Neoadjuvant therapy of early stage human epidermal growth factor receptor 2 positive breast cancer: latest evidence and clinical implications

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***Abstract*:** Neoadjuvant therapy in human epidermal growth factor receptor 2 (HER2)-positive breast cancer is exactly the paradigm of targeted therapy and a suitable setting to develop and test rapidly novel therapies in early stages. Moreover, neoadjuvant approaches provide a significant source of tumour tissue to identify molecular heterogeneity and potential predictive biomarkers of response. The addition of trastuzumab to primary chemotherapy revolutionized the treatment of this tumour subtype, increasing pathological complete response rate (pCR) that, even with its limitations, has also been shown to be an early marker of survival in HER2-positive disease. HER2-positive breast cancer is a biological heterogeneous disease with different characteristics and clinical outcomes. Multiple promising anti-HER2 drugs with nonoverlapping mechanisms of action have recently been developed. Combined administration of two different HER2-targeted agents, that is, trastuzumab with lapatinib or pertuzumab, and primary chemotherapy shows enhanced antitumour activity, with an increase in pCR to values never reached in the past. Moreover, results of recent studies show that the combination of targeted therapy alone (dual HER2 blockade with or without endocrine therapy) also has activity in a substantial percentage of patients, eradicating HER2-positive tumours without chemotherapy and with a favourable toxicity profile. It is still necessary to be able to select the appropriate group of patients who can avoid chemotherapy (approximately 25%), and to establish robust predictive biomarkers of response or resistance to the anti-HER2 approach. Neoadjuvant therapy represents an enormous step forward in HER2-positive breast cancer. The results of the most relevant neoadjuvant studies and latest evidence are described in this review, though new questions have emerged.

Keywords: dual HER2 blockade, early breast cancer, HER2 positive, neoadjuvant therapy, pathological complete response, predictive biomarkers

Introduction

Human epidermal growth factor receptor 2 (HER2) is overexpressed or amplified in 20% of breast carcinomas, and confers a more aggressive behaviour with poor clinical outcome [Slamon *et al.* 1987; Ross *et al.* 2009]. However, the addition to systemic treatment of trastuzumab (Herceptin; Roche, Basel, Switzerland), a humanized monoclonal antibody that blocks the HER2 receptor, has significantly improved the prognosis of patients with metastatic [Slamon *et al.* 2001] and early HER2-positive breast cancer [Romond *et al.* 2005; Piccart-Gebhart *et al.* 2005; Slamon *et al.* 2011; Joensuu *et al.* 2006], changing its natural history. HER2-positive breast cancer is a biological heterogeneous disease with different characteristics and clinical outcomes [Staaf *et al.* 2010]. Gene expression analyses within HER2-positive disease identify all of the main intrinsic molecular subtypes of breast cancer [luminal A, luminal B, HER2 enriched (HER2-E), and basal like) but the HER2-E subtype, and the luminal A and B subtypes, are the predominant ones [Prat and Perou, 2011].

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Neoadjuvant therapy also known as primary systemic therapy is a well established approach to treatment of locally advanced or inflammatory breast cancer. Primary therapy can improve the surgical options in many patients; it offers the same survival benefit as postoperative treatment and provides prognostic information [Wolmark *et al.* 2001; Van der Hage *et al.* 2001; Hennessy *et al.* 2005]. Due to these potential advantages, neoadjuvant therapy is becoming increasingly used in the context of operable disease. Patients who achieve a pathological complete response (pCR) with primary therapy have an improved survival [Bear *et al.* 2006; Rastogi *et al.* 2008; Cortazar *et al.* 2014]. This has recently been demonstrated in patients with HER2-positive breast cancer treated with primary chemotherapy plus anti-HER2 therapy [Untch *et al.* 2011; Cortazar *et al.* 2014; Piccart-Gebhart *et al.* 2013]. In fact, the highest rates of pCR have been reported in HER2-positive disease, mostly in the subset of HER2-positive/estrogen receptor (ER)-negative tumours [Baselga *et al.* 2012; Gianni *et al.* 2012; Schneeweiss *et al.* 2013]. Regulatory agencies might grant accelerated new drug approval in early breast cancer on the basis of a pCR, defined as 'the absence of any residual invasive cancer on haematoxylin and eosin evaluation of the resected breast specimen and all sampled ipsilateral lymph nodes following completion of neoadjuvant systemic therapy' as a good surrogate endpoint to potentially predict for long-term outcome [Prowell and Pazdur, 2012]. However, the magnitude of the effect on pCR might differ according to the intrinsic breast cancer subtypes: pCR is a good surrogate endpoint for triple-negative, luminal B/HER2-negative, and HER2-positive (nonluminal) breast cancer, but not for luminal A and luminal B/HER2-positive disease [von Minckwitz *et al.* 2012].

