

# Human Epidermal Growth Factor Receptor Family-Targeted Therapies in the Treatment of HER2-Overexpressing Breast Cancer

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Disclosures of potential conflicts of interest may be found at the end of this article.

**Key Words.** Epidermal growth factor receptor • Human epidermal growth factor receptor tyrosine kinase receptor family • Breast cancer • Tyrosine kinase inhibitor

## Learning Objectives

Describe the role of HER2 in breast cancer pathogenesis.

List the approved and investigational agents targeting the HER receptor family and downstream signaling pathways with focus on overcoming resistance to HER2-targeted therapies.

Describe ongoing clinical trials evaluating the efficacy and safety of agents targeting HER and downstream pathways in breast cancer patients.

## ABSTRACT

Breast cancer characterized by overexpression of human epidermal growth factor receptor 2 (HER2) has been associated with more aggressive disease progression and a poorer prognosis. Although an improved understanding of breast cancer pathogenesis and the role of HER2 signaling has resulted in significant survival improvements in the past 20 years, resistance to HER2-targeted therapy remains a concern. A number of strategies to prevent or overcome resistance to HER2-targeted therapy in breast cancer are

being evaluated. This article provides a comprehensive review of (a) the role of HER2 signaling in breast cancer pathogenesis, (b) potential receptor and downstream therapeutic targets in breast cancer to overcome resistance to HER2-targeted therapy, and (c) clinical trials evaluating agents targeting one or more members of the HER family and/or downstream pathways for the treatment of breast cancer, with a focus on metastatic disease. *The Oncologist* 2014;19:135–150

**Implications for Practice:** Although a greater understanding of the role of human epidermal growth factor receptor 2 (HER2) signaling in breast cancer pathogenesis has led to significant survival improvements, resistance to HER2-targeted therapy remains an important concern. Several treatment strategies are being evaluated to overcome resistance to HER2-targeted therapy, including targeting one or more members of the HER family and/or downstream signaling pathways. With the many ongoing investigations of HER family agents across various treatment settings in breast cancer, this is an exciting but challenging research area with rapidly evolving data.

## INTRODUCTION

The human epidermal growth factor family of tyrosine kinase receptors includes human epidermal growth factor receptor 1 (HER1; also known as epidermal growth factor receptor [EGFR]), HER2, HER3, and HER4. Most of these receptors can be activated by ligand-dependent dimerization—the pairing of two of the same receptors (homodimerization) or two different receptors (heterodimerization)—within the plasma membrane. Although there are no known endogenous ligands for HER2, when HER2 is overexpressed, it can undergo ligand-independent activation via spontaneous homodimerization and autoactivation [1] and may facilitate heterodimerization with HER1 and HER3, resulting in the activation of multiple signal transduction

pathways. HER2 overexpression (HER2 positivity) in breast cancer has been associated with poorer clinical outcomes compared with HER2-negative disease [2–6].

## TRASTUZUMAB

Trastuzumab (Herceptin; Genentech, South San Francisco, CA, <http://www.gene.com>) is an anti-HER2 monoclonal antibody initially approved as first-line treatment for metastatic breast cancer (MBC) in combination with paclitaxel in 1998 that is currently indicated by the U.S. Food and Drug Administration (FDA) for the treatment of HER2-positive breast cancer [7]. It appears to work by a variety of different mechanisms, including

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prevention of HER2 cleavage and downstream proliferative signaling, antiangiogenic effects, cellular cytotoxicity, and interference with DNA repair [8]. The original FDA approval of trastuzumab for MBC was based on a phase III trial comparing chemotherapy plus trastuzumab with chemotherapy alone in previously untreated HER2-positive MBC, with significantly greater time to progression (TTP), overall survival (OS), and overall response rate (RR) seen with trastuzumab plus chemotherapy [9]. However, an increase in heart failure rate was observed with the addition of trastuzumab (10% when given with chemotherapy vs. 2% with chemotherapy alone). Trastuzumab has also been investigated as a single agent in the metastatic setting, but RRs have been relatively low (approximately 20%–25%) [10, 11]. The combination of trastuzumab with antiestrogen therapies such as aromatase inhibitors has also been evaluated and was associated with improvement in progression-free survival (PFS) in two randomized trials (TAnDEM and eLECTRA) of postmenopausal women with HER2-overexpressing and estrogen receptor (ER)-positive MBC (Table 1) [12, 13].

Trastuzumab was subsequently approved in the adjuvant setting, and its benefits have been demonstrated in multiple phase III trials including HERA, FinHer, Breast Cancer International Research Group 006, and the National Surgical Adjuvant Breast and Bowel Project (NSABP) B31 [7, 14–17]. These randomized trials showed that trastuzumab therapy (in combination or subsequent to chemotherapy) was associated with improvements in disease-free survival (DFS) in patients with HER2-positive breast cancer [14–16]. The HERA trial demonstrated improvements in DFS and OS at a median follow-up of 8 years with both 1 and 2 years of trastuzumab maintenance; however, 2 years was not superior to 1 year of adjuvant trastuzumab [18]. Data on 9 weeks of maintenance trastuzumab are less substantiated [19], and the PHARE trial was inconclusive for non-inferiority when comparing 6 versus 12 months of trastuzumab adjuvant maintenance therapy, suggesting that 12 months of adjuvant trastuzumab is to remain the standard [20].

Trastuzumab has also been evaluated in the neoadjuvant setting combined with chemotherapy, with pathologic complete response (pCR) rates of 60% when given with paclitaxel and subsequent fluorouracil/epirubicin/cyclophosphamide (FEC) [21] and approximately 55% in a study of different sequencing of paclitaxel and FEC [22]. In the phase III GeparQuattro trial, HER2-positive patients received neoadjuvant chemotherapy with trastuzumab, with a pCR rate of 31.7% compared with 15.7% in the HER2-negative reference group that received chemotherapy alone [23]. As in many similar trials, pCR rates were higher in hormone receptor-negative versus hormone receptor-positive HER2-positive cancers. The higher pCR rate following neoadjuvant trastuzumab-containing therapy seems to translate to better overall outcome: in the NOAH study of HER2-positive locally advanced or inflammatory breast cancer, patients treated with neoadjuvant/adjuvant trastuzumab-containing therapy had improved event-free survival at 3 years (71% vs. 56% for chemotherapy alone;  $p = .013$ ) [24] and 5 years (57.5% vs. 43.3%;  $p = .016$ ) [25], with a trend toward longer 5-year OS (73.5% vs. 62.9%;  $p = .055$ ) [25].

### EGFR INHIBITORS

Limited clinical studies in breast cancer have assessed the efficacy and safety of erlotinib (Tarceva; Genentech), a reversible

oral EGFR-targeted tyrosine kinase inhibitor (TKI) [26, 27]. A phase II study of erlotinib in 23 HER2-positive MBC patients showed only one partial response (PR) [28]. Combining erlotinib with trastuzumab as first-line therapy for HER2-positive MBC in a phase I/II trial revealed four PRs and TTP of 9.03 months in 12 patients [29]. This trial did not meet its accrual goal; hence, the efficacy of this regimen is unclear. Phase I/II studies also added gefitinib (Iressa; AstraZeneca, Wilmington, DE, <http://www.astrazeneca.com>), another EGFR-targeted TKI [27, 30], to trastuzumab in HER2-positive MBC. A study of trastuzumab and gefitinib resulted in 9% RR, and median TTP was 3 months in patients with no prior metastatic therapy and 5.3 months in those with prior therapy; median OS was 27 months [31]. A phase I/II study evaluating the addition of gefitinib to docetaxel and trastuzumab in MBC reported PFS of 12.7 months and RR of 64% [32].

