# Chapter 6

# Emerging targeted agents for HER2-positive breast cancer

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## Challenges and unmet needs

Advances in understanding the biology of human epidermal growth factor receptor 2 (HER2)-positive breast cancer has led to the successful clinical development of HER2-targeted agents. Trastuzumab represents the archetype of molecular-targeted agents in the setting of solid tumors, with efficacy proven in metastatic, neoadjuvant, and adjuvant settings of HER2-positive breast cancer [1]. Lapatinib, a dual epidermal growth factor receptor (EGFR)/HER2 reversible tyrosine kinase inhibitor (TKI), is currently approved in combination with either capecitabine for patients with HER2-positive metastatic breast cancer refractory to taxanes, anthracycline, and trastuzumab, or with letrozole for postmenopausal women with HER2-positive metastatic breast cancer for whom hormonal therapy is indicated [2]. Pertuzumab, a monoclonal antibody targeting the dimerization domain II of HER2, is an approved agent for the first-line treatment of HER2-positive metastatic breast cancer, in combination with docetaxel and trastuzumab [3]. Lastly, a conjugate of trastuzumab with the microtubule inhibitory agent emtansine (T-DM1) was approved in 2013 for the treatment of patients with HER2-positive metastatic breast cancer who previously received trastuzumab and a taxane, separately or in combination, as well as in the neoadjuvant setting [4].

Meanwhile, there is a subset of patients with early-stage disease who experience recurrence, despite the administration of adjuvant trastuzumab. Moreover, in the metastatic setting, primary or secondary resistance to HER2-targeted



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agents inevitably develops, with affected patients experiencing continued disease progression [5]. This clinical reality mandates the development of additional targeted compounds. The elucidation of the different molecular mechanisms mediating resistance to HER2 blockade (Table 6.1) [6–11] holds the promise to effectively address this gap in treatment. In this chapter, we will provide a thorough overview of the emerging targeted agents that block HER2 (and other signaling pathways) that are currently under clinical development. The biologic rationale, as well as available clinical efficacy data, will be provided.

| Class of mechanism resistance | Molecular mediator | Description |
|---|---|---|
| Impaired trastuzumab access to HER2 | HER2 p95/HER2 shedding | HER2 shedding results in the formation of soluble ECD and HER2 p95. The latter is a constitutively active, truncated form of HER2, lacking the ECD (the binding site for trastuzumab) while retaining its signaling activity [6,7] |
| | Transmembrane masking proteins (MUC4 and CD44) | Transmembrane molecules that mask the HER2 epitope recognized by trastuzumab interfere with trastuzumab's antibody-binding capacity [8] |
| Alternative oncogenic signaling pathway activation | PI3K pathway | PIK3CA mutations and/or PTEN loss activate PI3K signaling, despite trastuzumab administration [8] |
| | IGF pathway | A selective IGF-1R and HER2 crosstalk has been documented in trastuzumab resistance [8]. Additionally, HER2/HER3/IGF-1R heterotrimers have been associated with trastuzumab resistance [9] |
| | Met pathway | Prevention of trastuzumab-mediated p27 induction [10] |
| | Src | Src activation functions as a common node downstream of multiple resistance mechanisms [11] |
| | HER3 | HER3/EGFR heterodimerization occurs upon HER2 blockade. HER3 signaling is not fully abrogated by dual HER2 blockade |

**Table 6.1 Mechanisms of trastuzumab resistance in HER2-positive breast cancer.** CD44, cluster of differentiation 44, ECD, extracellular domain; EGFR, epidermal growth factor receptor; HER2/3, human epidermal receptor 2/3; IGF, insulin growth factor; IGF-1R, IGF receptor 1; MUC4, mucin-4; PI3K, phosphoinositide 3-kinase; PTEN, phosphatase and tensin homolog.