Additionally, primary treatment of HER-2 positive breast cancer has become one of the most important and suitable scenarios to develop and test rapidly novel targeted therapies in contrast to these very long, large adjuvant trials that take years. The neoadjuvant approach also provides a significant source of tumour tissue to identify molecular heterogeneity, potential predictive biomarkers of response and mechanisms of resistance in residual tumours. This paper reviews the latest clinical trials in this regard and their impact in clinical practice.

Randomized phase III trials of chemotherapy and trastuzumab in the neoadjuvant setting

Randomized phase III trials of chemotherapy and trastuzumab in the neoadjuvant setting are summarized in Table 1. The addition of trastuzumab to primary chemotherapy has significantly improved the pCR rate in HER2-positive breast cancer. A number of clinical trials have evaluated the impact of adding trastuzumab to primary chemotherapy, varying the type of chemotherapy, timing of introduction of trastuzumab and duration of treatment with trastuzumab. Since 2005, major progress has been made in the neoadjuvant treatment of HER2-positive disease. The first randomized trial reported was the study [Buzdar *et al.* 2005]; 42 patients were randomized to receive or not receive weekly trastuzumab for a total of 24 weeks with concurrent administration of taxanes and anthracycline-based chemotherapy. Patients treated with trastuzumab achieved one of the highest pCRs in breast and nodes ever reported (65.2% *versus* 26%), although with small sample size because the study was discontinued early. No clinical cardiac dysfunctions or other relevant toxicities were observed. The updated safety and efficacy data from this trial [Buzdar *et al.* 2007] supported the initial findings and showed that disease-free survival was significantly better among trastuzumab-treated patients. The efficacy and safety observed in the MD Anderson Cancer Center trial were reproduced in a non-clinical trial setting, in which 51 of 83 consecutive patients (61.4%) achieved a pCR in the breast and lymph nodes [95% confidence interval (CI) 50–72%], and no symptomatic heart failure was seen at a median follow up of 50.2 months [Pernas *et al.* 2012].

Larger randomized phase III trials also supported the addition of trastuzumab to anthracycline- and taxane-based primary chemotherapy. In the NOAH trial [Gianni *et al.* 2010], 235 patients who were HER2 positive with locally advanced or inflammatory breast cancer were randomly assigned to neoadjuvant chemotherapy based on doxorubicin, paclitaxel, cyclophosphamide, methotrexate and fluorouracil with or without trastuzumab. Trastuzumab-treated patients had a substantial improvement in the pCR rate in breast (43% *versus* 22%; $p = 0.0007$) and of event-free survival at 3 years (71% *versus* 56%, HR 0.59; $p = 0.013$). The updated analysis reported recently [Gianni *et al.* 2013] confirms the significant event-free survival benefit and shows a strong trend towards improved overall survival with the

Table 1. Randomized phase III trials of chemotherapy and trastuzumab in the neoadjuvant setting.

Study [ref.]	*n*	Stage	Neoadjuvant therapy	pCR rate % (definition)
MDAnderson [Buzdar *et al.* 2005] 2nd cohort [Buzdar *et al.* 2007]	42 22	II-IIIA	P/3 weeks (4C) → FEC (4C) ± concomitant weekly trastuzumab	65% *versus* 26% 55% (ypT0/is ypN0)
NOAH [Gianni *et al.* 2010]	235	Only LABC (26% cT4d)	A + P/3 weeks (3C) → P/3 weeks (4C) → CMF (3C) ± concomitant 3 weeks trastuzumab	38% *versus* 19% (ypT0/is ypN0)
GeparQuattro [Untch *et al.* 2010]	445	II-III (10% cT4d)	EC × 4 → D [X] + concomitant trastuzumab/3 weeks	40% (ypT0/is ypN0)
ACOSOG Z1041 [Buzdar *et al.* 2013]	282	II-III (8% cN3)	FEC-75 (4C) → weekly P + trastuzumab × 12 weeks *versus* Weekly P × 12 weeks → FEC-75 (4C) + concomitant weekly trastuzumab	56.5% *versus* 54.2% (ypT0/is)
HannaH [Ismael *et al.* 2012]	596	II-III	D (4C) → FEC-75 (4C) + concomitant 3 weeks trastuzumab intravenously or subcutaneously	34% *versus* 39% (ypT0/is ypN0)