### DUAL EGFR AND HER2 INHIBITORS

#### Lapatinib

Dual TKIs that interact with several EGFR members have shown more promising results than EGFR inhibition alone. Lapatinib (Tykerb; GlaxoSmithKline, London, U.K., <http://www.gsk.com/uk>) is an oral small-molecule reversible inhibitor of both EGFR and HER2 [33]. Lapatinib was first approved by the FDA in 2007 in combination with capecitabine for the treatment of HER2-positive MBC after prior trastuzumab/chemotherapy [34, 35] (Table 2). Approval was based on a phase III study comparing lapatinib/capecitabine with capecitabine alone in patients with MBC progressing after chemotherapy/trastuzumab, as TTP improved from 4.4 to 8.4 months [36]. A phase III trial testing lapatinib in combination with antiestrogen therapy also showed improvement, with a median PFS of 3.0 and 8.2 months with letrozole/placebo versus lapatinib/letrozole, respectively, in hormone receptor-positive HER2-positive MBC [37]. However, a phase III study of HER2-positive MBC patients demonstrated longer PFS with trastuzumab compared with lapatinib (11.4 vs. 8.8 months) when combined with first-line taxane-based therapy; no significant difference in OS was observed, and more grade 3 to 4 events of diarrhea and rash were observed with lapatinib [38]. Trastuzumab was also superior to lapatinib in the neoadjuvant setting in the phase III GeparQuinto trial, with pCR of 30.3% versus 22.7%, respectively ( $p = .04$ ) [39]. In the adjuvant setting, the TEACH phase III study evaluated lapatinib versus placebo, with improved DFS observed with lapatinib in patients with confirmed HER2-positive disease [40]. Thus, lapatinib may be an alternative in patients for whom adjuvant trastuzumab is contraindicated.

#### Combination of Lapatinib and Trastuzumab

In a phase III study, lapatinib plus trastuzumab significantly prolonged median PFS compared with lapatinib alone in HER2-positive MBC patients progressing on prior trastuzumab, although RRs were not significantly different [41]. An international phase III study is evaluating lapatinib/trastuzumab plus chemotherapy in the first-line setting for MBC [42], and another phase III study is examining whether addition of lapatinib improves PFS in MBC patients receiving

**Table 1.** Completed phase III clinical trials of trastuzumab in HER2-positive breast cancer

Trail	Patient population	Treatment	PFS	OS	TTP	RR	DFS	pCR
<b>Metastatic</b>								
Slamon 2001; phase III; n = 469 [9]	HER2-positive MBC without prior chemotherapy	A: AC or paclitaxel + trastuzumab B: AC or paclitaxel	NR	A: 25.1 mo B: 20.3 mo	A: 7.4 mo B: 4.6 mo	A: 50% B: 32%	NR	NR
TAnDEM; phase III; n = 207 [12]	Postmenopausal HER2-positive MBC	A: Anastrozole B: Anastrozole + trastuzumab	A: 2.4 mo B: 4.8 mo	A: 23.9 mo B: 28.5 mo	A: 2.4 mo B: 4.8 mo	A: 6.8% B: 20.3%	NR	NR
eLEcTRA; phase III; n = 93 [13]	HER2-positive, ER-positive MBC with no prior therapy	A: Letrozole B: Letrozole + trastuzumab	NR	NS	A: 3.3 mo B: 14.1 mo	A: 13% B: 27%	NR	NR
<b>Adjuvant</b>								
HERA; phase III; n = 3,387 [14, 18]	HER2-positive early breast cancer, adjuvant setting	A: Trastuzumab (1 yr) B: Observation Disease free at 1 yr → trastuzumab 1 yr or 2 yr	NR	A: 96.0% B: 95.1%	NR	NR	A: 85.8% B: 77.4%	NR
NSABP B-31 and NCCTG N9831, phase III; n = 3,351 [15]	Operable HER2-positive breast cancer, adjuvant setting	A: Chemotherapy B: Chemotherapy + trastuzumab	NR	A: 86.6% B: 91.4%	NR	NR	A: 67.1% B: 85.3%	NR
Slamon 2011; phase III; n = 3,222 [16]	HER2-positive breast cancer, adjuvant setting	A: AC + docetaxel B: AC + docetaxel + trastuzumab C: docetaxel + carboplatin + trastuzumab	NR	NR	NR	NR	A: 75% B: 84% C: 81%	NR
FinHer; phase III; n = 1,010 [17, 19]	HER2-positive cohort (n = 232)	A: Chemotherapy + 9 wk trastuzumab B: Chemotherapy	NR	A: 91.3% B: 82.3%	NR	NR	NR	NR
PHARE; phase III; n = 3,382 [20]	HER2-positive early breast cancer	A: Trastuzumab 6 mo B: Trastuzumab 12 mo	NR	NR	NR	NR	NR	NR
<b>Neoadjuvant</b>								
NOAH; phase III; n = 235 HER2 positive cohort [24] [25]	HER2-positive locally advanced or inflammatory breast cancer, neoadjuvant setting	A: 1 yr neoadjuvant trastuzumab + chemotherapy B: Chemotherapy alone	NR	A: 73.5% B: 62.9%	NR	A: 87% B: 74%	NR	A: 43% B: 22%
GeparQuattro; phase III; n = 1,509 [23]	Operable, locally advanced breast cancer, neoadjuvant setting	A: AC → docetaxel + trastuzumab B: AC → docetaxel + capecitabine + trastuzumab C: AC → docetaxel → capecitabine + trastuzumab D: HER2-negative reference cohort	NR	NR	NR	NR	NR	A: 32.9% B: 31.3% C: 34.6% D: 15.7%
Buzdar 2007; phase III; n = 64 [21]	Operable HER2-positive breast cancer, neoadjuvant setting	A: P + FEC + trastuzumab (randomized) B: P + FEC + trastuzumab (assigned) C: P + FEC (randomized)	NR	NR	NR	NR	A: 100% B: NR C: 85.3% (at 3 yr)	A: 65.2% B: 54.5% C: 26.3%
ACOSOG Z1041 (Alliance); phase III; n = 282 [22]	Operable HER2-positive breast cancer, neoadjuvant setting	A: FEC → paclitaxel + trastuzumab B: Paclitaxel + trastuzumab → FEC + trastuzumab	NR	NR	NR	NR	NR	A: 55.1% B: 54.2%

Abbreviations: AC, anthracycline + cyclophosphamide; DFS, disease-free survival; ER, estrogen receptor; HER2, human epidermal growth factor receptor 2; MBC, metastatic breast cancer; NR, not reported; NS, not significant; OS, overall survival; pCR, pathologic complete response; P + FEC, paclitaxel + fluorouracil + epirubicin + cyclophosphamide; PFS, progression-free survival; RR, response rate; TTP, time to progression.

trastuzumab maintenance (NCT00968968). The ALTERNATIVE phase III study will randomize hormone receptor-positive HER2-positive MBC patients to letrozole and trastuzumab with and without lapatinib (NCT01160211) [43]. The phase III ALTO study (NCT00490139) is evaluating lapatinib, trastuzumab, trastuzumab followed by lapatinib, and both together in the adjuvant setting. The phase III NeoALTO trial evaluated neoadjuvant/adjuvant lapatinib, trastuzumab, and the combination with chemotherapy in women with early-stage disease [44]. The combination of trastuzumab and lapatinib resulted in a higher pCR (51.3%) compared with trastuzumab (29.5%) or lapatinib (24.7%) alone [44]. NSABP B-41 involved 529 patients who received chemotherapy plus neoadjuvant trastuzumab (pCR, 52.5%), lapatinib (53.2%), or trastuzumab/lapatinib (62%;  $p = .075$ ), followed by a year of adjuvant trastuzumab [45]. Based on presented results of CALGB 40601, there was no significant difference in the pCR rate between weekly paclitaxel plus trastuzumab (40%) versus weekly paclitaxel plus trastuzumab/lapatinib (51%;  $p = .11$ ) [46]. A prior meta-analysis of neoadjuvant trastuzumab and lapatinib trials found that pCR was highest with trastuzumab plus lapatinib [47], suggesting that the optimal use of lapatinib may be in conjunction with trastuzumab through dual blockade of HER2 and EGFR, as lapatinib alone appears less effective than in combination with trastuzumab in the first-line setting. Neoadjuvant lapatinib and trastuzumab along with hormonal therapy were also administered to hormone receptor-positive/HER2-positive patients in a phase II study, with a substantial pCR of 21% despite no concurrent chemotherapy [48].