## Novel HER2 tyrosine kinase inhibitors

### Neratinib

Currently under clinical development, neratinib is an orally available, irreversible TKI that blocks HER1, HER2, and HER4. A Phase II study evaluated neratinib in patients with HER2-positive metastatic breast cancer, either trastuzumab-pretreated (cohort A, n=66) or trastuzumab-naïve (cohort B, n=70) [12]. Substantial antitumor activity was noted, with the 16-week progression free survival (PFS) rate reaching 59% and 78% and median PFS reaching 22.3 and 39.6 weeks for cohorts A and B, respectively. Toxicities were manageable; diarrhea, nausea, vomiting, and fatigue were the most common grade 3/4 adverse events (AEs), and no neratinib-related grade 3/4 cardiotoxicity was reported [12].

A Phase I/II trial evaluated neratinib in combination with trastuzumab in 45 trastuzumab-pretreated patients with HER2-positive metastatic breast cancer and produced promising antitumor activity [13]. The 16-week PFS rate was 47% and the overall response rate (ORR) was 27%; median PFS was 19 weeks. Treatment with neratinib was relatively well tolerated [13]. Additionally, the triple combination of neratinib + trastuzumab + paclitaxel was evaluated in a Phase I trial of ten patients with HER2-positive metastatic breast cancer pretreated with trastuzumab and a taxane. One complete response (CR) and three partial responses (PR) were reported; diarrhea was the most frequent grade 3/4 AE [14].

Neratinib monotherapy was compared to lapatinib + capecitabine as a second-line treatment option for HER2-positive metastatic breast cancer in a randomized Phase II clinical trial. Despite its high antitumor activity, with an ORR of 29%, neratinib was inferior to the combination of lapatinib + capecitabine in terms of median PFS (the primary endpoint of the trial), which was 4.5 and 6.8 months for neratinib and lapatinib + capecitabine, respectively (hazard ratio [HR]=1.19; 95% CI, 0.89–1.60) [15].

Furthermore, a Phase I/II study assessed the combination of neratinib with capecitabine in 72 patients with HER2-positive breast cancer [16]. Promising antitumor activity was reported: the ORR reached 57% and 64% for patients with or without lapatinib pre-treatment, respectively, with the respective median PFS reaching 35.9 and 40.3 weeks. The toxicity reported was manageable, with the most common drug-related AEs being diarrhea (88%) and palmar–plantar erythrodysesthesia syndrome (48%) [16]. It should be noted

that neratinib has been recently evaluated in the adjuvant setting for patients with early-stage HER2-positive breast cancer, through the Phase III Extended Adjuvant Breast Cancer (ExteNET) trial [17]. This was a Phase III study that randomized 2,840 women with HER2-positive breast cancer and prior adjuvant trastuzumab and chemotherapy to receive either 240 mg/day of neratinib for 1 year or placebo (1,420 patients in each arm). The primary endpoint of the trial was invasive disease-free survival (iDFS). At 24 months, patients who received neratinib had an iDFS rate of 93.9%, as compared to 91.6% in the placebo group (HR=0.67, 95% CI, 0.50–0.91; $P$=0.009). No data were presented concerning overall survival because of the short follow-up period of the ExteNET study, and a follow-up is anticipated [17].

Currently, there are several ongoing trials evaluating neratinib in HER2-positive breast cancer (Table 6.2) [18–20]. Notably, a Phase III study investigating neratinib + capecitabine compared with lapatinib + capecitabine in patients

| Trial | Phase | Description | Patients (n) | Primary endpoint(s) | Secondary endpoint(s) |
|---|---|---|---|---|---|
| NCT01494662 [18] | II | Efficacy of neratinib in the treatment of CNS metastases | Patients with CNS metastases; any prior systemic therapy (45) | ORR | PFS, OS, CNS response, first site of disease progression, safety, association of CTCs and OS, clinical outcomes |
| NEFERTT (NCT00915018) [19] | II | Safety and efficacy of neratinib + paclitaxel vs trastuzumab + paclitaxel | Trastuzumab-naïve (for metastatic disease) (480) | PFS | OS, ORR, duration of OR, CBR, AEs, HRQoL, frequency of CNS lesions, time to CNS lesions |
| NALA (NCT01808573) [20] | III | Efficacy of neratinib + capecitabine compared with lapatinib + capecitabine | Patients who have received two or more prior HER2-targeted regimens | PFS | ORR, CBR, duration of response, safety, health outcomes assessments |

