British Journal of Cancer (2012) 106, 6–13

www.bjcancer.com

Minireview

Beyond trastuzumab: novel therapeutic strategies in HER2-positive metastatic breast cancer

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The use of trastuzumab, a monoclonal antibody that targets the human epidermal growth factor receptor 2 (HER2) alteration present in 25 to 30% of breast cancers, has been associated with improved survival outcomes in both the adjuvant and metastatic settings. However, despite the robust clinical efficacy of trastuzumab in HER2-positive metastatic breast cancer (MBC), primary and secondary resistance remains a clinical challenge. Although lapatinib has demonstrated modest activity in this setting, trials reported to date have yet to demonstrate improvements in overall survival with its use. Novel therapeutic strategies to circumvent trastuzumab resistance are warranted, and agents targeting the HER, vascular endothelial growth factor, heat shock protein 90, phosphoinositide 3 kinase/Akt/mammalian target of rapamycin, and insulin-like growth factor-1 receptor pathways represent rational approaches in the management of HER2-positive disease. In this review, early-phase and emerging trial data surrounding the use of these promising agents in HER2-positive MBC will be discussed.
British Journal of Cancer (2012) **106,** 6–13. doi:10.1038/bjc.2011.516 www.bjcancer.com

Keywords: HER2; breast cancer; afatinib; trastuzumab; T-DM1; lapatinib

The human epidermal growth factor receptor 2 (HER2) oncogene is amplified and overexpressed in 25 to 30% of breast cancers and has provided a basis for targeted therapeutics of this originally poor-prognostic phenotype (Slamon *et al*, 1987; Slamon *et al*, 1989). Amplification of HER2 (ErbB2), a member of the ErbB/HER family of receptor tyrosine kinases that also include epidermal growth factor receptor (EGFR)/HER1/ErbB1, HER3/ErbB3 and HER4/ErbB4, leads to increased receptor homo- and hetero-dimerisation and subsequent activation of downstream signaling pathways associated with cell proliferation, differentiation, survival and angiogenesis (Yarden and Sliwkowski, 2001). Trastuzumab (Herceptin; Genentech, South San Francisco, CA, USA), the first available HER2-directed therapy, is a humanised monoclonal antibody that targets the HER2 extracellular domain, and was approved by the US Food and Drug Administration in 1998 for the management of HER2-positive metastatic breast cancer (MBC) in combination with chemotherapy. The anti-proliferative and cytotoxic effects of trastuzumab likely result from a combination of antibody-dependent cellular cytotoxicity, inhibition of extracellular domain cleavage, decreased DNA repair, decreased intracellular signal transduction and anti-angiogenic effects (Spector and Blackwell, 2009). The pivotal phase-III trial of trastuzumab plus chemotherapy in the first-line management of HER2-positive MBC demonstrated robust improvements in response rates (RRs; 50% *vs* 32%), median time to progression (TTP; 7.4 *vs* 4.6 months) and median overall survival (25 *vs* 20 months) with the addition of trastuzumab (Slamon *et al*, 2001).

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Received 15 July 2011; revised 25 October 2011; accepted 31 October 2011

Nonetheless, primary and secondary resistance are frequently encountered. Possible mechanisms include HER2 crosstalk with other ErbB members or insulin-like growth factor-1 receptor (IGF-1R) (Nahta *et al*, 2004), presence of p95-HER2, a truncated receptor that lacks the extracellular binding domain for trastuzumab (Scaltriti *et al*, 2007), PTEN deficiency (phosphatase and tensin homologue deleted on chromosome 10) (Nagata *et al*, 2004), increased phosphoinositide 3 kinase (PI3k)/Akt pathway activation (Berns *et al*, 2007) and, more recently, presence of Rac1, a Ras-like small GTPase that affects trastuzumab-mediated endocytosis of the ErbB2 receptor (Dokmanovic *et al*, 2009). Still, no one mechanism has been defined in the clinic and it is not clear that resistance to trastuzumab means a complete loss of dependence on HER2 signaling.

Developing novel targeted agents for use in HER2-positive breast cancer remains clinically significant. In this review, strategies to overcome resistance to trastuzumab therapy (Figure 1) will be discussed including novel antibody-based approaches against HER2, newer ErbB-family tyrosine kinase inhibitors (TKIs), anti-angiogenic therapies, heat shock protein 90 (hsp90) inhibitors, PI3K and mammalian target of rapamycin (mTOR) inhibitors, and IGF-1R inhibitors (Tables 1 and 2).

