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HER2-Positive Breast Cancer

Current and Future Treatment Strategies

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Abstract

In the year 2006, breast cancer was estimated to affect >200 000 American women and cause nearly 56 000 deaths. Furthermore, breast cancer is the most common cancer diagnosed and second most common cause of cancer-related deaths in women. The current treatment armamentarium for breast cancer includes chemotherapy, endocrine therapy and biological therapy. Treatment has become more individualised based on characteristics of the tumour including overexpression of the human epidermal growth factor receptor (HER)-2. Between 20 and 30% of all breast cancers overexpress HER2, which means 40 000–60 000 patients will have this type of cancer.

Previously, overexpression of HER2 was a negative prognostic and predictive risk factor for survival; however, with the advent of trastuzumab, patients' prognosis is improving in all treatment settings. Much controversy exists in the use of trastuzumab, including (i) the sequence of adjuvant trastuzumab (concurrent with chemotherapy or sequential); (ii) the treatment duration (<1 year, 1 year or 2 years); and (iii) the treatment choice upon disease progression (whether to continue or not with trastuzumab and add another cytotoxic agent). Current trials are ongoing to help answer these questions.

Furthermore, there has been interest in predicting which HER2-positive patients would require anthracycline therapy, and which could avoid anthracycline therapy and its toxicities. Novel therapeutics, such as lapatinib, an oral

tyrosine kinase inhibitor, which blocks both the epidermal growth factor receptor and HER2 receptor has recently been approved by the US FDA. Whereas pertuzumab, a humanised monoclonal antibody, directed against heterodimerisation of HER2 and HER3 has entered phase II and III clinical trials.

In the year 2006, breast cancer was estimated to affect >200 000 American women and cause approximately 56 000 deaths.[1] Although the incidence of breast cancer is increasing, the 5-year survival rate has continued to improve. This improvement to overall survival (OS) is largely a result of new chemotherapeutic and biological agents. Human epidermal growth factor receptor (HER)-2 belongs to the family of epidermal growth factor receptors (EGFRs) and is overexpressed in 20–30% of all breast cancers [2,3] Furthermore, the overexpression of HER2 is a negative prognostic and predictive risk factor.[4,5] Patients with HER2 positivity have more aggressive disease, greater likelihood of lymph node involvement, decreased estrogen receptor (ER) expression and increased resistance to endocrine therapy, although they have recently been found to have an increased responsiveness to anthracycline treatment.[6-10]

Initially approved by the US FDA in 1998 for treatment of metastatic breast cancer, trastuzumab has been recently approved in the adjuvant setting after pivotal trials revealed statistically significant improvement in the risk of recurrence by nearly 50% and improvement of the OS by one-third.[11-14] There are still many unanswered questions as to the best sequence, the optimal treatment duration, and whether to continue trastuzumab upon disease progression. Randomised trials presented at national meetings and published have attempted to answer these questions.

1. Trastuzumab

Trastuzumab is a recombinant, humanised IgG monoclonal antibody that targets the extracellular domain of the HER2 receptor.[15] The exact mechanism of action of trastuzumab remains unclear, although several theories have been proposed.[16,17] Trastuzumab may decrease the concentration of HER2 at the cellular membrane; thus, preventing homodimerisation and heterodimerisation.[16] Trastuzumab also appears to cause arrest of the cell cycle through the inhibition of phosphatidylinositol 3-kinase (PI3K) and mitogen-activated protein kinase (MAPK) cascades by the downmodulation of the HER2 receptor. In addition, trastuzumab activates phosphatase and tensin homologue (PTEN) causing dephosphorylation of Akt resulting in cessation of growth.[16,18] Additionally, trastuzumab has been shown to sensitise HER2 overexpressed breast cancer cell lines to cytotoxic therapy (i.e. taxanes and etoposide) through reducing the levels of Mcl-1, an antiapoptotic protein, thus promoting cell death.[19] As seen in *in vivo* studies, trastuzumab may also inhibit angiogenesis.[20] Another mechanism of action is through antibody-dependent cellular cytoxicity (ADCC) by which the Fc domain of trastuzumab activates natural killer cells to attack and destroy cells expressing HER2.[21]