A, adriamicine; C, cycle; D, docetaxel; FEC, 5 fluorouracil + epirubicin + cyclophosphamide; LABC, locally advanced breast cancer; P, paclitaxel; pCR, pathological complete response; CMF, cyclophosphamide, methotrexate and fluorouracil; X, capecitabine.

addition of trastuzumab to primary chemotherapy, with a median follow up of 5.4 years (73.5 *versus* 62.9; HR 0.66; $p = 0.055$). The German GeparQuattro study [Untch *et al.* 2010] evaluated the incorporation of capecitabine into an anthracycline/taxane-based regimen and the concurrent use of trastuzumab in patients who were HER2 positive. Of 1509 patients included, 445 had HER2-positive tumours and all received trastuzumab concomitantly with all chemotherapy; pCR rate (defined as no invasive or *in situ* residual tumours in the breast) was higher in HER2-positive disease compared with the HER2-negative reference group (31.7% *versus* 15.7%; $p < 0.001$), without clinically significant toxicity. Cardiac events were low in these trials, despite concurrent administration of trastuzumab and anthracyclines. A negative hormone-receptor status was the only independent predictor for pCR.

The recent published phase III ACOSOG Z1041 trial [Buzdar *et al.* 2013] answers the question of the effect of the timing of trastuzumab administration in relation to an anthracycline- and taxane-based therapy. No improvement in breast pCR was found in patients treated with concurrent paclitaxel and trastuzumab followed by concurrent 5 fluorouracil + epirubicin + cyclophosphamide (FEC-75) and trastuzumab compared with FEC alone followed by concurrent paclitaxel and trastuzumab (54.2% *versus* 56.5%). The duration of neoadjuvant trastuzumab (24 weeks *versus* 12 weeks) did not significantly affect the pCR. Although substantial asymptomatic decreases in left ventricular ejection fraction occurred in similar percentages of patients in each group, concurrent administration of trastuzumab with anthracyclines offers no additional benefit, and the authors conclude that this is not warranted.

In the HannaH study [Ismael *et al.* 2012], 596 patients were randomly assigned to a 24-week regimen of trastuzumab administered either intravenously or at a fixed dose of 600 mg subcutaneously every 3 weeks concurrently with docetaxel followed by FEC. After surgery, patients continued trastuzumab to complete 1 year of treatment. A total of 40.7% in the intravenous group and 45.4% in the subcutaneous group achieved breast pCR. The difference between groups in breast pCR was 4.7% (95% CI −4.0 to 13.4). Subcutaneous trastuzumab was noninferior to intravenous trastuzumab in terms of pharmacokinetics and pCR. Subcutaneous treatment can be given in about 5 min, without loading

dose, providing substantial time saving for patients, physicians and nursing staff.

Novel anti-HER2 drugs and dual anti-HER2 blockade in the neoadjuvant setting

Lapatinib

Lapatinib (Tykerb; GlaxoSmithKline, Research Triangle Park, NC, USA) is an oral dual tyrosine kinase inhibitor which reversibly inhibits both epidermal growth factor receptor (EGFR) and HER2, and is currently approved for patients with HER2-positive advanced breast cancer whose disease has progressed on trastuzumab [Geyer *et al.* 2006]. In addition, lapatinib leads to an accumulation of HER2 at the cell surface, enhancing trastuzumab-dependent antibody-dependent cell-mediated cytotoxicity (ADCC) [Scaltriti *et al.* 2009]. In heavily pretreated patients, the combination of trastuzumab and lapatinib without chemotherapy significantly prolonged progression-free and overall survival compared with lapatinib alone [Blackwell *et al.* 2012]. In the neoadjuvant setting, lapatinib has been tested as a single agent or in combination with trastuzumab.