### Neratinib

Neratinib (PB-272; Puma Biotechnology, Inc., Los Angeles, CA, <http://www.pumabiotech.com> [licensed October 2011 from Pfizer, New London, CT, <http://www.pfizer.com>] [49]) is an oral small-molecule irreversible TKI of EGFR, HER2, and HER4 [50, 51]. In a phase II study, neratinib showed a median PFS of 22.3 and 39.6 weeks in 136 HER2-positive MBC patients with or without prior trastuzumab, respectively [52]. In another phase II MBC study with neratinib/vinorelbine, RRs were 41% (no prior lapatinib) and 8% (prior lapatinib) [53]. In a phase I/II trial of neratinib/paclitaxel, the RR among patients with HER2-positive MBC was 71% in those who had received up to one prior non-lapatinib-containing chemotherapy regimen and 77% in those pretreated with two or three chemotherapy regimens, which may have included lapatinib [54]. As with earlier trials, diarrhea was the most frequent toxicity observed with neratinib/vinorelbine and neratinib/paclitaxel, affecting >90% of patients [53, 54]. Another phase II study found no significant difference with neratinib versus lapatinib/capecitabine (median PFS was 4.5 and 6.8 months, and OS was 19.4 and 19 months, respectively) in 233 HER2-positive MBC patients [55]. Preliminary data from a phase I study (NSABP FB-8) of neratinib with trastuzumab and paclitaxel in HER2-positive MBC patients revealed one complete response (CR) and three PRs in 10 patients [56]. Multiple trials are ongoing examining the potential role of neratinib in the neoadjuvant (NCT01008150; NSABP FB-7), adjuvant (NCT00878709 [ExteNET]), and metastatic settings (NCT00915018 [NEFERTT]; NCT01808573 [NALA]).

### Afatinib

Afatinib (BIBW 2992; Boehringer Ingelheim, Ingelheim, Germany; <http://www.boehringer-ingelheim.com>) is an oral irreversible ErbB family inhibitor (EGFR, HER2, and HER4; Table 2) [57, 58]. Four of 41 patients had PR and 15 had stable disease in a phase II study of afatinib in heavily pretreated HER2-positive MBC [59]. Afatinib compared favorably with lapatinib and trastuzumab in 29 patients in the neoadjuvant setting, with RRs of 80%, 75%, and 36.4%, respectively [60]. There is an ongoing phase II study evaluating afatinib combined with trastuzumab-based therapy in the neoadjuvant setting (NCT01594177; DAFNE). Other phase II/III trials in the MBC setting include LUX-Breast 2 (NCT01271725) [61] and LUX-Breast 3 (NCT01441596) [62] (Table 2).

## NEWER ANTI-HER2 ANTIBODIES

### Pertuzumab

The monoclonal antibody pertuzumab (Perjeta; Genentech) binds to a different region of the HER2 extracellular domain than trastuzumab and can block dimerization of HER2 with other HER family receptors [63]. Pertuzumab was FDA approved in June 2012 in combination with docetaxel/trastuzumab as first-line therapy for HER2-overexpressing MBC [64]. This approval was based on the CLEOPATRA phase III study, in which 808 HER2-positive MBC patients were randomized to receive pertuzumab/trastuzumab/docetaxel or placebo/trastuzumab/docetaxel (Table 3) [65, 66]. At interim analysis, the addition of pertuzumab increased PFS from 12.4 months to 18.5 months ( $p < .001$ ), and OS analysis favored pertuzumab, with 17.2% deaths with pertuzumab versus 23.6% with control [65]. After an additional year of follow-up, median OS was 37.6 months with placebo/trastuzumab/docetaxel and had not been reached with pertuzumab/trastuzumab/docetaxel [66]. The addition of pertuzumab resulted in more diarrhea and febrile neutropenia, but did not increase cardiac dysfunction [65].

In the NeoSphere phase II trial, neoadjuvant pertuzumab/trastuzumab/docetaxel had the highest pCR rate in breast at 45.8%, as compared with trastuzumab/docetaxel (29%), pertuzumab/docetaxel (24%), and trastuzumab/pertuzumab (16.8%) [67]. pCR rates inclusive of breast and lymph nodes were also the highest with the triple combination (39.3%) versus trastuzumab/docetaxel (21.5%), pertuzumab/docetaxel (17.7%), and pertuzumab/trastuzumab (11.2%), with HER2-overexpressive hormone receptor-negative tumors responding best with 54.4% pCR inclusive of breast and lymph nodes [64]. The number of serious adverse events was similar across the three chemotherapy-containing arms but lower with pertuzumab/trastuzumab [67]. TRYPHAENA was a randomized phase II cardiac safety study of pertuzumab and trastuzumab as a component of neoadjuvant chemotherapy, reporting low incidences of left ventricular systolic dysfunction with pCR rates of 55% in breast and lymph nodes in patients with locally advanced stage II-III breast cancer treated with pertuzumab, trastuzumab, and docetaxel following FEC, and 64% with a nonanthracycline containing regimen of pertuzumab, trastuzumab, carboplatin, and docetaxel [68]. As a result of these trials, pertuzumab has recently been FDA approved

**Table 2.** Completed and ongoing phase II and phase III clinical trials of approved and investigational multitargeted HER family tyrosine kinase inhibitors in HER2-positive breast cancer<sup>a</sup>