1.1 Trastuzumab in Metastatic Breast Cancer

Before the use of trastuzumab in the adjuvant setting, it was approved for the treatment of metastatic breast cancer (MBC) in patients who tested HER2 positive. Trastuzumab has shown single-agent activity in MBC with a response rate of approximately 30%.[22] However, in combination with chemotherapy, trastuzumab produces higher response rates. On the basis of preclinical data showing synergy of trastuzumab with taxanes and anthracyclines, a randomised trial was conducted where a total of 234 patients received chemotherapy alone (either an anthracycline plus cyclophosphamide [AnC] or paclitaxel) compared with 235 patients who received chemotherapy plus trastuzumab.[14] Patients who received the combination of chemotherapy plus trastuzumab had a median time to progression (TTP) of 7.4 months compared with 4.6

months for the chemotherapy alone arm ($p < 0.001$). Furthermore, there was an improvement in median OS for those patients receiving chemotherapy plus trastuzumab compared with those receiving chemotherapy alone (25.1 vs 20.3 months; $p = 0.046$). At the completion of therapy, crossover in patients not receiving trastuzumab as initial therapy was allowed. However, it was shown that even when comparing upfront trastuzumab with crossing over, there was an improvement in outcome with the upfront use of trastuzumab. Interestingly, 39 patients (27%) in the AnC plus trastuzumab treatment arm had symptomatic or asymptomatic cardiac dysfunction, whereas in the AnC alone arm only 8% of patients had cardiac dysfunction. As a result of the relatively high incidence of cardiotoxicity in the anthracycline-containing arm (AnC plus trastuzumab), the FDA approved the combination of paclitaxel plus trastuzumab for use in MBC.

After the feasibility of combining trastuzumab with chemotherapeutic agents had been evaluated in preclinical studies,[23,24] several phase II and III trials using as agents such as docetaxel, vinorelbine (navelbine), capecitabine[25-29] or carboplatin and paclitaxel[30,31] were completed in patients with MBC. In a recent phase III trial comparing the combination of trastuzumab and paclitaxel plus carboplatin (HPCarbo) with trastuzumab and paclitaxel (HP), patients in the HPCarbo arm had significantly higher overall response rate compared with the HP arm (52% vs 36%, respectively) [$p = 0.04$] and a significantly improved progression-free survival (PFS) [11.2 vs 6.9 months, respectively; $p = 0.007$].[31] Toxicity was comparable with increased neutropenia and thrombocytopenia in the HPCarbo arm. Other trials combined trastuzumab in the metastatic setting with docetaxel (T) administered weekly or every third week.[25,26] Patients when treated with trastuzumab plus docetaxel (TH) every third week had a significantly improved overall response rate, OS, TTP, time to treatment failure and duration of response compared with docetaxel alone.[26] However, results of the Breast Cancer International Research Group (BCIRG 007) did not show any significant differences in median TTP (11.1 vs 10.4 months), overall response rates, rate of distant recurrence and clinical benefit when carboplatin (area under the concentration time curve [AUC] of 6) was added to docetaxel plus trastuzumab (TH vs TCarboH).[32]

The combination of trastuzumab (4 mg/kg on day 1 followed by 2 mg/kg on day 8) with weekly vinorelbine (30 mg/m^2) was evaluated in a phase II study as first-line chemotherapy in HER2-positive metastatic breast cancer.[33] The regimen was safely used with an overall response rate of 62.9%, a median duration of response of 17.5 months, median PFS of 9.9 months (95% CI 5.6, 12.1) and the 1-year PFS was 39.1%. The median survival for all patients was 23.7 months (95% CI 18.4, 32.6).[33] Moreover, oral vinorelbine combined with trastuzumab was found to be well tolerated and efficacious in a small observational trial.[34]

Another novel combination of liposomal doxorubicin with trastuzumab has been studied in a phase II study without the cardiotoxicity previously seen when trastuzumab was combined with an anthracycline.[35] After a median follow-up of 13.9 months, the median PFS was 12.0 months with only 10% of patients experiencing an asymptomatic decline in left ventricular function of ≥15%. Furthermore, no patients had symptomatic decline of left ventricular ejection fraction (LVEF) >10% from baseline and a value below the lower limit of normal.[35]