NEW ANTIBODY-BASED TARGETING OF HER2 – PERTUZUMAB, TRASTUZUMAB DM1 (T-DM1)

Pertuzumab

Like trastuzumab, pertuzumab (Omnitarg, 2C4; Genentech) targets the HER2 extracellular domain but at a different epitope, resulting in inhibited dimerisation of HER2 with other HER family receptors

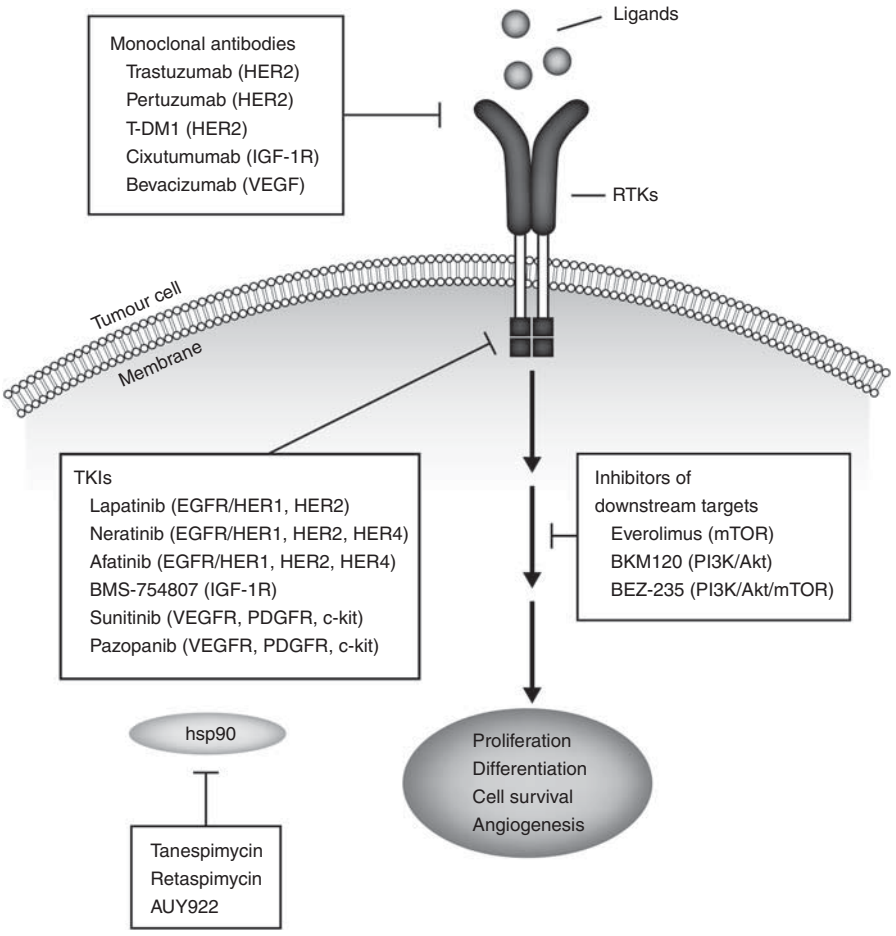

Figure 1 Targeted agents for HER2-positive MBC. Diagram depicting the molecular targets of approved and investigational agents for HER2-positive MBC. Abbreviations: Akt, protein kinase B; c-kit, stem cell factor receptor; EGFR, epidermal growth factor receptor; HER, human epidermal growth factor receptor; hsp90, heat shock protein 90; IGF-1R, insulin-like growth factor-1 receptor; MBC, metastatic breast cancer; mTOR, mammalian target of rapamycin; PDGFR, platelet-derived growth factor receptor; PI3K, phosphoinositide-3-kinase; RTK, receptor tyrosine kinase; TKI, tyrosine kinase inhibitor; VEGF, vascular endothelial growth factor; VEGFR, VEGF receptor.