**Table 6.2 Selected ongoing clinical trials with neratinib.** AEs, adverse events; CBR, clinical benefit rate; CNS, central nervous system; CTCs, circulating tumor cells; DLTs, dose-limiting toxicities; HRQoL, health-related quality of life; OR, objective response; ORR, overall response rate; OS, overall survival; PFI, progression-free interval; PFS, progression-free survival.

with HER2-positive metastatic breast cancer who have received two or more prior HER2-targeted regimens has recently been initiated [20].

## Afatinib

Afatinib is another oral, irreversible, pan-HER TKI that blocks EGFR/ HER1, HER2, and HER4. Afatinib has been assessed as monotherapy in a Phase II single-arm trial in 41 trastuzumab-pretreated patients with HER2-positive metastatic breast cancer who received a median of three prior chemotherapy lines in the metastatic setting (range 0–15) [21]. Promising antitumor activity was noted: 19 patients (46%) had clinical benefit (CR, PR, or stable disease [SD]); median PFS reached 15.1 weeks (95% CI, 8.1–16.7 weeks); and median overall survival (OS) was 61.0 weeks (95% CI, 56.7 weeks to 'not evaluable'). Its toxicity profile was consistent with the findings reported from the other targeted agents of the same family, with diarrhea and rash being the most common AEs [21].

A Phase I study assessed the concept of dual HER2 blockade in the setting of HER2-positive metastatic breast cancer, through the combination of afatinib with trastuzumab, with 18 heavily pre-treated patients receiving the combination [22]. The maximum tolerated dose (MTD) of afatinib was 20 mg daily, combined with weekly administered standard dosed trastuzumab, with diarrhea being the dose-limiting toxicity (DLT). The most frequently reported AEs were diarrhea (94%), skin rash (56%), and fatigue (56%). In terms of antitumor efficacy, the ORR and disease control rates reached 11% and 39%, respectively, with the median PFS being 111 days (95% CI, 56–274) [22].

In a randomized, open-label, neoadjuvant Phase II study, afatinib (n=10) was compared with trastuzumab (n=11) and lapatinib (n=8) in 29 patients with locally advanced HER2-positive breast cancer (median tumor size = 6.4 cm). After the 6-week treatment period, objective response was assessed using Response Evaluation Criteria for Solid Tumors (RECIST 1.0) and the best ORRs were 80%, 75%, and 36.4% for the patients treated with afatinib, lapatinib, and trastuzumab, respectively [23]. Concerning toxicities observed during the trial, all patients treated with afatinib experienced drug-related AEs, with the most common being diarrhea and cutaneous toxicities such as dermatitis acneiform and paronychia, compared to 6 out of 8 treated with lapatinib (most common AEs: diarrhea and rash) and 5 out of 11 treated with trastuzumab (vomiting and arthralgia).

There are several ongoing trials evaluating afatinib in HER2-positive breast cancer (Table 6.3) [24–27]. Unfortunately, a Phase III randomized study (LUX-Breast 1) comparing afatinib plus vinorelbine to trastuzumab plus vinorelbine as first- or second-line treatment in the metastatic setting was prematurely terminated.

| Trial | Phase | Description | Patients (n) | Primary endpoint(s) | Secondary endpoint(s) |
|---|---|---|---|---|---|
| LUX-Breast 1 (NCT01125566) [24] | III | Efficacy of afatinib + vinorelbine vs trastuzumab + vinorelbine | Trastuzumab-pretreated (508)<br>Prematurely stopped by an independent data monitoring and safety committee at n≈500 | PFS | Best RECIST assessment, OS, tumor shrinkage, time to deterioration, HRQoL, safety, PK |
| LUX-Breast 2 (NCT01271725) [25] | II | Safety and efficacy of afatinib, alone or in combination with paclitaxel or vinorelbine | Trastuzumab-pretreated in the (neo) adjuvant setting (85) | OR assessed by RECIST 1.1 | Best OR, duration of OR, PFS, safety |
| LUX-Breast 3 (NCT01441596) [26] | II | Safety and efficacy of afatinib<br>vs afatinib + vinorelbine<br>vs investigator's choice of treatment | CNS metastases, pretreated with trastuzumab and/or lapatinib (120) | Patient benefit at 12 weeks | PFS, OS |
| DAFNE (NCT01594177) [27] | II | Safety and efficacy of afatinib + trastuzumab followed by afatinib + trastuzumab + paclitaxel in patients receiving taxane + anthracycline neoadjuvant chemotherapy | Neoadjuvant (65) | pCR | RR by physical examination, conservation rate, safety, TR |