1.2 Use of Trastuzumab in Early Stage Breast Cancer

1.2.1 NSABP B31 and NCCTG 9831

Because trastuzumab was effective in improving response rate, duration of response and OS in patients with MBC, large randomised adjuvant trials were initiated in patients with early stage breast cancer.[13,36] The results from these trials were recently published and presented at national meetings. The National Surgical Adjuvant Breast and Bowel Project (NSABP)-B31 published a joint analysis with the North Central Cancer Treatment Group (NCCTG) B9831.[13] These trials were closed prematurely because of the superiority of the tras-

tuzumab arm. The B31 protocol enrolled 2043 node-positive, HER2-positive patients with early stage breast cancer and randomised them to four cycles of doxorubicin (A) 60 mg/m^2 and cyclophosphamide 600 mg/m^2 every 21 days followed by paclitaxel 175 mg/m^2 taken every 3 weeks for four cycles (ACP; group 1) or the same chemotherapy with weekly trastuzumab 4 mg/kg loading dose, then 2 mg/kg weekly for 52 weeks starting with the paclitaxel (ACPH; group 2). The protocol was later amended to allow weekly paclitaxel similar to the N9831 trial. The N9831 randomised 3505 HER2-positive (immunohistochemistry 3+ intensity or HER2 overamplified by fluorescence *in situ* hybridisation [FISH]), node-positive (the protocol was amended at a later point to include high-risk node-negative [>2cm ER-positive or >1cm if ER-negative]) patients into three groups. The control group, group A, received four cycles of AC followed by weekly paclitaxel 80 mg/m^2 for 12 weeks; group B received four cycles of AC followed by 12 weekly doses of paclitaxel followed by sequential weekly trastuzumab for 52 weeks; and group C received four cycles of AC, followed by 12 weekly doses of paclitaxel concomitantly with weekly trastuzumab, which would be continued for 40 more weeks after completion of paclitaxel. The combined analysis grouped the control groups (group 1 and group A from B31 and N9831, respectively) and compared them with group 2 and group C from B31 and N9831, respectively. Since there was no group in the B31 trial that evaluated sequential trastuzumab, group B from the N9831 trial was not included in the combined analysis.

Patients in both studies were excluded if they had any history of coronary disease, arrhythmias, cardiomegaly, chronic heart failure or cardiomyopathy, or required medications for angina pectoris or valvular heart disease. Furthermore, to ascertain any compromise in LVEF, either multiple-gated acquisition scanning (MUGA) and/or echocardiography ECHO were obtained (B31 used MUGA scanning only). Before receiving trastuzumab, patients had to have an LVEF ≥ lower limit of normal (LLN) for the institution and not have a decrease of >16% from their previous baseline (prior to starting AC). Furthermore, if any patient developed symptoms of chronic heart failure at any time of therapy (during AC or during trastuzumab), therapy was terminated. Patients were required to have either MUGA or ECHO prior to and after completing AC, and at 6, 9, and 18 months of therapy. If the LVEF declined ≥16% from baseline or 10–15% from their baseline to below the LLN, trastuzumab was withheld for 1 month. Upon re-evaluation, if the LVEF remained below the set limits, trastuzumab was discontinued.

Both trials were terminated early by the independent data-monitoring committee based on the significant benefits trastuzumab had in the adjuvant setting compared with the control arm. The primary endpoint, disease-free survival (DFS), was reached and at a median follow up of 2 years there was a statistically significant reduction in recurrence of 52% (hazard ratio [HR] 0.48; 95% CI 0.30, 0.59; $p < 0.0001$) with an absolute distant recurrence in the trastuzumab-containing arm at 3 and 4 years of 8.8% and 15.9%, respectively. Furthermore, OS was improved by a third; (HR 0.67; 95% CI 0.48, 0.93; $p = 0.015$). The absolute survival difference was 2.5% (94.3% vs 91.7%) at 3 years and 4.8% (91.4% vs 86.6%) at 4 years. Interestingly, brain metastases were more commonly seen as a first site of recurrence in the trastuzumab-treated group than the control. Possible theories include delayed failures at local sites compared with distant sites and the limitation that trastuzumab has in crossing the blood-brain-barrier.[37-43]

The incidence of symptomatic chronic heart failure (New York Heart Association [NYHA] class III or IV) or other cardiac-related deaths at 3 years in the B31 study was 0.8% in the control group compared with 4.1% in the trastuzumab-treated group. Moreover, 14% of trastuzumab-treated patients had to discontinue therapy as a result of asymptomatic decreases in LVEF, whereas 4% stopped as a result of symptomatic cardiotoxicity.[44] In the N9831 trial, the 3-year cumulative incidence of NYHA class III or class IV chronic heart failure was 2.9% in the trastuzumab-treated group compared with 0% in the control group. Interestingly, interstitial pneumonitis,

albeit rare, occurred more commonly in the trastuzumab-treated group compared with the control. Patients with stage I-IIA breast cancer who required radiation therapy did not have any increased incidence of radiation adverse events when radiation was given concurrently with trastuzumab.[45]