(EGFR/HER1, HER3 and HER4) (Agus *et al*, 2002). Preclinical studies in HER2-positive breast cancer have demonstrated promising antitumour efficacy with associated downregulation of PI3K/Akt and MAPK signaling pathways both as a single-agent and synergistically with trastuzumab (Nahta *et al*, 2004; Agus *et al*, 2002; Franklin *et al*, 2004; Scheuer *et al*, 2009). In a single-arm, phase-II study of trastuzumab plus pertuzumab (every 3 weeks) in 11 patients with HER2-positive MBC and disease progression on trastuzumab, an objective RR of 18% was reported, with partial responses in 2 patients and stable disease in 3 patients (Portera *et al*, 2008). The median TTP was 6 weeks. Using a lower limit left ventricular ejection fraction (LVEF) cut-off of 55%, six cases of left ventricular systolic dysfunction were seen (three grade 1, two grade 2 and one grade 3) in this trial, raising concerns over cardiotoxicity (Portera *et al*, 2008). In another single-arm phase-II trial of trastuzumab (weekly or every 3 weeks (q3 weekly)) plus pertuzumab (every 3 weeks) in 66 patients with trastuzumab-refractory, HER2-positive MBC, a similar objective RR was also demonstrated at 24.2%, including 5 patients with complete response (7.6%) and 11 with partial response (16.7%) (Baselga *et al*, 2010). Furthermore, stable disease $\geqslant 6$ months was seen in an additional 17 patients (25.8%), yielding a 50% clinical benefit rate (CBR). The median progression-free survival (PFS) was 5.5 months in this cohort. In contrast to the previous phase-II study, cardiotoxicity was less of an issue despite the use of identical drug doses in the q3 weekly regimen, with only three patients experiencing an asymptomatic LVEF decline $\geqslant 10\%$ and an absolute LVEF $< 50\%$. In two out of the three patients, recovery in LVEF was seen without treatment interruption, whereas the third patient withdrew from the study because of progressive disease. Overall, the trastuzumab/pertuzumab combination was well tolerated, with diarrhoea (64%), fatigue (33%), nausea (27%) and rash (26%) being the most common toxicities (Baselga *et al*, 2010). Ongoing trials are currently evaluating combinations of pertuzumab with trastuzumab and chemotherapy in HER2-positive MBC, including a phase-II trial of trastuzumab/capecitabine ± pertuzumab in the second-line setting (PHEREXA; NCT01026142) and a large global phase-III trial of trastuzumab/docetaxel ± pertuzumab in the first-line setting (CLEOPATRA; NCT00567190). Combinations of pertuzumab with T-DM1 (discussed below) are also under investigation in multiple phase-I–III studies.

T-DM1

T-DM1 (Genentech) represents a novel approach to drug delivery in which the monoclonal antibody trastuzumab is conjugated to an anti-microtubule agent (emtansine) (Lewis Phillips *et al*, 2008; Junttila *et al*, 2010). In preclinical models, potent antitumour activity was observed with T-DM1, including in trastuzumab- and lapatinib-resistant states (Lewis Phillips *et al*, 2008; Junttila *et al*, 2010). In a phase-I study of T-DM1 in a heavily pretreated

Table 1 Overview of novel therapeutic strategies under investigation for HER2-positive metastatic breast cancer

Agent	Type	Phase of development[a]	Clinical setting(s)
HER-targeted			
Pertuzumab (Omnitarg)	Monoclonal antibody	III	First-line and relapsed; combination with trastuzumab+chemotherapy
T-DM1	Antibody-drug conjugate	III	First-line and relapsed (after trastuzumab+taxane, after progression with ⩾2 prior HER2-targeted therapies); monotherapy, combination with pertuzumab
Lapatinib (Tykerb)	Reversible TKI	Approved	Relapsed (after anthracycline, taxane, and trastuzumab); combination with capecitabine
		III	First-line; combination with letrozole (if hormone receptor positive)
			First-line and relapsed; combination with chemotherapy or trastuzumab
Neratinib (HKI-272)	Irreversible TKI	III	First-line and relapsed (after trastuzumab ± chemotherapy); combination with chemotherapy or trastuzumab
Afatinib (BIBW 2992)	Irreversible TKI	III	First- and second-line (after trastuzumab+chemotherapy, after trastuzumab and/or lapatinib progression); monotherapy, combination with chemotherapy
Anti-angiogenic			
Bevacizumab (Avastin)	Monoclonal antibody	III	First-line; combination with chemotherapy and trastuzumab
Sunitinib (Sutent)	TKI	II	First- and second-line; combination with trastuzumab ± chemotherapy
Pazopanib (Votrient)	TKI	II	First-line; combination with lapatinib
Other strategies			
Tanespimycin (17-AAG)	Hsp90 inhibitor	II[b]	First- and second-line (after trastuzumab progression); combination with trastuzumab
Retaspimycin (IPI-504)	Hsp90 inhibitor	II	Relapsed (after trastuzumab progression); combination with trastuzumab
AUY922	Hsp90 inhibitor	I/II	Relapsed (after trastuzumab progression); monotherapy, combination with trastuzumab
BKM120	Pan-PI3K inhibitor	I/II	Relapsed (after trastuzumab progression); combination with trastuzumab
BEZ-235	PI3K/mTOR dual inhibitor	I/II	Relapsed (after trastuzumab progression); monotherapy, combination with trastuzumab; in *PIK3CA* and/or *PTEN* mutation-positive patients only
Everolimus (RAD001, afinitor)	mTOR inhibitor	III	First-line and relapsed (after trastuzumab resistance+taxane); combination with chemotherapy+trastuzumab
BMS-754807	IGF-1R inhibitor	I/II	Relapsed (after trastuzumab failure); combination with trastuzumab
Cixutumumab (IMC-A12)	IGF-1R inhibitor	II	Relapsed (after trastuzumab and chemotherapy); combination with capecitabine/lapatinib

Abbreviations: HER = human epidermal growth factor receptor; hsp90 = heat shock protein 90; IGF-1R = insulin-like growth factor-1 receptor; mTOR = mammalian target of rapamycin; TKI = tyrosine kinase inhibitor; T-DM1 = trastuzumab DM1. [a]Specific to breast cancer only, unless otherwise indicated. [b]Further clinical development has been halted.