**Table 6.3 Selected ongoing clinical trials with afatinib.** CNS, central nervous system; HRQoL, health-related quality of life; OR, objective response; OS, overall survival; pCR, pathologic complete response; PFS, progression-free survival; PK, pharmacokinetics; RECIST, Response Evaluation Criteria in Solid Tumors; RR, response rate; TR, translational research.

## PI3K/Akt/mTOR inhibition

### mTOR inhibitors

The phosphoinositide 3-kinase (PI3K)/Akt/mammalian target of rapamycin (mTOR) pathway is one of the most commonly deregulated oncogenic signaling pathways in the setting of breast cancer, with aberrations affecting most of its molecular components [28], primarily:

- overexpression of PI3K-activating receptor tyrosine kinases (RTKs), including HER2;
- activating events of positive PI3K pathway regulators (eg, *PIK3CA* mutations); and
- inactivating events of negative PI3K pathway regulators (eg, loss of phosphatase and tensin homologue [PTEN]).

Activation of this intracellular transduction system has been shown to mediate resistance to HER2 blockade. A study employing a large-scale RNA interference genetic screen in a HER2-overexpressing breast cancer cell line treated with trastuzumab revealed that PI3K pathway activation, conferred by either loss of PTEN or *PIK3CA* mutations, mediates resistance to trastuzumab [29]. Confirmatory clinical results were presented in the same study, from a cohort of 55 patients with HER2-positive metastatic breast cancer who received trastuzumab-based regimens; patients with tumors bearing either PTEN loss or *PIK3CA* mutations had a poorer clinical outcome [29].

Presently, the mTOR inhibitor everolimus is the most clinically advanced compound targeting this signaling pathway in patients with HER2-positive metastatic breast cancer. A Phase Ib clinical trial assessed the triple regimen of everolimus, paclitaxel, and trastuzumab administered weekly in 33 patients with HER2-positive metastatic breast cancer who had received a median of two prior lines of chemotherapy in the metastatic setting (range, 0–17 lines) [30]. Encouraging efficacy results were presented, with a median PFS of 34 weeks (95% CI, 29.1–40.7 weeks) and an overall clinical benefit rate (CBR) ≥24 weeks reaching 74%. Grade 3/4 neutropenia was the most frequently reported AE related to study treatment (52%) [30]. A different Phase Ib study evaluated the combination of everolimus, vinorelbine, and trastuzumab in 50 heavily pretreated patients with HER2-positive metastatic breast cancer [31]. Antitumor activity was noted, with an ORR of 19.1%, a disease control rate ≥6 months of 83%, and a median PFS of 30.7

weeks (95% CI, 28.0–44.9 weeks). In terms of toxicity, neutropenia (92%) and stomatitis (70%) of any grade were the two most frequently reported AEs [31].

Two Phase I/II studies evaluating the combination of everolimus + trastuzumab in the metastatic setting of HER2-positive breast cancer were conducted concurrently, with their results presented in a pooled analysis (n=47). The CBR was 34% and median PFS reached 4.1 months, with fatigue, infection, and mucositis being the most frequent AEs [32].

