trial of weekly docetaxel (Taxotere) alone or in combination with trastuzumab (Herceptin) in patients with metastatic breast cancer. *Proceedings of the American Society of Clinical Oncology* **19** 148a (abstract 1697).

Mass RD, Press M, Anderson S & Slamon D 2001*a* Improved survival benefit from Herceptin (trastuzumab) and chemotherapy in patients selected by fluorescence *in situ* hybridisation. *Breast Cancer Research and Treatment* **69** 213 (abstract 18).

Mass RD, Sanders C, Charlene K, Johnson L, Everett T & Anderson S 2001*b* The concordance between the clinical trials assay and flouresence *in situ* hybridisation in the Herceptin pivotal trials. *Proceedings of the American Society of Clinical Oncoloy* **20** 75a (abstract 291).

Pauletti G, Godolphin W, Press MF & Slamon DJ 1996 Detection and quantitation of HER-2/neu gene amplification in human breast cancer archival material using fluorescence *in situ* hybridization. *Oncogene* **13** 63–72.

Pegram MD, Lipton A, Hayes DF, Weber BL, Baselga JM, Tripathy D, Baly D, Baughman SA, Twaddell T, Glaspy JA & Slamon DJ 1998 Phase II study of receptor-enhanced chemosensitivity using recombinant humanized anti-p185HER2/neu monoclonal antibody plus cisplatin in patients with HER2/neu-overexpressing metastatic breast cancer refractory to chemotherapy treatment. *Journal of Clinical Oncology* **16** 2659–2671.

Pegram M, Hsu S, Lewis G, Pietras R, Beryt M, Sliwkowski M, Coombs D, Baly D, Kabbinavar F & Slamon D 1999 Inhibitory effects of combinations of HER-2/neu antibody and chemotherapeutic agents used for treatment of human breast cancers. *Oncogene* **18** 2241–2251.

Pegram MD, Lopez A, Konecny G & Slamon DJ 2000 Trastuzumab and chemotherapeutics: drug interactions and synergies. *Seminars in Oncology* **27** 21–25.

Petit AM, Rak J, Hung MC, Rockwell P, Goldstein N, Fendly B & Kerbel RS 1997 Neutralizing antibodies against epidermal growth factor and ErbB-2/neu receptor tyrosine kinases down-regulate vascular endothelial growth factor production by tumor cells *in vitro* and *in vivo*: angiogenic implications for signal transduction therapy of solid tumors. *American Journal of Pathology* **151** 1523–1530.

Piccart MJ, Di Leo A & Hamilton A 2000 HER2: a 'predictive factor' ready to use in the daily management of breast cancer patients? *European Journal of Cancer* **36** 1755–1761.

Pietras RJ, Fendly BM, Chazin VR, Pegram MD, Howell SB & Slamon DJ 1994 Antibody to HER-2/neu receptor blocks DNA repair after cisplatin in human breast and ovarian cancer cells. *Oncogene* **9** 1829–1838.

Pietras RJ, Pegram MD, Finn RS, Maneval DA & Slamon DJ 1998 Remission of human breast cancer xenografts on therapy with humanized monoclonal antibody to HER-2 receptor and DNA-reactive drugs. *Oncogene* **17** 2235–2249.

Pietras RJ, Poen JC, Gallardo D, Wongvipat PN, Lee HJ & Slamon DJ 1999 Monoclonal antibody to HER-2/neureceptor modulates repair of radiation-induced DNA damage and enhances radiosensitivity of human breast cancer cells overexpressing this oncogene. *Cancer Research* **59** 1347–1355.

Press MF, Bernstein L, Thomas PA, Meisner LF, Zhou JY, Ma Y, Hung G, Robinson RA, Harris C, El-Naggar A, Slamon DJ, Phillips RN, Ross JS, Wolman SR & Flom KJ 1997 HER-2/neu gene amplification characterized by fluorescence *in situ* hybridization: poor prognosis in node-negative breast carcinomas. *Journal of Clinical Oncology* **15** 2894–2904.

Roche 2001 Herceptin product monograph. Macclesfield, Cheshire, UK: Gardiner-Caldwell Communications Ltd.

Ross JS & Fletcher JA 1998 The HER-2/neu oncogene in breast cancer: prognostic factor, predictive factor, and target for therapy. *Stem Cells* **16** 413–428.

Ross JS & Fletcher JA 1999 HER-2/neu (c-erb-B2) gene and protein in breast cancer. *American Journal of Clinical Pathology* **112** S53–S67.