Table 2 Ongoing phase III clinical trials of investigational agents in HER2-positive metastatic breast cancer

Trial	Setting	Treatment regimen	Target accrual	Status[a]
Pertuzumab				
CLEOPATRA (NCT00567190)	First-line	Trastuzumab/docetaxel/pertuzumab vs trastuzumab/docetaxel/placebo	808	Active, no longer recruiting
T-DM1				
MARIANNE (NCT01120184)	First-line	Trastuzumab+taxane (docetaxel or paclitaxel) vs T-DM1+pertuzumab vs T-DM1+placebo	1,092	Recruiting
EMILIA (NCT00829166)	Relapsed (after trastuzumab+taxane)	T-DM1 vs lapatinib+capecitabine	980	Recruiting
NCT01419197	Relapsed (progression after ⩾2 prior HER2-targeted therapies)	T-DM1 vs treatment of physician's choice	795	Recruiting
Afatinib				
LUX-Breast 1 (NCT01125566)	Second-line (after trastuzumab progression)	Afatinib+vinorelbine vs trastuzumab+vinorelbine	780	Recruiting
Everolimus				
BOLERO-1 (NCT00876395)	First-line	Trastuzumab/paclitaxel/everolimus vs trastuzumab/paclitaxel/placebo	717	Recruiting
BOLERO-3 (NCT01007942)	Relapsed (after trastuzumab resistance+taxane)	Trastuzumab/vinorelbine/everolimus vs trastuzumab/vinorelbine/placebo	572	Recruiting

Abbreviations: HER = human epidermal growth factor receptor; T-DM1 = trastuzumab DM1. [a]ClinicalTrials.gov accessed October 14, 2011.

population of HER2-positive MBC in which patients had received a median of four prior systemic agents, an encouraging clinical RR of 44% was reported (Krop *et al*, 2010). At a maximum tolerated dose of 3.6 mg kg^{-1} IV q3 weekly, T-DM1 was well-tolerated overall with only mild toxicities (thrombocytopenia, transaminitis, fatigue, nausea and anemia) and no reported cardiotoxicities. In two phase-II trials of single-agent T-DM1 in a heavily pretreated population, impressive RRs were similarly reported (Krop *et al*, 2009b; Burris *et al*, 2010). The first trial enrolled 112 patients with HER2-positive MBC who had received a median of three prior chemotherapy agents; the overall RR was 25.9%, and 24.2% in those who had previously received both trastuzumab and lapatinib (Burris *et al*, 2010). T-DM1 was well tolerated, with grade 3 or 4 hypokalemia (8.9%) and thrombocytopenia (8%) being the most

common. The second trial enrolled 110 patients who had previously received a median of seven prior systemic agents (including an anthracycline, taxane, capecitabine, trastuzumab and lapatinib); an objective RR of 32.7% and a CBR of 44.5% were reported (Krop *et al*, 2009a). Good patient tolerability to T-DM1 was seen, with thrombocytopenia, fatigue, and nausea being the most common adverse events (AEs), and no dose-limiting cardiotoxicities. Recently, preliminary results from a randomised phase-II trial of T-DM1 *vs* trastuzumab/docetaxel in first-line, HER2-positive MBC were presented (Perez *et al*, 2010). In 137 patients with a median follow-up of 6 months, single-agent T-DM1 achieved an objective RR of 47.8%, as compared with 41.4% for the trastuzumab/docetaxel arm. A more favorable safety profile was observed with the T-DM1 arm, with a lower incidence of grade 3 and 4 AEs (37.3% *vs* 75.0%). A recent update of these data also demonstrated a significant increase in investigator-reported PFS with T-DM1 compared with the control arm (14.2 *vs* 9.2 months, respectively (Hurvitz *et al*, 2011). Preliminary data from a single-arm phase-Ib/II trial evaluating the combination of pertuzumab and T-DM1 in patients with previously untreated ($n=21$) and relapsed ($n=46$) HER2-positive MBC showed a RR of 57.1% in previously untreated patients (majority had received trastuzumab (86%), taxanes (71%) and anthracyclines (62%) in the adjuvant setting) and a RR of 34.8% in patients with relapsed disease (Dieras *et al*, 2010). T-DM1 plus pertuzumab appeared to be well-tolerated overall, although cardiotoxicity was observed with LVEF declines in two patients. The results of large global phase-III trials of T-DM1, including T-DM1 *vs* lapatinib plus capecitabine in patients previously treated with a taxane and trastuzumab (EMILIA; NCT00829166), as well as a three-arm trial evaluating T-DM1 *vs* T-DM1/pertuzumab *vs* trastuzumab/taxane in the first-line setting (MARIANNE; NCT01120184), are eagerly awaited.