Trial	Patient population	Study design	PFS	OS	RR	pCR
<b>Approved</b>						
<b>Lapatinib</b>						
Geyer 2006; phase III; n = 324; completed [36]	HER2-positive MBC with prior treatment	A: Lapatinib + capecitabine	A: 8.4 mo	TTP: A: 8.4 mo	A: 22%	NR
		B: Capecitabine	B: 4.1 mo	B: 4.4 mo	B: 14%	
Gelmon 2010; phase III; n = 652; ongoing [38]	HER2-positive MBC	A: Lapatinib + taxane-based chemo → lapatinib	A: 8.8 mo	NR	NR	NR
		B: Trastuzumab + taxane-based chemotherapy → trastuzumab	B: 11.4 mo			
TEACH, phase III; n = 3,161; completed [40]	HER2-positive early breast cancer, adjuvant	A: Lapatinib	DFS: A: 13%	A: 6%	NR	NR
		B: Placebo	B: 17%	B: 6%		
GeparQuinto; phase III; n = 620; completed [39]	HER2-positive early breast cancer, neoadjuvant	A: AC + docetaxel + trastuzumab	NR	NR	A: 90.1%	A: 30.3%
		B: AC + docetaxel + lapatinib			B: 90.2%	B: 22.7%
<b>Investigational</b>						
<b>Lapatinib + trastuzumab</b>						
Blackwell 2010; phase III; n = 296; completed [41]	HER2-positive MBC with prior trastuzumab	A: Paclitaxel + lapatinib	A: 8.1 wk	A: 39.0 wk	A: 6.9%	NR
		B: Paclitaxel + lapatinib + trastuzumab	B: 12.0 wk	B: 51.6 wk	B: 10.3%	
Crown 2012; phase III; n = 600 <sup>b</sup> ; ongoing [42]	HER2-positive MBC, no prior systemic therapy	A: Paclitaxel + trastuzumab	Primary endpoint	NR	NR	NR
		B: Paclitaxel + trastuzumab + lapatinib				
HALT: LPT112515; phase III; n = 280 <sup>b</sup> ; ongoing [122]	HER2-positive MBC	A: Lapatinib + trastuzumab maintenance	Primary endpoint	NR	NR	NR
		B: Trastuzumab maintenance alone				
ALTERNATIVE; phase III; n = 525 <sup>b</sup> ; ongoing (NCT01160211)	HER2-positive MBC, prior trastuzumab and endocrine therapy, (neo) adjuvant	A: Lapatinib + trastuzumab + aromatase inhibitor	NR	Primary endpoint	NR	NR
		B: Trastuzumab + aromatase inhibitor				
		C: Lapatinib + aromatase inhibitor				
ALTTO; phase III; n = 8,381; ongoing (NCT00490139)	HER2-positive primary breast cancer, adjuvant	A: Trastuzumab	DFS primary endpoint	NR	NR	NR
		B: Lapatinib				
		C: Trastuzumab followed by lapatinib				
		D: Lapatinib + trastuzumab				
NSABP B-41; phase III, n = 529; ongoing [45]	HER2-positive early breast cancer, neoadjuvant	A: AC → paclitaxel + trastuzumab	NR	NR	NR	A: 52.5%
		B: AC → paclitaxel + lapatinib				B: 53.2%
		C: AC → paclitaxel + trastuzumab + lapatinib				C: 62.0%
NeoALTTO; phase III; n = 455; ongoing [44]	HER2-positive early breast cancer, neoadjuvant	A: Lapatinib	NR	NR	NR	A: 24.7%
		B: Trastuzumab				B: 29.5%
		C: Lapatinib + trastuzumab				C: 51.3%
CALGB 40601; phase III; n = 305; ongoing [46]	HER2-positive primary breast cancer, neoadjuvant	A: Paclitaxel + trastuzumab	NR	NR	NR	A: 40%
		B: Paclitaxel + trastuzumab + lapatinib				B: 51%
		C: Trastuzumab + lapatinib (closed early)				C: 32%
<b>Neratinib</b>						
Burstein 2010; phase II; n = 136; completed [52]	HER2-positive locally advanced or MBC	A: Neratinib (prior trastuzumab therapy)	A: 22.3 wk	NR	A: 24%	NR
		B: Neratinib (no prior trastuzumab therapy)	B: 39.6 wk		B: 56%	
			16 wk PFS:			
			A: 59%			
			B: 78%			
Martin 2011; phase II, n = 233; ongoing [55]	HER2-positive MBC with prior trastuzumab and taxane	A: Neratinib	A: 4.5 mo	A: 19.4 mo	A: 29%	NR
		B: Lapatinib + capecitabine	B: 6.8 mo	B: 19.0 mo	B: 41%	

(continued)



**Table 2.** (continued)

Trial	Patient population	Study design	PFS	OS	RR	pCR
Awada 2012; phase II; <i>n</i> = 79; ongoing (NCT00706030) [53]	HER2-positive MBC with prior trastuzumab	A: Neratinib + vinorelbine (prior lapatinib) B: Neratinib + vinorelbine (no prior lapatinib)	A: 22.7 wk B: 48.0 wk	NR	A: 8% B: 41%	NR
Chow 2013; phase II; <i>n</i> = 102; completed [54]	HER-2 positive MBC with prior trastuzumab and up to 3 prior chemotherapy regimens	A: Neratinib + paclitaxel ( $\leq 1$ prior chemotherapy regimen; no lapatinib) B: Neratinib + paclitaxel (2 or 3 prior chemotherapy regimens; lapatinib permitted)	A: 63.1 wk B: 52.1 wk	NR	A: 71% B: 77%	NR
NALA; phase III; <i>n</i> = 600 <sup>b</sup> ; ongoing (NCT01808573)	HER2-positive MBC with $\geq 2$ prior HER2-directed regimens	A: Neratinib + capecitabine B: Lapatinib + capecitabine	Coprimary endpoint	Coprimary endpoint	NR	NR
ExteNET; phase III; <i>n</i> = 2,842; ongoing (NCT00878709)	Early-stage HER2-positive breast cancer with prior trastuzumab, adjuvant	A: Neratinib B: Placebo	DFS primary endpoint	NR	NR	NR
NSABP FB-7; phase II; <i>n</i> = 126 <sup>b</sup> ; ongoing (NCT01008150)	HER2-positive locally advanced breast cancer, neoadjuvant	A: Paclitaxel + neratinib $\rightarrow$ AC B: Paclitaxel + trastuzumab $\rightarrow$ AC C: Paclitaxel + trastuzumab + neratinib $\rightarrow$ AC	NR	NR	NR	Primary endpoint
NEFERTT; phase II; <i>n</i> = 480; ongoing (NCT00915018)	HER2-positive MBC	A: Neratinib + paclitaxel B: Trastuzumab + paclitaxel	Primary endpoint	NR	NR	NR
<b>Afatinib</b>						
Lin 2012; phase II; <i>n</i> = 41; completed [59]	HER2-positive MBC progressing after trastuzumab	Afatinib	15.1 wk	61.0 wk	10%	NR
Rimawi 2012; phase II; <i>n</i> = 29; completed [60]	HER2-positive locally advanced breast cancer, neoadjuvant	A: Afatinib B: Lapatinib C: Trastuzumab	NR	NR	A: 80% B: 75% C: 36.4%	NR
LUX-Breast 2; phase II; <i>N</i> = 120 <sup>b</sup> ; recruiting [61]	HER2-positive MBC progressing on neoadjuvant trastuzumab and/or lapatinib	A: Afatinib until progression followed by afatinib/vinorelbine B: Afatinib until progression followed by afatinib/paclitaxel	NR	NR	Primary endpoint	NR
LUX-Breast 3; phase II; <i>N</i> = 120 <sup>b</sup> ; ongoing [62]	HER2-positive MBC with brain metastases after prior HER2-targeted therapy	A: Afatinib B: Afatinib + vinorelbine C: Investigator's choice of treatment	NR	NR	Benefit at 12 wk primary endpoint	NR
DAFNE; phase II; <i>n</i> = 65; recruiting (NCT01594177)	HER2-positive early breast cancer, neoadjuvant	Afatinib + trastuzumab $\rightarrow$ afatinib + paclitaxel + trastuzumab $\rightarrow$ AC + trastuzumab	NR	NR	NR	Primary endpoint
Swanton 2012; phase II; <i>n</i> = 40 <sup>b</sup> ; ongoing [123]	HER2-positive metastatic inflammatory breast cancer	A: Trastuzumab naive patients; afatinib until progression $\rightarrow$ afatinib + vinorelbine on progression B: Patients with prior trastuzumab; afatinib until progression $\rightarrow$ afatinib + vinorelbine	NR	NR	Clinical benefit rate primary endpoint	NR

<sup>a</sup>Per ClinicalTrials.gov, June 2013.

<sup>b</sup>Estimated enrollment.

AC, anthracycline + cyclophosphamide; DFS, disease-free survival; HER2, human epidermal growth factor receptor 2; MBC, metastatic breast cancer; NR, not reported; OS, overall survival; pCR, pathologic complete response; PFS, progression-free survival; RR, response rate; TTP, time to progression.

for use as part of neoadjuvant therapy for patients with HER2-positive locally advanced, inflammatory, or early-stage breast cancer [64]. A phase III study (APHINITY) of trastuzumab and chemotherapy with or without pertuzumab in the adjuvant setting is ongoing (NCT01358877), as are phase II trials of pertuzumab with trastuzumab/paclitaxel as neoadjuvant therapy for inflammatory breast cancer (NCT01796197) and of pertuzumab and trastuzumab with or without chemotherapy in MBC (NCT01835236).