The combined analysis revealed the significant benefit in the reduction of recurrence and death. However, the benefit of trastuzumab in the 191 node-negative patients still needs to be evaluated. As presented at a national meeting, an unplanned interim analysis revealed concurrent use of trastuzumab with paclitaxel was more effective than sequential use in both DFS and OS; however, the results from the planned analysis are still pending (table I).[46]

1.2.2 HERceptin Adjuvant Trial

Another large, phase III international, multi-centre trial, the HERA (HERceptin Adjuvant) trial, conducted by the Breast International Group (BIG) 1-01, sequenced trastuzumab after primary surgery and after a minimum of four cycles of adjuvant or neoadjuvant chemotherapy.[12] Over 5000 HER2-positive patients with early stage breast cancer were randomised to receive trastuzumab or observation. If randomised to receive trastuzumab, patients received an initial dose of 8 mg/kg followed by maintenance doses of 6 mg/kg every 3 weeks for 1 or 2 years. Eligible patients had to have node-positive disease or if node negative, a tumour >1cm. Major exclusion criteria included distant metastases, T4 tumours (including inflammatory breast cancer), cumulative doses of doxorubicin >360 mg/m^2 or epirubicin >720 mg/m^2, prior stem cell transplant, LVEF <55% based on ECHO or MUGA, history of chronic heart failure, coronary artery disease or unstable arrhythmias. Cardiac monitoring via MUGA or ECHO was completed at baseline and 3, 6, 12, 18, 24, 30, 36 and 60 months after randomisation. Trastuzumab was withheld if any National Cancer Institute Common Toxicity Criteria nonhaematological grade 3 or 4 event occurred until the recovery to at least grade 2 toxicity; however, if the recovery was >5 weeks, trastuzumab was definitively withheld. Furthermore, trastuzumab was stopped in any patient with LVEF ≤45% or who developed a 10% absolute decrease in their baseline LVEF and LVEF <50%. Trastuzumab was permanently discontinued if the LVEF did not return to above LLN per protocol within 3 weeks.

At a median follow-up of 1 year, an interim analysis of 3387 patients (1694 with trastuzumab and 1693 with placebo) revealed 127 new events in the trastuzumab-treated group compared with 220 in the control group, with a risk reduction of 46% (HR 0.54; 95% CI 0.43, 0.67; $p < 0.0001$) and absolute 2-year DFS of 8.4% (95% CI 2.1, 14.8). The 2-year OS was not statistically significant, (HR 0.76, 95% CI 0.47, 1.24; $p = 0.26$). As seen in the joint analysis, brain metastases occurred more frequently in the trastuzumab-treated group compared with the placebo. The incidence of symptomatic heart failure in the trastuzumab-treated group (1.7%) was lower than that seen in the concurrent arms of combined analysis. Unlike the joint analysis, in which 191

Table I. Results of adjuvant trastuzumab trials

Endpoint (% patients)	B31 (Joint)[13]	N9831[13,46]	HERA[12]	BCIRG[47]	FINher[48]
Improvement in disease-free survival	52 ($p = 3 \times 10^{-12}$)	Sequential: 13 ($p = 0.2936$) Concurrent: 36 ($p = 0.0114$)	Arm 2: 46 Arm 3: u/k	Arm 2: A-T-H: 51 ($p = 4.8 \times 10^{-7}$) Arm 3: T-Carbo-H: 39 ($p = 1.5 \times 10^{-4}$)	58 ($p = 0.01$)
Improvement in overall survival	33 ($p = 1.5 \times 10^{-2}$)	Sequential: 15 ($p = 0.4752$) Concurrent: 26 ($p = 0.26$)	36	Endpoint not reached	59 ($p = 0.07$)
Cardiotoxicity	4.1	2.9	0.5	AC-T: 1.2 AC-TH: 2.3 T-Carbo-H: 1.2	No decrease in LVEF or cardiac function

A = doxorubicin; **BCIRG** = Breast Cancer International Research Group; **C** = cyclophosphamide; **Carbo** = carboplatin; **FINher** = Finland herceptin; **H** = trastuzumab; **HERA** = HERceptin Adjuvant; **LVEF** = left ventricular ejection fraction; **T** = docetaxel; **u/k** = unknown.

patients were node negative,[13] one-third (550 patients) of the patients in HERA had node-negative disease and only 26% of the randomised group received a taxane with an anthracycline compared with 100% in the joint analysis. However, the same gains were seen in the node-negative population and the anthracycline-naive patients as was seen in the joint analysis.[13]

After the interim analysis, patients in the placebo arm were able to crossover and receive trastuzumab therapy. Of the 1698 placebo-treated patients, 861 elected to take trastuzumab. Now, after 2 years since the first arm was initially presented, benefit in DFS has been maintained (HR 0.64; 95% CI 0.54, 0.76).[49] Furthermore, the OS was also significantly improved (HR 0.66; 95% CI 0.47, 0.91).