Temsirolimus, another mTOR blocking agent, has been assessed in combination with neratinib in a Phase I study for patients with HER2-positive metastatic solid tumors, including breast cancer [33]. The most common drug-related toxicity was diarrhea, reaching 93% for all grades, while it constituted also the most frequent DLT observed in the study. Grade 3 diarrhea was reported in 22% of the patients, with other common AEs being nausea (53%), vomiting (42%), stomatitis (53%), hypokalemia (30%), rash (38%), and some cases of cytopenias (≥ grade 3 toxicity rates were 8% for anemia, 18% for lymphopenia, 5% for neutropenia, and 7% for thrombocytopenia). Concerning drug efficacy, of 15 patients with this histology enrolled in the trial, there were two objective responses in patients with HER2-amplified breast cancer [33].

BOLERO-3 randomized 569 women with HER2-positive locally advanced or metastatic breast cancer who were previously treated with a taxane and were resistant to trastuzumab to receive everolimus (n=284) or placebo (n=285) in combination with trastuzumab and vinorelbine (Figure 6.1) [34]. Final PFS results showed that the addition of everolimus to trastuzumab and vinorelbine reduced the risk of disease progression by 22% after a median follow-up of 20.2 months (HR=0.78; 95% CI, 0.65–0.95; $P<0.0067$), with median time to progression being 7.00 months in the everolimus arm and 5.78 months in the placebo arm [34]. OS findings were not reported. Regarding toxicity, the most common AEs reported were neutropenia (73% vs 62% of the patients in the everolimus and placebo group respectively), leucopenia (38% versus 29%), anemia (19% vs 6%), febrile neutropenia (16% vs 4%), stomatitis (13% vs 1%), and fatigue (12% vs 4%).

The results of BOLERO-1, international collaborative Phase III trial assessing everolimus in the first-line setting of HER2-positive metastatic breast cancer, were recently published [35]. BOLERO-1 randomized 719

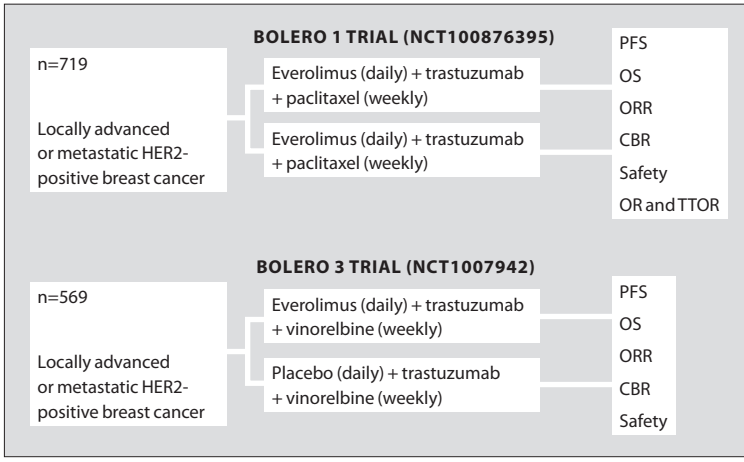


**Figure 6.1 Everolimus in HER2-positive breast cancer: the BOLERO-1 and -3 clinical trials.** CBR, clinical benefit rate; OS, overall survival; ORR, overall response rate; PFS, progression-free survival; PRO, patient-reported outcome; TTOR, time to objective response.

patients with newly diagnosed HER2-positive metastatic breast cancer 2:1 to receive trastuzumab and paclitaxel with or without everolimus. The study did not meet its primary endpoint because the PFS was comparable between the two arms: 14.95 and 14.49 months for the arms with and without everolimus, respectively (HR=0.89; $P$=0.1166) [35]. In a subgroup analysis based on the hormone receptor status, there was a 7-month improvement in the PFS with the addition of everolimus for patients with hormone receptor negative, HER2-positive disease (PFS 20.27 months vs 13.08 months; HR=0.66; $P$=0.0049). This improvement in PFS did not meet the prespecified level of significance, which was set at $P$<0.0044. Concerning the reported toxicities, they were similar to the BOLERO-3 trial [34,35]. In particular, stomatitis and diarrhea were the most frequently reported AEs, observed more frequently in the everolimus arm (67% vs 32% and 56% vs 47%, respectively).