### MM-111

MM-111 (Merrimack Pharmaceuticals, Cambridge, MA, <http://merrimackpharma.com>) is a monoclonal antibody that targets HER2 and HER3, preventing their dimerization. In preclinical studies, MM-111 was shown to decrease HER2/HER3-phosphatidylinositol 3-kinase (PI3K) signaling and was synergistic with trastuzumab and lapatinib in HER2-positive cancers [69]. A phase I study is investigating MM-111 in combination with trastuzumab in HER2-positive MBC progressing on previous

therapies (NCT01097460), and results are awaited from a phase I study of MM-111 as monotherapy in HER2-positive solid tumors (NCT00911898).

### Trastuzumab-DM1

Trastuzumab-DM1 (T-DM1; Kadcyca; Genentech) is an antibody-drug conjugate, with trastuzumab delivering the antimicrotubule agent DM1 (emtansine) to HER2-positive tumor cells [70, 71]. In preclinical models, T-DM1 has demonstrated inhibition of cellular proliferation and promotion of cell death in trastuzumab-resistant breast cancer cells [70]. Data from two separate single-arm phase II trials in MBC patients progressing on prior HER2-targeted therapy reported RRs of 33.6% and 26.9% with T-DM1 [72]. A phase III trial (EMILIA) in HER2-positive locally advanced or MBC patients previously treated with trastuzumab and a taxane demonstrated a PFS and OS benefit for TDM-1 (9.6 and 30.9 months, respectively) compared with lapatinib/capecitabine (6.4 and 25.1 months) [73]. The T-DM1 arm also had fewer grade  $\geq 3$  adverse events. This led to FDA approval of T-DM1 for HER2-positive MBC patients previously treated with trastuzumab and a taxane. T-DM1 also showed PFS improvement as first-line therapy in MBC patients versus trastuzumab/docetaxel in a phase II study [74]. MARIANNE (NCT01120184) is an ongoing phase III study of HER2-positive MBC patients randomized to receive first-line trastuzumab/taxane, T-DM1/placebo, or T-DM1/pertuzumab; T-DM1 is also being studied in the adjuvant and neoadjuvant settings (NCT01196052 [75]; NCT01745965; NCT01853748).

### TARGETING HER FAMILY DOWNSTREAM SIGNALING PATHWAYS

Targeting cellular signaling pathways downstream of HER family receptors is an emerging strategy for potential breast cancer therapies (Fig. 1).

#### The PI3K/Akt Pathway

The PI3K/protein kinase B (Akt) pathway is associated with cellular proliferation/survival, motility, angiogenesis, inhibition of apoptosis, and metastasis [76–79]. Phosphorylated PI3K and Akt have been detected in circulating breast cancer cells from approximately 80% of patients with metastatic disease [80], and Akt is associated with overexpression of HER2 in breast tumors [81]. A biomarker analysis of the phase III CLEOPATRA study showed that patients with mutated *PIK3CA* had shorter PFS in both arms, suggesting that PI3K inhibitors could be combined with HER2-targeted therapy in these patients [82].

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GDC-0941 (Genentech), XL147 (SAR245408; Sanofi, Paris, France, <http://en.sanofi.com>), and BKM120 (NVP-BKM120; Novartis, Basel, Switzerland, <http://www.novartis.com>) are PI3K inhibitors, and BEZ235 (NVP-BEZ235; Novartis) is a dual PI3K/mammalian target of rapamycin (mTOR) inhibitor [83–85] that are currently being evaluated in phase I/II clinical trials in combination with trastuzumab with or without chemotherapy. Results from a phase Ib/II study of BKM120 plus trastuzumab in trastuzumab-resistant metastatic HER2-positive patients suggested preliminary activity [84], and the phase Ib/II PIKHER2 trial of BKM120 plus lapatinib in HER2-positive, PI3K-activated, trastuzumab-resistant disease is ongoing [86]. In MBC patients who progressed on prior trastuzumab, a phase I trial is ongoing to investigate GDC-0941 plus trastuzumab or T-DM1 (NCT00928330), and XL147 was given in combination with trastuzumab or trastuzumab/paclitaxel in another phase I/II study (NCT01042925). However, these trials do not limit patients to those with *PIK3CA* mutations.

In a phase I study, BEZ235 was evaluated in HER2-positive MBC patients with molecular alterations of *PI3KCA* or *PTEN* who progressed on prior trastuzumab; 1 PR and 4 stable disease were seen in 15 patients [87]. In phase I studies, the Akt inhibitor MK-2206 (Merck & Company, Inc.; Whitehouse Station, NJ, <http://www.merck.com>) was combined with trastuzumab and lapatinib in refractory HER2-positive breast and gastroesophageal tumors (one CR and one PR in 24 patients) [88] and with paclitaxel and trastuzumab in patients with HER2-positive solid tumors, including but not limited to breast cancer (two CRs and seven PRs in 14 patients) [89]. In the ongoing neoadjuvant I-SPY 2 phase II trial, HER2-positive patients receive trastuzumab/paclitaxel with MK-2206; other arms include neratinib or the insulin-like growth factor 1 receptor (IGF-1R) antibody, ganitumab (AMG 479; Amgen, Thousand Oaks, CA, <http://www.amgen.com>; NCT01042379).

#### The mTOR Pathway

mTOR is a key component of the PI3K/Akt pathway and is involved in the control of cell cycle progression [77]. The mTOR inhibitors temsirolimus (Torisel; Pfizer), everolimus (Afinitor; Novartis), and ridaforolimus (Merck & Company, Inc.) have been evaluated in breast cancer, and everolimus was approved by the FDA for postmenopausal women with advanced hormone receptor-positive HER2-negative breast cancer in combination with exemestane after failure of letrozole or anastrozole [90]. Pooled data from two phase I/II studies in HER2-positive MBC patients who progressed on trastuzumab reported an RR of 15% and median PFS of 4.1 months with everolimus plus trastuzumab [91]. Two phase Ib studies of MBC patients with prior trastuzumab therapy showed PFS of 30.1 and 34 weeks with everolimus in combination with trastuzumab/vinorelbine or trastuzumab/paclitaxel, respectively [92, 93]. BOLERO-1 (NCT00876395) and BOLERO-3 (NCT01007942) are phase III studies of everolimus in HER2-positive MBC (Table 4). BOLERO-3 compared the combination of trastuzumab, vinorelbine, and everolimus versus placebo in trastuzumab-resistant stage IV breast cancer, resulting in a small clinical PFS benefit of 7.00 versus 5.78 months, respectively ( $p = .0067$ ) [94].

**Table 3.** Completed and ongoing phase II and phase III clinical trials of trastuzumab-DM1 and pertuzumab in HER2-positive breast cancer<sup>a</sup>