The HERA trial evaluated the use of sequential trastuzumab in order to circumvent added cardiotoxicity of combining an anthracycline with trastuzumab as well as to determine the optimal duration of trastuzumab therapy. Sequential therapy appears to improve DFS by nearly 50%. Cardiotoxicity was lower than seen in the joint analysis. There are multiple reasons for the lower incidence of cardiotoxicity including: more frequent monitoring, different modalities of monitoring, sequential therapy, as well as fewer patients exposed to anthracycline therapy. The third arm, in which patients received 2 years of sequential trastuzumab, will help determine the optimal treatment duration.

1.2.3 Breast Cancer International Research Group 006

The BCIRG initiated a phase III, multicentre trial to evaluate the adjuvant use of trastuzumab concurrently with a nonanthracycline after multiple phase II trials confirmed the feasibility of this approach.[36,50] The BCIRG 006 randomised 3222 HER2-positive patients (FISH only) with axillary lymph node positive or high-risk lymph node negative (tumour size >2cm, estrogen and progesterone receptor status negative, histological and/or nuclear grade 2–3, or <35 years of age) breast cancer to two arms containing adjuvant AC followed by docetaxel (100 mg/m^2 every 21 days for four cycles) with or without trastuzumab (weekly during chemotherapy then every 21 days), while the third arm included docetaxel 75 mg/m^2 and carboplatin (TCarbo; AUC of 6 every 3 weeks × 6) with trastuzumab for 1 year.[47] The interim analysis after 23 months of treatment revealed statistically significant risk reduction of first recurrence of 51% (HR 0.49; $p = 4.8 \times 10^{-7}$) with AC-TH (arm 2) compared with control and a risk reduction of 39% (HR 0.61; $p = 1.5 \times 10^{-4}$) with TCarboH (arm 3) compared with the control arm. OS, a secondary endpoint, has not been evaluated because of limited events. However, the incidence of cardiac events in arm 1 was 1.2%, whereas in arm 2 it was 2.3% ($p = 0.046$). In arm 3, the incidence of cardiac events was similar to the control arm at 1.2% ($p = 1.00$).

The BCIRG 006 results were similar to the combined analysis in highlighting the benefit from adjuvant trastuzumab.[13] Further, the BCIRG trial showed that nonanthracycline therapy concurrently with trastuzumab was effective.[51] However, the anthracycline arm was superior in DFS to the nonanthracycline arm (77 events in the anthracycline arm compared with 98 events in the nonanthracycline arm), although not reaching statistical significance ($p = 0.16$).

1.2.4 Finland Herceptin Trial

The last adjuvant trastuzumab trial, the FINher (Finland Herceptin) trial, tested whether an abbreviated course of trastuzumab was effective.[48,52] The FINher trial included patients with early stage breast cancer (axillary node positive or tumour >2cm with negative axillary nodes and negative progesterone receptor). The trial included two randomisations: the first was a randomisation between docetaxel (100 mg/m^2 every 21 days for three cycles) and vinorelbine (25 mg/m^2 day 1, 8, 15 of 21-day schedule); the second included only HER2-positive patients (HER2 overamplified [2+ or 3+] detected by chromogenic *in situ* hybridisation [CISH]) who were randomised to weekly trastuzumab for a total of 9 weeks. A total of 232 HER2-positive patients were randomised to receive either adjuvant docetaxel every 3 weeks for three cycles or vinorelbine on days 1, 8, 15 of 21-day cycle for three cycles with or without concurrent weekly trastuzumab (4 mg/kg

loading dose, then 2 mg/kg weekly). All patients then received fluorouracil 600 mg/m^2, epirubicin 60 mg/m^2 and cyclophosphamide 600 mg/m^2 (FEC every 21 days for 18 weeks for six cycles after their initial therapy). Recurrence-free survival (RFS) was the primary endpoint, while the secondary endpoints evaluated LVEF, adverse events and OS. The docetaxel dose was amended to 80 mg/m^2 because of a high incidence of neutropenic fevers.