Sarup JC, Johnson RM, King KL, Fendly BM, Lipari MT, Napier MA, Ullrich A & Shepard HM 1991 Characterization of an anti-p185HER2 monoclonal antibody that stimulates receptor function and inhibits tumor cell growth. *Growth Regulation* **1** 72–82.

Schechter AL, Stern DF, Vaidyanathan L, Decker SJ, Drebin JA, Greene MI & Weinberg RA 1984 The neu oncogene: an erb-B-related gene encoding a 185 000-Mr tumour antigen. *Nature* **312** 513–516.

Semba K, Kamata N, Toyoshima K & Yamamoto T 1985 A v-erbB-related protooncogene, c-erbB-2, is distinct from the c-erbB-1/epidermal growth factor-receptor gene and is amplified in a human salivary gland adenocarcinoma. *PNAS* **82** 6497–6501.

Seshadri R, Firgaira FA, Horsfall DJ, McCaul K, Setlur V & Kitchen P 1993 Clinical significance of HER-2/neu oncogene amplification in primary breast cancer. The South Australian Breast Cancer Study Group. *Journal of Clinical Oncology* **11** 1936–1942.

Shih C, Padhy LC, Murray M & Weinberg RA 1981 Transforming genes of carcinomas and neuroblastomas introduced into mouse fibroblasts. *Nature* **290** 261–264.

Slamon DJ 2001 Update on Taxotere/Platinum/Herceptin combinations. *Satellite meeting of the 24th San Antonio Breast Cancer Symposium*. San Antonio, TX, USA.

Slamon DJ, Clark GM, Wong SG, Levin WJ, Ullrich A & McGuire WL 1987 Human breast cancer: correlation of relapse and survival with amplification of the HER-2/neu oncogene. *Science* **235** 177–182.

Slamon DJ, Godolphin W, Jones LA, Holt JA, Wong SG, Keith DE, Levin WJ, Stuart SG, Udove J, Ullrich A *et al.* 1989 Studies of the HER-2/neu proto-oncogene in human breast and ovarian cancer. *Science* **244** 707–712.

Slamon DJ, Leyland-Jones B, Shak S, Fuchs H, Paton V, Bajamonde A, Fleming T, Eiermann W, Wolter J, Pegram M, Baselga J & Norton L 2001 Use of chemotherapy plus a monoclonal antibody against HER2 for metastatic breast cancer that overexpresses HER2. *New England Journal of Medicine* **344** 783–792.

Sliwkowski MX, Lofgren JA, Lewis GD, Hotaling TE, Fendly BM & Fox JA 1999 Nonclinical studies addressing the mechanism of action of trastuzumab (Herceptin). *Seminars in Oncology* **26** 60–70.

Tripathy D, Seidman A, Hudis C, Pierri MK, Keefe D, Murphy M & Stewart S 2001. Effect of cardiac dysfunction on treatment outcome in the herceptin (trastuzumab) pivotal trial. *Proceedings of the American Society of Clinical Oncology* **20** 49a (abstract 191).

Uber KA, Nicholson BP, Thor AD, Merkel DE, Goldstein LJ, Gradishar WJ & Sledge GW 2001 A phase II trial of weekly docetaxel (D) and Herceptin (H) as first- or second-line treatment in HER-2 overexpressing metastatic breast cancer. *Proceedings of the American Society of Clinical Oncology* **20** 50b (abstract 1949).

Vogel C, Cobleigh MA, Tripathy D, Gutheil JC, Harris LN, Fehrenbacher L, Slamon DJ, Murphy M, Novotny WF, Burchmore M, Shak S, Stewart SJ & Press M 2002 Efficacy and safety of trastuzumab as single agent first-line treatment of HER2-overexpressing metastatic breast cancer: *Journal of Clinical Oncology* **3** 719–726.

Winer E, Batist G, Belt R, Gutheil J, Park Y & Wells L 2000 Reduced cardiotoxicity of liposome-encapsulated doxorubicin (TLC D-99) compared to free doxorubicin in first line-therapy of metastatic breast cancer in patients at increased risk for anthracycline-induced cardiac toxicity. *Proceedings of the American Society of Clinical Oncology* **19** 84a (abstract 323).

Yarden Y & Sliwkowski MX 2001 Untangling the ErbB signalling network. *Nature Reviews in Molecular and Cellular Biology* **2** 127–137.