Trial	Patient population	Study design	PFS	OS	RR	pCR
<b>Trastuzumab-DM1</b>						
Burriss; phase II; <i>n</i> = 112; completed [124]	HER2-positive MBC, prior anti-HER2 therapy and chemotherapy	T-DM1	4.6 mo	NR	25.9%	NR
MARIANNE; phase III; <i>n</i> = 1,095; ongoing [125]	HER2-positive MBC, first-line	A: Trastuzumab + taxane B: T-DM1 + pertuzumab C: T-DM1 + placebo	Primary endpoint	NR	NR	NR
TH3RESA; phase III; <i>n</i> = 600; recruiting (NCT01419197)	HER2-positive MBC with ≥2 prior HER2-targeted regimens	A: T-DM1 B: investigator's choice	Primary endpoint	Primary endpoint	NR	NR
EMILIA; phase III; <i>n</i> = 991; ongoing [73]	HER2-positive MBC, prior trastuzumab and taxane	A: T-DM1 B: Lapatinib + capecitabine	A: 9.6 mo B: 6.4 mo	A: 30.9 mo B: 25.1 mo	A: 43.6% B: 30.8%	NR
Hurvitz; phase II; <i>n</i> = 137; completed [74]	Previously untreated HER2-positive MBC	A: T-DM1 B: Trastuzumab + docetaxel	A: 14.2 mo B: 9.2 mo	NR	A: 64.2% B: 58.0%	NR
Phase II; <i>n</i> = 153; ongoing [75]	HER2-positive early stage inflammatory, neoadjuvant or adjuvant	T-DM1 Primary endpoint: cardiac safety	NR	NR	NR	NR
Phase II; <i>n</i> = 380; ongoing (NCT01745965)	HER2-positive, ER-positive early breast cancer, neoadjuvant	A: T-DM1 B: T-DM1 + endocrine therapy C: Trastuzumab + endocrine therapy	NR	NR	NR	Primary endpoint
ATEMP; phase II; <i>n</i> = 500 <sup>b</sup> ; ongoing (NCT01853748)	HER2-positive early breast cancer, adjuvant	A: T-DM1 B: Trastuzumab + paclitaxel	DFS primary endpoint	NR	NR	NR
<b>Pertuzumab</b>						
CLEOPATRA; phase III; <i>n</i> = 808; completed [65] [66]	HER2-positive locally recurrent, unresectable, or MBC	A: Trastuzumab + docetaxel + placebo B: Trastuzumab + docetaxel + pertuzumab	A: 12.4 mo B: 18.7 mo	A: 37.6 mo B: not reached	A: 69.3% B: 80.2%	NR
Phase II; <i>n</i> = 208 <sup>b</sup> ; ongoing (NCT01835236)	HER2-positive MBC	A: Trastuzumab + pertuzumab B: Trastuzumab + pertuzumab + paclitaxel or vinorelbine (T-DM1 at progression in each arm)	NR	Primary endpoint	NR	NR
NeoSphere; phase II; <i>n</i> = 417; completed [67]	HER2-positive early breast cancer with no previous therapy, neoadjuvant	A: Trastuzumab + docetaxel B: Trastuzumab + docetaxel + pertuzumab C: Trastuzumab + pertuzumab D: Docetaxel + pertuzumab	NR	NR	A: 79.8% B: 88.1% C: 67.6% D: 71.4%	A: 29.0% B: 45.8% C: 16.8% D: 24.0%
Phase II; <i>n</i> = 30 <sup>b</sup> ; ongoing (NCT01796197)	HER2-positive inflammatory breast cancer, neoadjuvant	Pertuzumab + trastuzumab + paclitaxel	NR	NR	NR	Primary endpoint
APHINITY; phase III; <i>n</i> = 4,800 <sup>b</sup> ; recruiting (NCT01358877)	HER2-positive early breast cancer, adjuvant	A: Chemotherapy + trastuzumab + pertuzumab	Invasive DFS primary endpoint	NR	NR	NR

(continued)



**Table 3.** (continued)

Trial	Patient population	Study design	PFS	OS	RR	pCR
		B: Chemotherapy + trastuzumab + placebo				
TRYPHAENA; phase II; <i>n</i> = 225; completed [68]	HER2-positive early breast cancer, neoadjuvant	A: FEC + trastuzumab + pertuzumab × 3 → THP × 3 B: FEC × 3 → THP × 3 C: Carboplatin + THP × 6	NR	NR	NR	A: 61.6% B: 57.3% C: 66.2%

<sup>a</sup>Per ClinicalTrials.gov, June 2013.

<sup>b</sup>Estimated enrollment.

Abbreviations: DFS, disease-free survival; ER, estrogen receptor; FEC, 5-fluorouracil + epirubicin + cyclophosphamide; HER2, human epidermal growth factor receptor 2; MBC, metastatic breast cancer; NR, not reported; OS, overall survival; pCR, pathologic complete response; PFS, progression-free survival; RR, response rate; THP, docetaxel + trastuzumab + pertuzumab TTP, time to progression.

A phase II study evaluating two doses of temsirolimus in patients with heavily pretreated MBC showed an RR of only 9.2% [95]. A phase I/II study (NCT01111825) is ongoing that assesses temsirolimus and neratinib in trastuzumab-refractory HER2-amplified MBC. Ridaforolimus was combined with trastuzumab in the same patient population, with two PRs in 14 patients [96]. MLN0128 (INK128; Millennium Pharmaceuticals, Inc., Cambridge, MA, <http://www.millennium.com>) is another mTOR inhibitor in phase I study, with an expansion cohort with paclitaxel and weekly trastuzumab in HER2-positive patients (NCT01351350).

### OTHER TARGETS

Overexpression of IGF-1R can lead to trastuzumab resistance [97], and results are forthcoming from phase II trials of the IGF-1R inhibitors BMS-754807 (Bristol-Myers Squibb, Princeton, NJ, <http://www.bms.com>) in combination with trastuzumab (NCT00788333) and cixutumumab (IMC-A12; Eli Lilly and Company, Indianapolis, IN, <http://www.lilly.com>) in combination with lapatinib/capecitabine in MBC (NCT00684983). Heat shock protein 90 (HSP90) inhibitors have also been tested in HER2-positive trastuzumab-refractory MBC. In a phase II trial of tanespimycin (Bristol-Myers Squibb) and trastuzumab, 6 of 27 patients had PR and a median PFS of 6 months [98]; however, there are no ongoing studies with tanespimycin in breast cancer. In a phase II study of AU922 (Novartis) with trastuzumab in trastuzumab-refractory HER2-positive MBC, 5 PRs were seen in 22 patients [99].

Telomerase inhibitors have shown synergy with trastuzumab in inhibiting HER2-positive cancer cell growth and restoring trastuzumab sensitivity in trastuzumab-resistant cell lines [100]. Preliminary results with imetelstat (GRN163L; Geron Corporation, Menlo Park, CA, <http://www.geron.com>) and trastuzumab in women with trastuzumab-resistant breast cancer showed no significant toxicity, although no responses were seen [101].

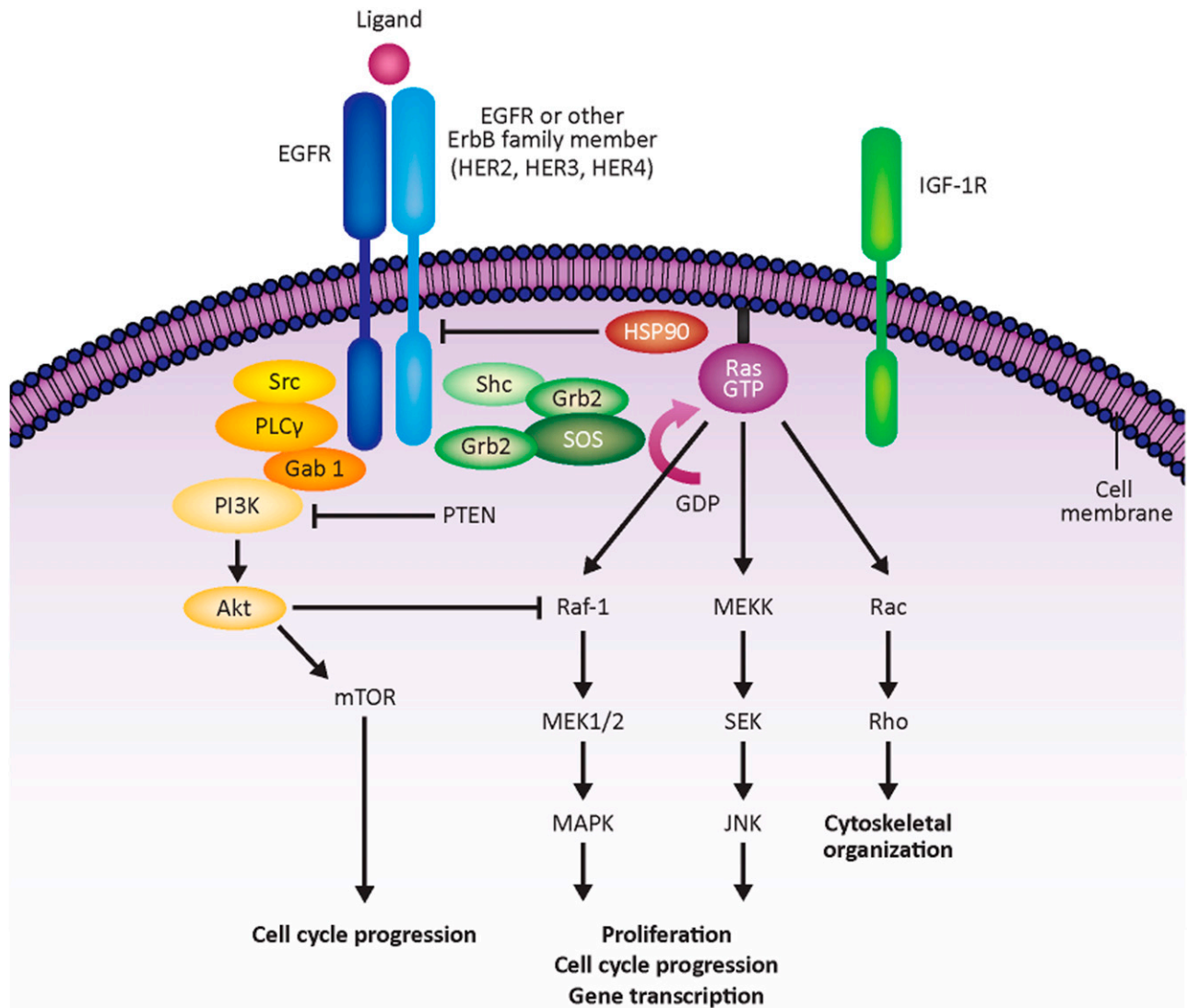
Vascular endothelial growth factor (VEGF) inhibitors have also been combined with HER2-targeted therapy, given the possible association between HER2 and VEGF expression [102]. Pazopanib (Votrient; GlaxoSmithKline) is a multiple receptor TKI against VEGF receptor (VEGFR) 1-3, platelet-derived growth factor receptor, and stem cell factor receptor