After a median follow-up of 3 years, HER2-positive patients treated with trastuzumab had a significantly improved RFS (HR 0.42; 95% CI 0.21, 0.83; p = 0.01) and decreased distant recurrence (HR 0.29; 95% CI 0.13, 0.64; p = 0.002) compared with HER2-positive patients not receiving trastuzumab. Moreover, a nonsignificant improvement in OS was seen in the trastuzumab-treated arm (HR 0.41; 95% CI 0.16, 1.08, p = 0.07). The HR for recurrence in HER2 overamplified, trastuzumab-treated patients did not significantly change with the type of chemotherapy, number of lymph nodes involved or centre providing therapy. Furthermore, HER2-positive patients treated with trastuzumab had a similar survival free of distant disease at 3 years compared with HER2-negative patients (HR 1.09; 95% CI 0.52, 2.29; p = 0.82); however, as expected, HER2-positive patients treated without trastuzumab did worse than HER2-negative patients. Regarding toxicities, there was no decline in LVEF in HER2-positive patients treated with trastuzumab, but surprisingly they had a greater stabilisation of their LVEF compared to other therapies. The short course of trastuzumab therapy was effective in this small sample size; moreover, the abbreviated course appears to have less cardiotoxicity than a more pronged course evaluated in other adjuvant trials.

1.3 Molecular Predictors of Response

In an attempt to identify patient subgroups that may not benefit from the use of an anthracycline, the BCIRG evaluated the topoisomerase IIα gene (*TOP2A*).[53] *TOP2A* codes for topoisomerase IIa, an enzyme that is active in the cell cycle. Overexpression of *TOP2A* has been related to a more aggressive form of breast cancer.[54] However, topoisomerase IIα is inhibited by anthracyclines and the lack of amplification of *TOP2A* may negate anthracycline therapy. There is often coamplification of both the *HER2* gene and *TOP2A* gene based on the close proximity of the two genes (i.e. [17q21.1] for *HER2* and [17 q 21.2] for *TOP2A*).[53,55,56] In the BCIRG 006 study, patients with amplification of *TOP2A* had a higher likelihood of recurrence in the TCarboH arm than in the anthracycline arm.[53] Conversely, if *TOP2A* was nonamplified, the TCarboH arm had a nearly similar DFS compared with the AC-TH arm (45 events in AC-TH vs 54 events in TCarboH), possibly sparing the use of an anthracycline in that subgroup of patients. Further investigation is needed to confirm these findings.

Another gene found to be co-amplified with *HER2/neu* is the *c-MYC* gene, and it has been shown that patients in which both genes are co-amplified and are treated with trastuzumab have a better prognosis than patients without *c-MYC* amplification.[57] After examining nearly 1900 tissue blocks from patients treated with chemotherapy (no trastuzumab) from the NSABP B-28 trial, it was found through a multivariate analysis that amplification of three gene products (HER2, c-MYC and phosphatidic acid phosphatase type 2 domain containing 1B [HTPAP]) resulted in a worse prognosis. Kim et al.[57] then evaluated the response to trastuzumab in patients with co-amplification of *cMYC* and *HER2*. Their initial hypothesis was overexpression of c-MYC and HER2 would be a predictor of lack of response. However, patients with co-amplification of *c-MYC* and *HER2* treated with trastuzumab were found to have a reduction in recurrence rate by 76% (HR = 0.24; 2p < 0.0001) and an improved OS by 64% (HR = 0.36; 2p = 0.012) compared with patients without *c-MYC* co-amplification.

1.4 Resistance to Trastuzumab

Patients treated with trastuzumab eventually become refractory to trastuzumab within 1 year.[14,58] Multiple mechanisms of resistance have been hypothesised. First of all, the HER2 receptor may mutate so that trastuzumab can no longer attach. A mutation in exon 21 of the *HER2* gene has recently