In the NeoALTTO study [Baselga *et al.* 2012], a phase III trial, 455 women were randomized to receive weekly trastuzumab, lapatinib (1500 mg), or trastuzumab plus lapatinib (1000 mg). Initially, patients received anti-HER2 therapy alone for 6 weeks and then combined with weekly paclitaxel (80 mg/m^2) for a further 12 weeks. After surgery, patients received adjuvant chemotherapy with FEC followed by the same targeted therapy as in the neoadjuvant phase for a total of 52 weeks. Dual anti-HER2 therapy yielded a substantially higher pCR rate (defined as no evidence of infiltrating tumour in the breast) than the monotherapy arms (51% *versus* 29% with trastuzumab alone $p = 0.001$ and 25% with lapatinib alone $p = 0.13$). This trial confirmed the finding from previous studies of higher pCR rate in ER-negative tumours compared with ER-positive tumours. Lapatinib-containing arms were associated with more grade 3 and 4 adverse events, mainly diarrhoea (grade 3 in 23% *versus* 2%), and liver enzyme disorders (17.5% *versus* 7.4%) and higher rates of treatment discontinuation. In the CHER-LOB study [Guarneri *et al.* 2012], a noncomparative randomized phase II trial, 121 patients with operable HER2-positive breast cancer were randomized to receive weekly trastuzumab, lapatinib (1500 mg) or trastuzumab plus lapatinib (1000 mg), concurrently with chemotherapy (consisting of weekly paclitaxel for 12 weeks, followed by FEC). The results showed an increase in the pCR rate in the breast and nodes from 25% with trastuzumab (lower than expected) or 26.3% with lapatinib to 46.7% with dual HER2 blockade. The pCR rate was higher in the case of HR negativity (41.3% *versus* 28.8%). Diarrhoea, dermatologic and hepatic toxicities occurred more frequently in the lapatinib-containing arms as well as dose reductions and treatment discontinuations. No cases of congestive heart failure were observed. In the open-label, randomized phase III NSABP B-41 study [Robidoux *et al.* 2013], 529 women were randomized to receive trastuzumab, lapatinib (1250 mg) or trastuzumab plus lapatinib (750 mg) in combination with weekly paclitaxel and after having received four cycles of doxorubicin and cyclophosphamide. By contrast with the previous reported studies, although the pCR rate in the breast was increased from 52.5% to 62% with dual HER2 blockade, the difference was not statistically significant ($p = 0.095$). It should be noted that the trastuzumab control group did substantially better than anticipated. Moreover, only 63% of the patients in the dual-blockade arm completed the treatment (essentially due to gastroenteric toxicity) compared with 78% in the trastuzumab arm. Grade 3 diarrhoea was observed in 20% in the lapatinib group and in 27% in the combination group. In this trial, the group of lapatinib as a single HER2-targeting agent also showed a high percentage of breast pCR (53%), although seven of these patients (4%) crossed over to trastuzumab. The US Oncology study presented at the 2011 American Society of Clinical Oncology (ASCO) Annual Meeting [Holmes *et al.* 2013] evaluated the administration of trastuzumab, lapatinib or the combination of both for 2 weeks followed by four cycles of FEC-75 and then weekly paclitaxel for 12 weeks. The pCR rate for trastuzumab, lapatinib or the combination of both was 54%, 45% and 74% respectively. The results presented at the 2013 ASCO Annual Meeting from the CALGB 40601 study [Carey *et al.* 2013], a phase III trial with a similar design to NeoALTTO, showed a numerically higher pCR rate in the breast with dual blockade (51% *versus* 40% in the trastuzumab alone arm), but similar to B-41, the difference was not significant.

The head-to-head comparison of lapatinib to trastuzumab treatment in the phase III

randomized trial GeparQuinto, GBG 44 trial [Untch *et al.* 2012] showed a significantly lower pCR rate (defined as ypT0ypN0) with chemotherapy and lapatinib (23%) than with chemotherapy and trastuzumab (30%). In this trial, 620 patients who were HER2 positive with operable or locally advanced breast cancer were randomized to receive four cycles of epirubicin (90 mg/m^2) plus cyclophosphamide (600 mg/m^2) and four cycles of docetaxel (100 mg/m^2), every 3 weeks, with either trastuzumab or lapatinib (1000–1250 mg) throughout all cycles before surgery. Targeted agent dose reductions were again more common with lapatinib than with trastuzumab (32% *versus* 1%), mainly due to diarrhoea and skin rash; trastuzumab more frequently caused oedema and dyspnoea. Results in the phase II randomized GEICAM 2006-14 trial [Alba *et al.* 2011] are similar with a pCR rate in the trastuzumab group twice as high (48%) as that in the lapatinib group (24%), using the same chemotherapy regimen as in the GeparQuinto trial. Some explanations of this reduced activity of lapatinib as a single anti-HER2 agent, although not seen in the NSABP B-41 study [Robidoux *et al.* 2013], might be related to dose reductions or discontinuations. Another explanation could be a lower ability of lapatinib to block the HER2 pathway compared with trastuzumab, which might have an additional anti-tumour effect via ADCC. These results of lapatinib treatment in early breast cancer have led to the recommendation that it should not be used as a single (neo) adjuvant anti-HER2 target outside clinical trials.