HER-FAMILY TKIS – LAPATINIB, NERATINIB AND AFATINIB

Lapatinib

Lapatinib (Tykerb; GlaxoSmithKline, London, UK) is a small molecule, reversible, dual inhibitor of EGFR/HER1 and HER2, currently approved by the US Food and Drug Administration for use in MBC. Preclinical studies demonstrated potent antitumour effects in HER2-overexpressing models, including in cell lines with acquired trastuzumab resistance (Rusnak *et al*, 2001; Xia *et al*, 2002; Konecny *et al*, 2006). In phase-I and -II trials of single-agent lapatinib in patients with HER2-positive breast cancer refractory to trastuzumab, lapatinib exhibited modest clinical activity and a tolerable toxicity profile (diarrhoea and rash) (Burris *et al*, 2005; Burstein *et al*, 2008b; Blackwell *et al*, 2009). The pivotal phase-III trial evaluated the combination of lapatinib (1250 mg daily) plus capecitabine (2000 mg m^{-2} daily, given on days 1–14 of a 21-day cycle) *vs* capecitabine alone in patients with HER2-positive locally advanced or MBC who were treatment refractory to an anthracycline, taxane and trastuzumab (Geyer *et al*, 2006). In this trial of 324 patients, lapatinib plus capecitabine resulted in a 4-month improvement in median TTP (8.4 *vs* 4.4 months; hazard ratio (HR) = 0.49; $P<0.001$). A higher RR also favoured the combination arm (22% *vs* 14%), although this was not statistically significant. In the updated efficacy analyses, the improvement in median TTP was confirmed (6.2 *vs* 4.3 months; HR = 0.57; $P=0.00013$), although no statistical differences in overall survival were demonstrated (Cameron *et al*, 2008; Cameron *et al*, 2010). Lapatinib was reasonably well tolerated, with an increased incidence of diarrhoea and rash with the addition of capecitabine. The EGF30008 trial (Johnston *et al*, 2009) was a phase-III trial that evaluated letrozole plus lapatinib ($n=642$) *vs* letrozole plus placebo ($n=644$) in treatment-naive post-menopausal patients with hormone receptor-positive MBC. Among the 219 estrogen receptor-positive, HER2-positive patients, a 5.2-month improvement in the primary endpoint of median PFS was seen in the lapatinib/letrozole arm (8.2 *vs* 3.0 months; HR = 0.71; $P=0.019$). Overall survival data are awaited. Interestingly, a biomarker analysis of this study suggests there may be a role for HER-family targeting with letrozole in HER2-negative ER low-expressing tumours (Finn *et al*, 2009a). In the EGF30001 phase-III trial, which evaluated lapatinib plus paclitaxel *vs* paclitaxel alone in the first-line setting, a median TTP improvement of 11.3 weeks was observed in the HER2-positive population (36.4 *vs* 25.1 weeks; HR = 0.53), albeit on a subset analysis (Di Leo *et al*, 2008; Finn *et al*, 2009b). Total HER2 blockade with lapatinib and trastuzumab was also evaluated in a phase-III trial of lapatinib plus trastuzumab *vs* lapatinib alone in patients with MBC who had received a median of three prior trastuzumab-containing regimens, and an almost 4-week improvement in PFS (12.0 *vs* 8.1 weeks; HR = 0.73; $P=0.008$), as well as a doubling of CBR (24.7% *vs* 12.4%; $P=0.01$), were observed (Blackwell *et al*, 2010). Importantly, data thus far only suggest a trend to overall survival improvement (51.6 *vs* 39.0 weeks; HR = 0.75; $P=0.106$). Interim results of a phase-II trial evaluating the combination of lapatinib and trastuzumab in patients with HER2-positive MBC (cohort 1 ($n=40$): no prior lapatinib, trastuzumab or chemotherapy for metastatic disease and >1 year since adjuvant trastuzumab, if received; cohort 2 ($n=47$): one to two prior lines of chemotherapy, including trastuzumab, or relapse within 1 year of adjuvant trastuzumab) were recently presented and showed objective RRs of 41.7% and 25% in cohorts 1 and 2, respectively (Lin *et al*, 2011). Grade 3/4 treatment-related toxicities were described as uncommon, with grade 3 diarrhoea reported in 7% and all others (not specified) in $<3\%$ of patients.