been discovered in patients with intrinsic and acquired resistance.[59] In addition, trastuzumab could be blocked from attaching to HER2 by mucin-4 (MUC4), a membrane-associated glycoprotein, which may directly phosphorylate and activate HER2.[60] Cross-talk, a form of communication between different classes of receptors, occurs between HER2 and the insulin-like growth factor I receptor (IGF-IR) and has been responsible for phosphorylation of HER2 in trastuzumab-resistant cells.[61] Early preclinical trials revealed resistance to trastuzumab and a higher cellular proliferation in cell lines with overexpression to IGF-IR than cell lines without overexpression of IGF-IR.[62] In addition, other receptors of the HER family, by the creation of heterodimers (i.e. EGFR **:** HER2, HER2 **:** HER3), are able to activate downstream signals and promote cellular growth even in the presence of trastuzumab.[60] PTEN deleted on chromosome 10 (*PTEN*), a tumour suppressor gene, has been shown to suppress tumour growth and also to sensitise breast cancers to trastuzumab.[18] As previously mentioned, one mechanism of activity of trastuzumab is through the activation of PTEN and inhibition of the PI3K-Akt pathway; however, if *PTEN* is mutated or completely lost, cells have decreased sensitivity to trastuzumab.[18,63] Furthermore, PTEN activity has been investigated as a marker for the efficacy of trastuzumab as elevated levels of PTEN are related to a better response.[63] In other preclinical studies, the reduction of p27(kip1), a cyclin-dependent kinase inhibitor, led to resistance to trastuzumab, whereas higher levels enhanced cell death.[64]

2. Novel Agents

Novel modalities exist to circumvent resistance to trastuzumab by targeting more than one member of the EGFR family (table II). Lapatinib, previously known as GSK572016, is an oral, dual HER2 and EGFR tyrosine kinase inhibitor that has demonstrated preclinical and clinical activity in HER2-positive breast cancer.[65-67] As monotherapy in early clinical studies, lapatinib was clinically active and well tolerated in heavily pretreated, HER2-positive patients with advanced breast cancer.[65] A recently published, phase III, randomised, open-label, industry-sponsored study revealed lapatinib with capecitabine effective than monotherapy with

Table II. HER2-targeted agents and mechanism of action

Drug	Mechanism of action
Trastuzumab (Herceptin®; Genentech, San Francisco, CA, USA)	A humanised IgG monoclonal antibody that targets the extracellular domain of the HER2 receptor[15] Other mechanisms: HER2 downregulation[16] Inhibition of homodimerisation and heterodimerisation[16] Inhibition of PI3K and MAPK[16,18] Sensitize to chemotherapy[19] Activation of PTEN phosphatase[20] Inhibition of angiogenesis[20] ADCC[21]
Lapatinib (Tykerb®; Glaxo-SmithKline; Research Triangle Park, NC, USA)	Oral, dual HER1 and HER2 TKI[65-67]
Pertuzumab (Omnitarg™; Genentech, San Francisco, CA, USA)	A humanised monoclonal antibody directed against heterodimerisation of HER2 and HER3[71-73] Blocks the ability of the receptor to dimerise with other receptors of the EGFR family[72,74]
Geldanamycin (17-AAG) [various manufacturers, including the National Cancer Institute]	A heat-shock protein which destabilises HER2 protein and causes rapid depletion of HER2, thereby disrupting its association with β-catenin in SKBr3 human breast cancer cells[75]
BIBW-2992 (Boehringer Ingelheim, Ingelheim, Germany)	Oral, irreversible inhibitor of EGFR1 and HER2 receptor tyrosine kinases[76,77]

ADCC = antibody dependent cellular cytotoxicity; **EGFR** = epithelial growth factor receptor; **HER** = human epithelial growth factor receptor; **MAPK** = mitogen-activated protein kinase; **PI3K** = phosphoinositide-3 kinase; **PTEN** = phosphatase and tensin homologue; **TKI** = tyrosine kinase inhibitor.

capecitabine in HER2-positive patients previously treated with an anthracyline, a taxane and trastuzumab.[68] The combination of lapatinib (1250mg orally per day continuously) plus capecitabine (1000 mg/m^2 twice daily on days 1–14 every 3 weeks) in 163 patients was compared with capecitabine alone in 161 patients (1250 mg/m^2 twice daily on days 1–14 every 3 weeks).[68] The combination improved median TTP (8.4 vs 4.4 months; $p < 0.001$) and response rate compared with single-agent capecitabine (22% vs 14%; $p = 0.09$).[68] However, no overall survival advantage has been seen to date with combination therapy. Interestingly, a nonsignificant decrease in CNS metastases was seen in the combination arm compared with monotherapy. Lapatinib in combination with capecitabine is now FDA approved for use in HER2-positive patients previously treated with an anthracyline, a taxane and trastuzumab. Regarding toxicities, patients tolerated the combined therapy well, although there was a slightly higher incidence of diarrhoea and skin rash in the lapatinib arm. However, there were no significant declines in the LVEF. In a review of >2800 patients exposed to lapatinib, the incidence of symptomatic declines of LVEF was 1.3%, and in the majority of patients it was transient and lapatinib was able to be restarted.[69] An interesting finding was the lower incidence of brain metastases as the site of progression seen, because lapatinib may have better CNS penetration compared with trastuzumab. In support thereof, a phase II study has shown activity (two partial responses in 39 patients) with lapatinib monotherapy in the treatment of HER2-positive CNS metastases. [70]