[103]. Two phase II studies of pazopanib and lapatinib in HER2-positive advanced or MBC did not show improvement in 12-week disease progression rates or PFS compared with lapatinib alone, while demonstrating increased toxicity (Table 4) [104, 105]. Sunitinib (Sutent; Pfizer) is another multiple receptor TKI of VEGFR 1-3, platelet-derived growth factor receptor, stem cell factor receptor, and colony-stimulating factor-1 receptor [106]. A single-arm phase II study of sunitinib, docetaxel, and trastuzumab in HER2-positive MBC reported 16 PRs in 22 patients [107]. In the phase II AVANTHER study of neoadjuvant bevacizumab (Avastin; Genentech), a monoclonal anti-VEGF antibody, with trastuzumab and paclitaxel, pCR was achieved in 18 of 44 HER2-positive patients [108]. Another phase II study is evaluating neoadjuvant trastuzumab and chemotherapy with or without bevacizumab (NCT01367028). In the locally recurrent and MBC setting, a single-arm phase II study combined bevacizumab with trastuzumab/capecitabine as first-line therapy, with an RR of 73% and median PFS of 14.4 months [109]. In a phase II study of bevacizumab with trastuzumab/vinorelbine in HER2-positive MBC, the RR was 73% as first-line therapy and 71% as second-line therapy; median PFS durations were 9.9 and 7.8 months, respectively [110]. The phase III AVEREL study combined trastuzumab/docetaxel with or without bevacizumab as first-line therapy. RRs were similar; improved median PFS with bevacizumab (from 13.7 to 16.5 months per investigator assessment; *p* = .0775) did not reach statistical significance [111]. Another phase III trial (E1105) compared first-line chemotherapy/trastuzumab with or without bevacizumab, with no significant differences in PFS or RR observed between treatments [112].

### FUTURE DIRECTIONS

#### Defining Who May Benefit From HER2-Targeted Therapy

The Cancer Genome Atlas Network molecular analysis based on mRNA expression revealed the most frequent subtypes of human breast tumors are basal-like, luminal A and B, and HER2 enriched (HER2E) [113]. Luminal subtypes include ER-positive tumors, and the HER2E subtype has frequent *HER2*



**Figure 1.** Downstream effectors of HER signaling. Figure adapted from Atalay et al. [77] by permission of Oxford University Press. Abbreviations: Akt, protein kinase B; EGFR, epidermal growth factor receptor; Gab 1, GRB2-associated binding protein 1; GDP, guanosine 5'-diphosphate; Grb2, growth factor receptor-bound protein 2; GTP, guanosine-5'-triphosphate; HER, human epidermal growth factor receptor; HSP90, heat shock protein 90kDa  $\alpha$ ; IGF-1R, insulin-like growth factor-1 receptor; JNK, mitogen-activated protein kinase 8; MAPK, mitogen-activated protein kinase 1; MEK1, mitogen-activated protein kinase kinase 1; MEKK, mitogen-activated protein kinase kinase kinase 1; mTOR, mechanistic target of rapamycin; PI3K, phosphatidylinositol 3-kinase; PLC $\gamma$ , phospholipase C  $\gamma$ ; PTEN, phosphatase and tensin homolog; Rac, v-akt murine thymoma viral oncogene homolog 1; Raf1, v-raf-1 murine leukemia viral oncogene homolog 1; Ras, Ras kinase family; Rho, rhodopsin; SEK, simple epithelial keratin; Shc, SHC (Src homology 2 domain containing) transforming protein 1; SOS, son of sevenless homolog; Src, sarcoma virus oncogene.

amplification with a strong signal of EGFR, HER2, and phosphorylated HER2/EGFR. However, only approximately 50% of clinically HER2-positive tumors fell into this HER2E-mRNA subtype, and the rest were predominantly luminal mRNA subtypes. Several trials, including NeoALTTO, NOAH, NeoSphere, and NSABP B-41, have shown lower pCR rates with HER2-targeted therapy in patients with both ER and HER2 positivity compared with HER2-positive-only patients. It may be that some of these ER-positive/HER2-positive tumors are luminal and not of the HER2E mRNA subtype. In the neoadjuvant I-SPY 1 trial, pCR was highest in the HER2-enriched subset and lowest in the luminal A subset, and pCR

was not as prognostic for ER-positive/HER2-positive tumors as for ER-negative/HER2-positive tumors [114]. There may be interaction between the ER and HER2 receptors, as inhibition of HER2 may be associated with increased activity of ER [115, 116]. Another study of stage I to III HER2-positive patients showed that hormone receptor-negative patients presented with higher stage and grade of disease were more likely to recur first in the central nervous system and had worse survival than hormone receptor-positive/HER2-positive patients [117]. Molecular analysis may help identify this subtype of HER2-positive tumors that may behave more similarly to ER-positive tumors.