Neratinib

Neratinib (HKI-272; Pfizer, New York, NY, USA) is an irreversible, oral small-molecule TKI of EGFR/HER1, HER2 and HER4 (Rabindran *et al*, 2004). In preclinical HER2 models, antiproliferative effects were accompanied by G1 cell-cycle arrest and decreased downstream signal transduction (Rabindran *et al*, 2004). In a phase-I study of neratinib in advanced solid malignancies, partial response was seen in 8 out of 25 (32%) HER2-positive breast cancer patients who were previously treated with trastuzumab, anthracyclines and taxanes (Wong *et al*, 2009). Diarrhoea was the dose-limiting toxicity at a maximum tolerated dose of 320 mg once daily. An open-label, phase-II multicenter trial of single-agent neratinib in advanced HER2-positive breast cancer, which enrolled both trastuzumab-refractory ($n=66$) and trastuzumab-naive ($n=70$) patients, demonstrated modest clinical activity in both cohorts (Burstein *et al*, 2010). Objective RRs of 24% and 56% were seen in the trastuzumab-refractory and trastuzumab-naive groups, respectively, with a median PFS of 22.3 and 39.6 weeks. At a dose of 240 mg once daily, diarrhoea was the most common grade 3/4 AE, occurring in up to 30% of patients and necessitating dose reductions and/or symptomatic management. No cases of grade 3 or 4 cardiotoxicity were observed (Burstein *et al*, 2010). Currently, studies of single-agent neratinib (neratinib *vs* lapatinib/capecitabine, NCT00777101) and neratinib combinations (with capecitabine, NCT00741260; trastuzumab, NCT00398567; paclitaxel, NCT00445458; vinorelbine, NCT00706030; and neratinib/paclitaxel *vs* trastuzumab/paclitaxel, NCT00915018) are under evaluation in HER2-positive MBC. The clinical relevance of neratinib as a 'pan-HER' family inhibitor and it being irreversible is yet to be proven.

Afatinib

Afatinib (BIBW 2992; Boehringer Ingelheim, Ingelheim, Germany), an anilino–quinazoline-derived irreversible, oral small-molecule ErbB

family TKI (EGFR/HER1, HER2 and HER4), has also demonstrated activity in early-phase trials of advanced solid tumours and trastuzumab-refractory HER2-positive breast cancer (Hickish *et al*, 2009; Yap *et al*, 2010; Yamamoto *et al*, 2011). Preclinical data showed low nanomolar potency for EGFR, HER2 and HER4 kinases (Yamamoto *et al*, 2011), as well as anti-proliferative effects in HER2-dependent models (Li *et al*, 2008). In a phase-I trial of 53 patients with advanced solid tumours, antitumour efficacy was reported with continuous once-daily dosing of afatinib, including stable disease for $\geqslant 6$ months in 1 breast cancer patient (receptor status and prior therapy not specified) (Yap *et al*, 2010). Gastrointestinal (diarrhoea and nausea/vomiting) and dermatologic AEs (rash and dry skin), as well as fatigue, were most common. An open-label, single-arm phase-II study of afatinib in 41 patients with HER2-positive MBC following trastuzumab failure demonstrated partial responses in 4 patients and stable disease in 8 patients (maintained for at least four cycles) (Hickish *et al*, 2009). At a dose of 50 mg once daily, grade 3 rash (9.8%) and diarrhoea (22%) were most common. Recently, a global phase-III trial of afatinib in HER2-positive MBC was initiated (LUX-Breast 1; NCT01125566), which is evaluating vinorelbine/afatinib *vs* vinorelbine/trastuzumab in patients with prior trastuzumab therapy.

ANTI-ANGIOGENIC STRATEGIES – BEVACIZUMAB, SUNITINIB AND PAZOPANIB

Bevacizumab

Preclinical and clinical studies in HER2-positive breast cancer have reported positive associations between HER2 and vascular endothelial growth factor (VEGF) expression levels (Yen *et al*, 2000; Yang *et al*, 2002; Konecny *et al*, 2004). In a phase-II trial evaluating trastuzumab and the monoclonal anti-VEGF antibody bevacizumab (Avastin, Genentech) in the first-line metastatic setting, an objective clinical RR by the World Health Organization criteria of 48% and a CBR of 60% were reported (Hurvitz *et al*, 2009). Cardiovascular toxicities, including hypertension (most common AE), as well as declines in LVEF (15 grade 1/2, 1 grade 4), and ulcer perforation were seen. Two ongoing phase-III trials, AVEREL (docetaxel/trastuzumab ± bevacizumab; NCT00391092) and ECOG 1105 (carboplatin/paclitaxel/trastuzumab ± bevacizumab; NCT00520975), are evaluating the addition of bevacizumab to chemotherapy and trastuzumab as first-line therapy in HER2-positive MBC.