Pertuzumab

Pertuzumab (Perjeta; Roche, Basel, Switzerland) is a recombinant humanized monoclonal antibody that binds to the dimerization domain II of HER2, thus inhibiting heterodimerization of HER2 with other HER family members, including EGFR, HER3 and HER4. By blocking the pairing of HER2/HER3, the most potent signalling HER dimer, pertuzumab affects key signalling pathways as phosphoinositide-3-kinase (PI3K)/Akt/mammalian target of rapamycin (mTOR) that mediate cell proliferation and survival. Preclinical studies have shown that pertuzumab and trastuzumab have synergistic activity inhibiting breast tumour cell survival. Pertuzumab in combination with trastuzumab and docetaxel compared with trastuzumab plus docetaxel significantly prolonged progression-free survival when used as first-line treatment in the metastatic setting (18.5 *versus* 12.4 months) [Baselga *et al.* 2012] as well as overall survival [Swain *et al.* 2013]. These results have led to regulatory approval of pertuzumab in combination with trastuzumab and docetaxel and have placed this regimen as the standard of care in first-line HER2-positive advanced breast cancer. In the neoadjuvant setting, the NeoSphere trial [Gianni *et al.* 2012] is a phase II, randomized, four-arm study, with a unique design, as one of these arms included biological treatment without chemotherapy. In this study, 417 patients (a third of whom had locally advanced disease) were randomized to receive four cycles every 3 weeks of docetaxel + trastuzumab or docetaxel + trastuzumab + pertuzumab or docetaxel + pertuzumab or the doublet of the two monoclonal antibodies, trastuzumab + pertuzumab, without chemotherapy. After surgery, all patients received anthracyclines and trastuzumab. The pCR rate (defined by no invasive carcinoma in the breast) was 45.8% for the combined regimen of the dual blockade compared with 29% for the trastuzumab arm ($p = 0.014$) and 24% for the pertuzumab arm ($p = 0.003$). Surprisingly the pCR in the chemotherapy-free arm was almost 17%, with a favourable safety profile, thus raising the question of whether a subgroup of patients with HER2-positive breast cancer can be treated with biological agents alone and avoid chemotherapy. However, a third of the patients in this arm did not respond to the combination of both antibodies, highlighting the need for predictive biomarkers of response. As seen in previous studies, tumour eradication was higher in ER-negative than in ER-positive disease. This was also observed in the group of patients treated without chemotherapy (pCR rate of 27% *versus* 6%). In the phase II TRYPHAENA study [Schneeweiss *et al.* 2013], 225 patients with HER2-positive disease were randomized to three arms, all of them experimental, with the dual blockade of pertuzumab and trastuzumab. Arms A and B received three cycles of FEC followed by three cycles of docetaxel and they were randomized to start the combination of pertuzumab and trastuzumab with cycle 1 of FEC (arm A) or with cycle 1 of docetaxel (arm B). Arm C received six cycles of concurrent docetaxel, carboplatin and pertuzumab plus trastuzumab. The primary endpoint of cardiac safety was met, with low rates of symptomatic left ventricular systolic dysfunction across all arms. Pertuzumab did not increase the rate of cardiac dysfunction observed in combinations of trastuzumab plus standard chemotherapy, regardless of whether it was given sequentially or concurrently with anthracycline-based or combined with carboplatin-based

chemotherapy. A pCR in the breast (ypT0/is) was seen in 61.6% (arm A), 57.3% (arm B) and 66.2% (arm C). The duration of dual anti-HER2 therapy (9 weeks *versus* 18 weeks) did not significantly affect pCR, as seen with the duration of trastuzumab in the ACOSOG Z1041 trial [Buzdar *et al.* 2013]. Higher pCR rates were observed in patients with hormone-negative disease (79.4%, 65% and 83.8% for arms 1, 2 and 3, respectively).

The US Food and Drug Administration approved (on 30 September 2013) pertuzumab for use in combination with trastuzumab and docetaxel for the neoadjuvant treatment of patients with HER2-positive, locally advanced, inflammatory or early stage breast cancer, on the basis of significantly improved pCR (see http://www.fda.gov).