## Other PI3K/Akt/mTOR-blocking agents

mTOR inhibitors block the mTORC1 complex of the PI3K/Akt/mTOR signaling pathway, while the mTOCR2 complex remains uninhibited [36]. Moreover, mTORC1 inhibition downregulates ribosomal protein S6 kinase

α-1 (S6K-α-1)-dependent autoinhibitory feedback mechanisms. As a way to overcome these hurdles, various direct PI3K-blocking agents are under clinical development, including:

- dual PI3K/mTOR inhibitors;
- pan-PI3K inhibitors;
- isoform-selective PI3K inhibitors (p110α-selective inhibitors being the most relevant for breast cancer);
- AKT inhibitors;
- mTORC1/2 inhibitors; and
- 3-phosphoinositide-dependent protein kinase-1 (PDK1) inhibitors.

Preclinical studies have provided evidence of antitumor activity for these inhibitors in the setting of HER2-positive breast cancer, and some of these agents have now entered clinical trials (Table 6.4) [37–44]. For example, a study by Junttila et al showed that HER2-overexpressing breast cancer cells bearing PIK3CA mutations E545K and H1047R were sensitive to GDC-0941, a pan-PI3K inhibitor [45]. Another study found that the transduction of HER2-overexpressing breast cancer cell lines with either of these PIK3CA mutations rendered them resistant to lapatinib; this resistance was reversed upon administration of NVP-BEZ235, a dual PI3K/mTOR inhibitor [46].

Preliminary antitumor activity from early clinical trials has already been reported. A Phase I trial assessed the combination of MK-2206, an Akt inhibitor, with trastuzumab in 32 patients with trastuzumab and/or lapatinib-pretreated HER2-positive metastatic breast cancer; findings showed one patient with CR, one with PR (unconfirmed), and four with prolonged SD for ≥4 months [47]. Concerning toxicity findings, the most frequently reported treatment-related AEs were fatigue, hyperglycemia, and cutaneous toxicity (ie, rash), consistent with prior findings for this agent class. Another Phase I study evaluated three different doses of daily oral BEZ235, a dual PI3K/mTOR inhibitor, in combination with weekly trastuzumab in 19 patients with trastuzumab-resistant HER2-positive breast cancer bearing molecular alterations of PIK3CA and/or PTEN. Of the 19 patients, 15 were evaluable for efficacy; 4 had SD for ≥4 cycles (16 weeks), and there was 1 case of PR in a patient with pulmonary and brain metastases [48].

| Trial | Phase | Description | Patients (n) | Primary endpoint(s) | Secondary endpoint(s) |
|---|---|---|---|---|---|
| NCT02152943 [37] | I | Toxicity of everolimus combined with letrozole and trastuzumab | Pretreated with ER+/ HER2-positive metastatic | MTD, CBR of triple combination | N/A |
| PIKHER2 (NCT01589861) [38] | Ib/II | Safety and efficacy of buparsilib in combination with lapatinib | Trastuzumab-pretreated, HER2-positive/ PI3K-activated (106) | MTD, ORR | Safety, clinical benefit, PFS, PK |
| NCT01042925 [39] | I/II | Dose escalation of XL147 in combination with trastuzumab or trastuzumab + paclitaxel | Trastuzumab-pretreated (42) | MTD, objective tumor response | PFS, PK, PD |
| NCT02038010 [40] | Ib | Dose escalation of BYL719 with T-DM1 | Pretreated HER2-positive metastatic (28) | DLT and MTD of BYL-719 | PK data generation |
| NCT02167854 [41] | I | Dose escalation of BYL719 combined with LJM716 and trastuzumab | Pretreated HER2-positive metastatic (48) | MTD of BYL719 | Toxicity |
| NCT01132664 [42] | Ib/II | Safety and efficacy of buparsilib in combination with trastuzumab | Trastuzumab-pretreated (88) | AEs, DLT | ORR |
| NCT01285466 [43] | Ib | Dose escalation of BEZ235 and buparsilib in combination with paclitaxel or paclitaxel + trastuzumab | Metastatic or locally advanced HER2-positive (110) | DLTs | AEs, ORR, PK, impact of treatment on biomarkers of PI3K pathway |
| NCT00928330 [44] | Ib | Safety, tolerability, PK, and activity of GDC-0941 in combination with trastuzumab or T-DM1 | Trastuzumab-pretreated (57) | AEs, changes in cardiac function, changes in vital signs and clinical laboratory results | PK, PFS, ORR, DR |