**Table 4.** Completed and ongoing clinical trials of everolimus, pazopanib, and bevacizumab in HER2-positive breast cancer<sup>a</sup>

Trial	Patient population	Study design	PFS	OS	RR	pCR
<b>Everolimus</b>						
Andre; phase Ib; n = 33; completed [93]	HER2-positive MBC, prior trastuzumab	Everolimus + paclitaxel + trastuzumab	34 wk	NR	44%	NR
Jerusalem; phase Ib; n = 50 [92]	HER2-positive MBC, prior trastuzumab	Everolimus + vinorelbine + trastuzumab	30.1 wk	NR	19.1%	NR
Morrow; phase I/II; n = 47 [91]	HER2-positive MBC progressing after trastuzumab	Everolimus + trastuzumab	4.1 mo	NR	15%	NR
BOLERO-1; phase III; n = 719; ongoing [126]	HER2-positive MBC, no prior systemic therapy	A: Everolimus + paclitaxel + trastuzumab B: Paclitaxel + trastuzumab	Primary endpoint	NR	NR	NR
BOLERO-3; phase III; n = 569; ongoing [94]	HER2-positive MBC, prior taxane and resistance to trastuzumab	A: Everolimus + vinorelbine + trastuzumab B: Vinorelbine + trastuzumab	A: 7.00 mo B: 5.78 mo	NR	A: 40.8% B: 37.2%	NR
<b>Pazopanib</b>						
Cristofanilli; phase II; n = 163; completed [105]	HER2-positive inflammatory breast cancer	Cohort 1	Cohort 1	Cohort 1	Cohort 1	NR
		A: Lapatinib + placebo	A: 16.1 wk	A: 14.7 mo	A: 29%	
		B: Lapatinib + pazopanib	B: 14.3 wk	B: 16.2 mo	B: 45%	
		Cohort 2	Cohort 2	Cohort 2	Cohort 2	
		A: Lapatinib	A: 16.0 wk	A: 15.9 mo	A: 47%	
B: Lapatinib + pazopanib	B: 16.0 wk	B: NE	B: 58%			
C: pazopanib	C: 11.4 wk	C: NE	C: 31%			
Johnston; phase II; n = 190; completed [104]	HER2-positive MBC	Cohort 1	Cohort 1 wk 12 PDR:	Cohort 1	Cohort 1, wk 12	NR
		A: Lapatinib + pazopanib	A: 36.2%		A: 36.2%	
		B: Lapatinib	B: 38.9%		B: 22.2%	
		Cohort 2: lapatinib + pazopanib	PFS A vs. B: NS		Cohort 2, wk 12, 33.3%	
<b>Bevacizumab</b>						
AVANTHER; phase II; n = 44; completed [108]	Early HER2-positive breast cancer, neoadjuvant	Paclitaxel + trastuzumab + bevacizumab → trastuzumab + lipodox/cyclophosphamide	NR	NR	NR	42.9%
Phase II; n = 100 <sup>b</sup> ; recruiting (NCT01367028)	HER2-positive early breast cancer, neoadjuvant	A: Trastuzumab + docetaxel	NR	NR	NR	NR
		B: Trastuzumab + docetaxel + bevacizumab				
		C: Trastuzumab + docetaxel + NPLD				
		D: Trastuzumab + docetaxel + NPLD + bevacizumab				
		Primary endpoint: cardiac toxicity				
Martin et al.; phase II; n = 88; completed [109]	HER2-positive MBC without prior chemotherapy	Bevacizumab + trastuzumab + capecitabine	14.4 mo	NR	72.7%	NR
Lin et al.; phase II; n = 29; completed [110]	HER2-positive MBC without prior chemotherapy or 1 prior regimen	A: Bevacizumab + trastuzumab + vinorelbine (first-line)	A: 9.9 mo	NR	A: 73%	NR
		B: Bevacizumab + trastuzumab + vinorelbine (second-line)	B: 7.8 mo		B: 71%	

(continued)

**Table 4.** (continued)

Trial	Patient population	Study design	PFS	OS	RR	pCR
AVAREL; phase III; <i>n</i> = 424; completed [111]	HER-positive MBC without prior chemotherapy	A: Trastuzumab + docetaxel B: Trastuzumab + docetaxel + bevacizumab	A: 13.7 mo B: 16.5 mo	A: >38 moB: >38 mo	A: 69.9%B: 74.3%	NR
E1105; phase III; <i>n</i> = 96 evaluable (terminated due to low accrual) [112]	HER2-positive MBC	A: Paclitaxel/carboplatin + trastuzumab + placebo B: Paclitaxel/carboplatin + trastuzumab + bevacizumab	A: 11.1 moB: 12.1 mo	NR	A: 52%B: 52%	NR

<sup>a</sup>Per ClinicalTrials.gov, June 2013.

<sup>b</sup>Estimated enrollment.

Abbreviations: DLT, dose-limiting toxicity; HER2, human epidermal growth factor receptor 2; lipodox, liposomal doxorubicin; MBC, metastatic breast cancer; NE, not estimable; NPLD, nonpegylated liposome-encapsulated doxorubicin; NR, not reported; NS, not significant; OS, overall survival; PDR, progressive disease rate; PFS, progression-free survival; RR, response rate.

Several trials, including NeoALTO, NOAH, NeoSphere, and NSABP B-41, have shown lower pCR rates with HER2-targeted therapy in patients with both ER and HER2 positivity compared with HER2-positive-only patients. It may be that some of these ER-positive/HER2-positive tumors are luminal and not of the HER2E mRNA subtype.

In addition, except for early trastuzumab trials, which defined HER2 positivity criteria as immunohistochemistry (IHC) scores of 2+ or 3+, subsequent trials define positivity as 3+ by IHC or  $\geq 2.0$  by fluorescence in situ hybridization (FISH), or IHC 2+/FISH  $\geq 2.0$ . In the NSABP B-31 trial, for example, there was no direct correlation between *HER2* copy number by FISH and benefit from adjuvant trastuzumab in HER2-positive patients [118]. However, it is not known whether HER2-positive tumors that have lower activation of HER2/EGFR would be less likely to respond to HER2- and EGFR-targeted treatments than those with higher levels of activation.

There is also a small proportion of the HER2E mRNA subtype that is clinically considered triple-negative breast cancer [113]. It is not yet known whether HER2E triple-negative breast cancer benefits from HER2-targeted agents. There is also the possibility that HER2 expression level in a primary tumor may not be predictive of HER2 expression level in circulating tumor cells (CTCs). Several studies have shown HER2-positive CTCs in patients with HER2-negative tumor biopsy specimens and vice versa, with discordance rates of approximately 30% [119, 120]. In an ongoing phase III study (DEFECT III), patients with HER2-negative MBC and HER2-positive CTCs are randomized to receive standard therapy alone with or without lapatinib (NCT01619111).

Studies have also shown that patients can have different hormone receptor and HER2 status throughout tumor progression [121], although patients are not always rebiopsied at relapse. When tumor tissue from the NSABP B-31 trial was re-examined, 9.7% of women had breast cancer that should have been classified as HER2-negative [118]. The mRNA levels of HER2 in tumors that were HER2-negative were significantly lower than those in HER2-positive tumors. However, patients with these “HER2-negative/low” tumors, defined as IHC 1+ or 2+,

benefited from trastuzumab. These observations prompted an ongoing phase III NSABP study (NCT01275677) randomizing patients with “HER2-low” invasive breast cancer (IHC 1+ or 2+) to receive chemotherapy alone or with trastuzumab.

Ongoing and future studies will help answer questions regarding different combinations of targeted agents in HER2-positive breast cancer, how they may be combined with specific chemotherapeutics or endocrine therapy, and the use of biomarkers to assist in determining trastuzumab resistance and treatments that may offer maximum benefit in individual patients.

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#### For Further Reading:

Francisco J. Esteva, Sandra X. Franco, Maura K. Hagan et al. An Open-Label Safety Study of Lapatinib Plus Trastuzumab Plus Paclitaxel in First-Line HER2-Positive Metastatic Breast Cancer. *The Oncologist* 2013;18:661–666.

#### Implications for Practice:

Dual targeting of the HER2 receptor using trastuzumab and lapatinib has been shown to be effective in HER2-positive metastatic breast cancer. In this study, we evaluated the safety of paclitaxel in combination with trastuzumab and lapatinib. The main side effect was diarrhea, which occurred in the majority of patients at the standard dosing of all three drugs. A pharmacokinetic interaction was found between paclitaxel and lapatinib, resulting in increased exposure of both drugs. We evaluated three dose levels of lapatinib and paclitaxel (all patients received standard trastuzumab dosing). A dose of lapatinib 750 mg/day had the lowest incidence of diarrhea in combination with paclitaxel 80 mg/m<sup>2</sup> per week and trastuzumab 2 mg/kg per week. These doses should be used if the triplet is considered for further development in patients with HER2-positive metastatic breast cancer.