Endocrine therapy and dual anti-HER2 blockade in the neoadjuvant setting

Approximately 50% of HER2-positive breast cancers are also hormone receptor (HR) positive. HER2-positive/ER-positive tumours, may be relatively resistant to endocrine therapies. Preclinical and clinical data suggest that there is a complex bidirectional cross talk between HER2 and ER signalling pathways [Giuliano *et al.* 2013]. Therefore, treatment approaches targeting either pathway are associated with upregulation of the other one, as an escape mechanism, causing resistance to therapy. Combining targeted treatments that simultaneously block both signalling pathways is a promising approach to prevent or overcome either endocrine or anti-HER2 resistance in some HER2-positive/ER-positive tumours. The phase II TBCRC006 study [Rimawi *et al.* 2013] assessed the combination of lapatinib (1000 mg) plus trastuzumab in a chemotherapy-free regimen, administered for 12 weeks in 64 patients with stages I–III HER2-positive breast cancer (with a median tumour size of 6 cm). Women with ER-positive disease also received letrozole (plus luteinizing hormone-releasing hormone agonist if premenopausal). Overall in-breast pCR (ypT0/is) was 27% and once again HR negativity was associated with increased pCR rates (36% *versus* 21%). The surprising pCR rate of 21% achieved in patients who were HER2 positive/ER positive with the dual HER2 blockade and endocrine therapy is 3.5-fold higher than the pCR achieved in the NeoSphere chemotherapy and endocrine free arm and more similar to the 26% pCR rate achieved in HER2-positive/ER-positive tumours treated with trastuzumab plus pertuzumab and docetaxel [Gianni *et al.* 2012; Prat and Baselga, 2013]. These data support the hypothesis that a subset of patients with primary HER2-positive disease may not require cytotoxic chemotherapy, a hypothesis that requires further validation and identification of biomarkers to select the appropriate patients. Clinical trials with novel therapeutic approaches in primary HER2-positive breast cancer without chemotherapy are ongoing, as the 'PAM50 HER2-enriched Phenotype as a Predictor of Response to Dual HER2 Blockade in HER2-positive Early Breast Cancer (PAMELA)' study [ClinicalTrials.gov identifier: NCT01973660].

Predictive factors of response or resistance to neoadjuvant anti-HER2 therapy

Different predictive biomarkers of pCR or residual disease after neoadjuvant chemotherapy in HER2-positive breast cancer have been evaluated in many studies. Nonetheless, to the present day, no biomarker is clearly validated and only HER2 remains a suitable biomarker for therapeutic decisions in HER2-positive disease [Guiu *et al.* 2013].

Hormonal receptor status and pCR

ER-negative tumours are associated with higher pCR rates compared with ER-positive ones, including in HER2-positive disease [Cortazar *et al.* 2014]. This is regardless of the drug regimen or the duration of chemotherapy. Moreover, in patients treated with just dual blockade without chemotherapy tumour eradication is also higher in ER-negative disease [Gianni *et al.* 2012; Rimawi *et al.* 2013], thus indicating that the likelihood of response according to HR status is an intrinsic tumour characteristic (see Table 2). As mentioned before, a pooled analysis of German neoadjuvant studies evidenced a different prognostic value of pCR [von Minckwitz *et al.* 2012]. In patients with HER2-positive/ER-negative tumours, pCR was associated with significantly higher disease-free survival compared with no pCR, but in the group of HER2-positive/ER-positive tumours, no difference was observed. The ER pathway might be a relevant escape mechanism in ER-positive and HER2-positive tumours [Wang *et al.* 2011]. Thus, the importance of also blocking the ER in HER2-positive/ER-positive disease, as demonstrated in the TBCRC006 study [Rimawi *et al.* 2013], achieving a surprising 21% of pCR with the dual HER2 blockade and endocrine therapy in this subset of patients.

Table 2. Novel anti-HER2 drugs and dual anti-HER2 blockade in the neoadjuvant setting.