In a heavily pre-treated HER2-positive MBC population (median of four prior chemotherapies, three prior biological and two prior hormonal therapies) of which 47 out of 52 (90%) received prior trastuzumab, a phase-II study of lapatinib plus bevacizumab reported a modest overall RR of 13% (Dickler *et al*, 2008). Diarrhoea and rash, attributable to lapatinib use, were the most common AEs. Declines in LVEF were also observed in this trial (two grade 1 and three grade 2).

VEGFR TKIs

Sunitinib (Sutent; Pfizer) is an oral, multitargeted TKI against VEGFR, platelet-derived growth factor receptor and stem cell factor receptor (c-kit). In an open-label phase-II study of sunitinib monotherapy in patients with MBC previously treated with taxanes and anthracyclines, an overall RR of 11% was observed (Burstein *et al*, 2008a). However, no correlation between clinical response and ER or HER2 status was found. At present, two early-phase clinical trials are evaluating sunitinib/trastuzumab combinations in HER2-positive breast cancer, including a phase-I trial of sunitinib plus trastuzumab and docetaxel in the first-line setting (NCT00372424) and a phase-II trial of sunitinib plus trastuzumab in the second-line setting (NCT00243503).

Pazopanib (Votrient; GlaxoSmithKline) is an oral multitargeted TKI against VEGFR-1/2/3, platelet-derived growth factor receptor and c-kit. In a randomised phase-II study of pazopanib (400 mg per day) plus lapatinib (1000 mg per day) *vs* lapatinib alone (1500 mg per day) in HER2-positive, locally advanced or MBC in the first-line setting, an interim analysis of 114 evaluable patients (total $n = 141$) demonstrated modest efficacy with the dual TKI approach (Slamon *et al*, 2008). Pazopanib plus lapatinib yielded a 12-week progressive disease rate of 15.9% *vs* 36.8% for lapatinib monotherapy (by investigator assessment). A secondary endpoint of 12-week RR also favoured the combination arm at 44.9% *vs* 27.8% (by investigator assessment; 36.2% *vs* 22.2% by independent assessment). AEs of diarrhoea, nausea, transaminitis, hypertension, fatigue and dysgeusia were potentiated with the pazopanib/lapatinib combination, whereas hair color change was solely observed in the dual TKI arm. Notably, four patients experienced declines in LVEF (three asymptomatic and one symptomatic) with the combined anti-HER2/VEGF strategy.

HSP90 INHIBITORS

A novel therapeutic approach involves targeting the hsp90 molecular chaperone, whose function includes regulating the stability and maturation of various oncoproteins including HER2 (Trepel *et al*, 2010). Tanespimycin (17-AAG, KOS-953; Bristol-Myers Squibb, New York, NY, USA), a first-generation geldanamycin derivative, has demonstrated robust antitumour activity in preclinical models of HER2-positive breast cancer (Munster *et al*, 2001; Munster *et al*, 2002). A phase-I study of tanespimycin plus trastuzumab was encouraging, and antitumour activity was observed in patients with HER2-positive MBC (Modi *et al*, 2007). In a subsequent single-arm phase-II trial of tanespimycin (IV weekly) plus trastuzumab in patients with HER2-positive MBC and disease progression following trastuzumab, an overall RR of 22% and CBR of 59% were reported (Modi *et al*, 2011). Tanespimycin was well-tolerated overall, with diarrhoea, fatigue, nausea and headache as the most common toxicities. Although further clinical development of tanespimycin has been halted, other hsp90 inhibitors, including retaspimycin (IPI-504; Infinity Pharmaceuticals, Cambridge, MA, USA) and AUY922 (Novartis, Cambridge, MA, USA) are currently under evaluation in early-phase clinical trials as single agents or in combination with trastuzumab (NCT00817362, NCT00526045 and NCT01271920).

PI3K/AKT/MTOR PATHWAY MODULATION

Another strategy to combat trastuzumab resistance involves modulation of the PI3K/Akt/mTOR pathway (Nahta and O'Regan, 2010). Evaluation of the mTOR inhibitor everolimus (RAD001, Afinitor; Novartis) in HER2-positive breast cancer is the most advanced in clinical development to date. In a preclinical study of PTEN-deficient, trastuzumab-resistant *in vitro* and *in vivo* models, the combination of everolimus and trastuzumab resulted in enhanced antitumour effects (Lu *et al*, 2007). In phase-I trials, promising clinical activity was reported with everolimus when used in combination with paclitaxel/trastuzumab or vinorelbine/trastuzumab in HER2-positive MBC (Andre *et al*, 2010; Jerusalem *et al*, 2011). In the first trial, a phase-Ib dose-escalation study of everolimus with weekly paclitaxel and trastuzumab, an overall RR of 44% was reported among 27 evaluable patients (Andre *et al*, 2010). In all, 74% of patients experienced disease control for >6 months, with a median PFS of 34 weeks for the entire cohort. At the established dose of 10 mg per day, grade 3 and 4 neutropenia were the most common AEs (52%). In the second phase-Ib trial, which evaluated everolimus plus weekly vinorelbine and trastuzumab in HER2-positive MBC patients pretreated with trastuzumab, an overall RR of 19.1%, disease control rate of 83.0% and median