Recently, Iwata et al.[78] reported the results of a phase II study in abstract form showing lapatinib as monotherapy was active in heavily treated patients. In addition, monotherapy with lapatinib showed activity in inflammatory breast cancer (IBC) in patients expressing high levels of ErBb2, p-ErbB2 and IGF-IR.[79] Moreover, a phase II trial evaluating neoadjuvant lapatinib 1500mg on days 1–14 combined with weekly paclitaxel 80 mg/m^2 for 12 cycles in IBC has been presented at a national conference.[80] The preliminary data suggest the combination was active and well tolerated. Other randomised studies are currently under way in both the adjuvant and metastatic settings with lapatinib in combination with trastuzumab or directly comparing the two.[81]

Another novel agent, pertuzumab, is a humanised monoclonal antibody directed against heterodimerisation of HER2 and HER3.[71-73] Known as a dimerisation inhibitor, pertuzumab binds to HER2 at a different site than trastuzumab and near the dimerisation domain, and blocks the ability of the receptor to dimerise with other receptors of the EGFR family.[72,74] Preclinical studies of trastuzumab in combination with pertuzumab revealed increased growth inhibition of BT474 breast cancer cells with combined therapy compared to either agent by itself.[82] In a phase I study,[83] pertuzumab appeared safely used and well tolerated with asthenia, nausea and vomiting being the most common adverse effects; however, there was a rare grade 4 gastrointestinal haemorrhage in a patient with pre-existing oesophageal varices. Unlike prior experience with trastuzumab, there were no clinically significant reductions in LVEF reported, although the incidence of cardiotoxicity would need to be studied further. There has been interest in the concurrent use of pertuzumab and lapatinib.[84] In addition, pertuzumab has been studied in other solid tumours including pancreatic islet, ovarian and lung.[83,85,86]

3. Future Approaches

Future approaches for HER2-positive breast cancer patients are evolving. First of all, the combination of trastuzumab with anthracyclines is still being investigated. A phase II, multicentre trial of HER2 positive, metastatic breast cancer using pegylated liposomal doxorubicin with trastuzumab was well tolerated, effective and without any patient experiencing symptomatic heart failure.[35] Other studies have provided interest in the combination of trastuzumab with endocrine therapy[87] or trastuzumab with bevacizumab.[88] Other novel approaches include therapy with ADAM (A Disintegrin And Metalloprotease) metalloproteases.[89] *In vitro* find-

ings of ADAM metalloproteases have shown preclinical synergy with trastuzumab by inhibiting the cleavage of the extracellular domain (ECD) of HER2, thus turning off the transmembrane portion of HER2. Lastly, immunotherapy through vaccination against HER2/neu peptide to inhibit tumour growth has been recently investigated using a Ii-Key/Her2/neu MHC class II peptide-based vaccine.[90] A phase I/I trial has also shown the feasibility, safety and the immunogenic potential of the combination of trastuzumab with a HER2 peptide vaccine in HER2-positive breast cancer.[91] Furthermore, a phase I trial also showed the potential in creating an immune response when the HER2/neu peptide is incorporated into poly-lactide-co-glycolide (PLG) microspheres.[92]

In addition to novel therapeutic modalities, there has been a further desire to find biomarkers of response for HER2-positive disease. Serum ECD has been tested as a predictor for response to trastuzumab,[25,93] whereas the EGF100151 study[94] found a higher response to lapatinib with higher concentrations of the serum ECD expression by FISH. In addition, there has also been interest in evaluating the truncated form of the HER2 receptor, p95HER2, as a marker for metastasis and worse outcome.[95]

In the meantime, the optimal use of trastuzumab as well as the use of other novel agents, such as lapatinib and pertuzumab, is being studied in the next generation of clinical trials. The prevention of brain metastases with the potential use of an oral tyrosine kinase inhibitor has led to the design of studies combining the two agents. Furthermore, blocking not only a receptor but also its ability to heterodimerise has also raised the interest of investigators. Until then, a great success in the field of HER2-positive breast cancer is the ability to use a targeted therapy to convert an unfavourable subtype of breast cancer to a more favourable one.

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