Study [ref.]	Phase	*n*	Neoadjuvant therapy	pCR (%)		
				All	HR+	HR–
Neo-ALTTO [Baselga *et al.* 2012]	3	455	Lapatinib + paclitaxel Trastuzumab + paclitaxel Lapatinib + trastuzumab + paclitaxel (anti-HER2 therapy alone 6 weeks → concomitant with weekly paclitaxel × 12 weeks)	20 28 47 (ypT0/isypN0)	16 23 42	34 36 61
NSABP B-41 [Robidoux *et al.* 2013]	3	519	Trastuzumab + CHT Lapatinib + CHT Trastuzumab + lapatinib + CHT [CHT: AC (4C) → weekly P (day 1, 8, 15, 28 × 4C)]	49 47 60 (ypT0/isypN0) $p = 0.056$ (combination *versus* trastuzumab) $p = 0.78$ (lapatinib *versus* trastuzumab)	45 42 55	58 55 70
CHER-LOB [Guarneri *et al.* 2012]	2	121	Trastuzumab + CHT Lapatinib + CHT Trastuzumab + lapatinib + CHT [CHT: weekly P (12 weeks) → FEC75 (4C)]	25 26 47 (ypT0/isypN0)	25 23 36	27 36 56
US Oncology [Holmes, 2013]	2	100	Trastuzumab + CHT Lapatinib + CHT Trastuzumab + lapatinib + CHT [CHT: FEC75 (4C) → weekly P (12 weeks)]	54 45 74	38 23 71	69 63 78
CALGB 40601 [Carey *et al.* 2013]	3	305	Trastuzumab + paclitaxel Lapatinib + trastuzumab + paclitaxel Lapatinib + paclitaxel (closed early) (16 weeks of neoadjuvant treatment)	40 51 32 $p = 0.11$ (ypT0/is)		
NeoSphere [Gianni *et al.* 2012]	2	417	Trastuzumab + docetaxel Trastuzumab + pertuzumab + docetaxel Trastuzumab + pertuzumab Pertuzumab + docetaxel (12 weeks of neoadjuvant treatment)	29 46 17 24 (ypT0/is)	20 26 6 17	37 63 27 30
TRYPHAENA [Schneeweiss *et al.* 2013]	2	225	FEC + trastuzumab + pertuzumab × 3 → D + trastuzumab + pertuzumab × 3 FEC × 3 → D + trastuzumab + pertuzumab × 3 D + carboplatin + trastuzumab + pertuzumab × 6	62 57 66 (ypT0/is)	46 48 50	79 65 84
TBCRC 006 [Rimawi *et al.* 2013]	2	64	Lapatinib + trastuzumab ± letrozol (12 weeks of neoadjuvant treatment)	27 (ypT0/is)	21	36

A, adriamicine; C, cycle; D, docetaxel; FEC, 5 fluorouracil + epirubicin + cyclophosphamide; HER2, human epidermal growth factor receptor 2; P, paclitaxel; pCR, pathological complete response rate.

Activation of PI3K pathway

HER2 overexpression induces an activation of multiple signalling pathways, including the PI3K/Akt. Reported data are discrepant with regard to the prognostic or predictive value of *PIK3CA* mutations especially in HER2-positive breast cancer. In the adjuvant setting, the results from the FinHER trial showed that PIK3CA mutations were associated with a better outcome; however this effect disappeared after 3 years. There were no statistically significant associations with trastuzumab benefit [Loi *et al.* 2013a]. In the neoadjuvant scenario, some studies have shown that patients with low expression of phosphatase and tensin homolog (PTEN) or PI3K mutations have poorer response, with lower pCR rate and worse

clinical outcome with trastuzumab-containing treatment [Dave *et al.* 2011; Berns *et al.* 2007; Nagata *et al.* 2004]. In a prospective analysis of 737 participants of the GeparSixto and GeparQuinto studies, PI3KCA mutation was found to predict resistance to antiHER2/chemotherapy in primary HER2-positive/hormone-receptor-positive breast cancer. Within the HER2-positive/HR-positive subgroup, the patients with *PIK3CA* mutation had a pCR rate of only 6.5% compared with 30.8% in the wild type group ($p = 0.005$). In contrast, there was no difference in pCR (42.9% *versus* 46.1%) according to *PIK3CA* mutation status in the HER2-positive/HR-negative ($p = 0.825$) group [Loibl *et al.* 2013]. Low PTEN expression could be a potential biomarker to select patients with tumours resistant to trastuzumab but sensitive to lapatinib [Dave *et al.* 2011].

Truncated form of HER2 receptor (p95HER2)

In preclinical models, p95HER2 is associated with resistance to trastuzumab but responsiveness to lapatinib [Scaltriti *et al.* 2007]. The results of clinical studies evaluating p95HER2 are controversial. In the CHER-LOB study [Guarneri *et al.* 2012], p95HER2 was present in 30.7% of cases. However, the pCR rate was not different for p95HER2-positive or p95HER2-negative tumours in any treatment group. In the GeparQuattro study, p95HER2-positive tumours surprisingly showed a significantly higher pCR rate compared with that of p95HER2-negative tumours (59% *versus* 24%) [Loibl *et al.* 2011].