PFS of 30.7 weeks were reported for the 47 evaluable patients (Jerusalem *et al*, 2011). Neutropenia (92%) and stomatitis (70%) were the most common hematologic and nonhematologic toxicities, respectively. Additionally, three patients developed febrile neutropenia and four patients received G-CSF support. Based on these promising early-phase clinical data in HER2-positive MBC, phase-III trials of trastuzumab/paclitaxel ± everolimus in the first-line setting (BOLERO-1; NCT00876395) and trastuzumab/vinorelbine ± everolimus in the trastuzumab-refractory setting (BOLERO-3; NCT01007942) are in progress. Early-phase clinical trials are currently evaluating modulation of the PI3K/Akt/mTOR pathway with the use of PI3K/Akt inhibitors, including BKM120 (Novartis) and BEZ-235 (Novartis) (NCT00620594 and NCT01132664). Preclinical studies with PI3K/Akt inhibitors suggest increased activity in tumours with *PIK3CA* mutations (Brachmann *et al*, 2009), identified in approximately 20 to 30% of HER2-positive breast cancers (Saal *et al*, 2005; Stemke-Hale *et al*, 2008; Gonzalez-Angulo *et al*, 2011).

IGF-1R INHIBITORS

Crosstalk between HER2 and IGF receptor families leading to activation of alternative signaling pathways has also been implicated in trastuzumab resistance (Nahta *et al*, 2006). Preclinical models of trastuzumab-resistant, HER2-positive breast cancer have characterised restoration of trastuzumab sensitivity by disrupting the IGF-1R/HER2 heterodimer, synergistic interactions with trastuzumab and associated decreased downstream receptor signaling with IGF-1R inhibition (Lu *et al*, 2001; Nahta *et al*, 2005; Esparis-Ogando *et al*, 2008). In phase-I trials of IGF-1R monoclonal antibodies in advanced solid malignancies, these agents appear to be well-tolerated overall, although toxicities of thrombocytopenia and hyperglycemia were observed (Weroha and Haluska, 2008). Currently, phase-I and -II studies of anti-IGF-1R therapies are underway in patients with locally advanced or metastatic HER2-positive breast cancer after trastuzumab failure, including a phase-I/II study of the small-molecule inhibitor BMS-754807 (Bristol-Myers Squibb) in combination with trastuzumab (NCT00788333) and a phase-II study of capecitabine/lapatinib with or without the monoclonal antibody cixutumumab (IMC-A12; ImClone, Bridgewater, NJ, USA) in patients previously treated with trastuzumab and an anthracycline and/or a taxane (NCT00684983).

CONCLUSIONS

There is no doubt that trastuzumab has provided significant clinical benefit in patients with HER2-positive breast cancer. Still, primary (*de novo*) and secondary (acquired) resistance represents a real clinical challenge. Lapatinib has demonstrated modest clinical activity in this setting and highlights the importance of ongoing HER2 blockade in trastuzumab-refractory states. Newer HER-family TKIs that are both irreversible and target HER4 in addition to EGFR and HER2 are being evaluated and may provide superior outcomes in this population. Meanwhile, anti-VEGF strategies, such as bevacizumab, have demonstrated promising activity and phase-III results are eagerly awaited. Other investigational agents in HER2-positive MBC, including hsp90 and mTOR inhibitors, utilise novel approaches to combat trastuzumab resistance and have also shown promising activity in early-phase clinical trials. IGF-1R inhibition is supported by biologic rationale in the setting of trastuzumab resistance due to receptor crosstalk, but mature clinical data are lacking. The two antibody-based HER2-directed approaches, pertuzumab and T-DM1, have shown promising efficacy. Both agents are currently under phase-III evaluation and have the potential to establish new treatment paradigms. Finally, ongoing translational research is critical in the development of novel biomarkers predictive of clinical benefit with these evolving targeted agents in HER2-positive breast cancer in order to minimise untoward drug-related toxicities and ultimately enhance patient outcomes.

ACKNOWLEDGEMENTS

This work was supported by Boehringer Ingelheim Pharmaceuticals, Inc. (BIPI). Editorial assistance was provided by Lisa Shannon, PharmD, of MedErgy, which was contracted by BIPI for these services. The authors meet criteria for authorship as recommended by the International Committee of Medical Journal Editors, were fully responsible for all content and editorial decisions, and were involved at all stages of manuscript development. The authors received no compensation related to the development of the manuscript. The manuscript is the sole product of the authors and no writing assistance was obtained. RYT was a Research Fellow of the Terry Fox Foundation (award #020017), Canada.

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