HER2 serum levels

The extracellular domain of the HER2 protein can be cleaved from the surface by metalloproteases and detected in peripheral blood and measured by enzyme-linked immunosorbent assay. In *GeparQuattro* and *GeparQuinto* trials a positive association between higher prechemotherapy sHER2 levels and higher pCR was shown [Witzel *et al.* 2010, 2012]. A fall of HER2 serum levels over 20% during neoadjuvant therapy was a predictor of pCR only in the group treated with lapatinib [Witzel *et al.* 2012].

Tumour infiltrating lymphocytes

High levels of tumour infiltrating lymphocytes (TILs) predict trastuzumab and chemotherapy benefit in early-stage HER2-positive breast cancer, as seen in the adjuvant, phase III, FinHER study [Loi *et al.* 2013a]. Most recently, Loi and colleagues validated these findings in the GeparQuattro trial. In this trial, each 10% increment in TILs was associated with higher rates of pCR (adjusted odds ratio 1.14; 95% CI 1.01–1.29; $p = 0.037$) after neoadjuvant trastuzumab and chemotherapy [Loi *et al.* 2013b].

Other potential biomarkers

In the NOAH trial, c-Myc amplification was associated with higher probability of pCR and overexpression of insulin-like growth factor was associated with higher likelihood of residual disease after trastuzumab-based chemotherapy [Gianni *et al.* 2008]. Markers of resistance to trastuzumab as p-4E- BP1 (an activator of the mTOR pathway) and ALDH1 (a stem cell marker), were investigated in patients included in the GeparQuattro study [Huober *et al.* 2010]. Elevated circulating miR-210 levels [Jung *et al.* 2012] and lower expression of genes involved with CD40 signalling [Esteva *et al.* 2007] have been associated with a greater risk of residual disease. Molecular profiles have suggested that responders have disruption of HER2–HER3 linkages and downstream regulators of growth and transcription, and nonresponders' tumours might use stem cell related pathways [Holmes *et al.* 2013]. Polymorphisms on the FcγRIIIa appear to exert an effect on ADCC and trastuzumab activity [Musolino *et al.* 2008].

18F fluorodeoxyglucose positron emission tomography/computed tomography

Early metabolic assessment using 18F fluorodeoxyglucose positron emission tomography/computed tomography (18F-FDG PET/CT) can identify patients with an increased likelihood of pCR after neoadjuvant trastuzumab, lapatinib or their combination when given with chemotherapy, as seen in the Neo-ALTTO study [Gebhart *et al.* 2013]. Mean SUVmax reductions for pCR and non-pCR respectively were 54.3% *versus* 32.8% at week 2 ($p = 0.02$) and 61.5% *versus* 34.1% at week 6 ($p = 0.02$). The pCR rates were twice as high for 18F-FDG PET/CT responders than nonresponders (week 2: 42% *versus* 21%, $p = 0.12$; week 6: 44% *versus* 19%, $p = 0.05$).

Conclusion

HER2 breast cancer is a biological heterogeneous disease with different characteristics and clinical

outcomes. The introduction of trastuzumab to primary chemotherapy in HER2-positive disease has dramatically improved the pCR rate, a good surrogate marker for long-term efficacy, particularly for patients with HER2-positive/ER-negative breast cancer. Neoadjuvant treatment of HER2-positive breast cancer has become one of the most suitable scenarios to develop novel targeted therapies. Although there were differences between clinical trials with respect to duration of treatment, type of therapy, definitions of pCR and sample size, data from neoadjuvant randomized studies (see Table 2) indicate greater efficacy with the combination of two HER2-targeted agents with nonoverlapping mechanisms of action than with a single HER2-targeted therapy, as seen in the metastatic setting. Lapatinib or pertuzumab in combination with trastuzumab and chemotherapy provides the highest pCR. The subgroup of patients with HER2-positive/ER-negative tumours achieves higher pCR rates than those with ER-positive tumours. There is a subset of patients with HER2-positive breast cancer in whom chemotherapy could be omitted entirely, just with dual blockade with or without hormonal treatment. However, there are still many questions to be answered, such as do all HER2-positive breast cancer patients need multiple HER2-targeted agents; which is the optimal dual HER2 blockade and the best chemotherapy regimen to be combined with; how long should the neoadjuvant therapy last; and which patients can be treated with biological therapy exclusively and can avoid chemotherapy with dual HER2-targeted therapy with or without hormonal treatment? There is indeed an urgent need to find robust predictive biomarkers of response to each anti-HER2 agent or their combination in order to develop rationally individualized therapy.

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Conflict of interest statement

The author has no conflicts of interest.

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