**Table 6.4 Selected ongoing clinical trials with everolimus and other PI3K/Akt/mTOR blocking agents.** AE, adverse events; BCS, breast-conserving surgery; CBR, clinical benefit rate; CNS, central nervous system; DLT, dose-limiting toxicities; DR, duration of response; HRQoL, health-related quality of life; MTD, maximum-tolerated dose; OCRR, objective clinical response rate; ORR, overall response rate; OS, overall survival; pCR, pathologic complete response; PD, pharmacodynamics; PFS, progression-free survival; PK, pharmacokinetics; RR, response rate; TTP, time to progression.

## Other signaling pathways

### HER3 inhibition

HER3, the third member of the ErbB type I transmembrane RTKs, has recently emerged as a rational therapeutic target for HER2-positive breast cancer. While HER3 lacks kinase activity, it is the preferred dimerization partner with the other ErbB receptors, with the HER2/HER3 heterodimer being the most potent PI3K signaling pathway activator [49]. In HER2-overexpressing breast cancer cells, preferential phosphorylation of HER3 has been documented, with HER3 being as important as HER2 in terms of maintaining cellular proliferation [50]. A 2012 study showed that the administration of an anti-HER2 monoclonal antibody preventing HER2/HER3 heterodimerization stimulated the formation of EGFR/HER3 heterodimers in high and low HER2-expressing cancer cells [51].

Importantly, another study found that dual HER2 blockade with trastuzumab and lapatinib does not fully abrogate HER3 signaling, whereas the addition of an anti-HER3 monoclonal antibody to the trastuzumab + lapatinib combination in a HER2-positive breast cancer xenograft model resulted in reduced tumor growth and prolonged survival compared with trastuzumab + lapatinib alone [52]. Based on this evidence, early-phase clinical trials are currently ongoing with anti-HER3 monoclonal antibodies (eg, U3-1287/AMG 888) (Table 6.5) [53–56].

### Src inhibition

The Src family of tyrosine kinases fuels malignant progression through induction of cellular proliferation, metastatic dissemination, and angiogenesis [57,58]. Src has been shown to be an important mediator of intracellular transduction of HER2 signaling [59]. Additionally, there is evidence that Src is a modulator of response to trastuzumab, functioning as a common node downstream of different resistance pathways for both de novo and acquired trastuzumab resistance. The addition of a Src-blocking agent has reversed these two types of resistance in vivo [11]. A quantitative proteomics approach coupled with a focused siRNA screen revealed that proteins associated with the Src kinase pathway are upregulated in trastuzumab-resistant HER2-overexpressing breast cancer cell lines [60]. Early-phase clinical trials assessing the efficacy of Src small-molecule inhibitors (eg, dasatinib) are currently recruiting patients in the setting of HER2-positive breast cancer (Table 6.5).

| Trial | Phase | Description | Patients (n) | Primary endpoint(s) | Secondary endpoint(s) |
|---|---|---|---|---|---|
| **HER3-blocking agents** | | | | | |
| NCT01512199 [53] | Ib/II | Safety and efficacy of U3-1287 (AMG 888) in combination with trastuzumab + paclitaxel | Trastuzumab-naïve (86) | MTD, PFS | PK, ORR, DCR |
| **Src-blocking agents** | | | | | |
| NCT01306942 [54] | I/II | Safety and efficacy of dasatinib in combination with trastuzumab + paclitaxel | Trastuzumab-naïve (60) | MTD | Safety, CBR, TTP, PFS, DR |
| **IGF-blocking agents** | | | | | |
| NCT00684983 [55] | II | Safety and efficacy of capecitabine and lapatinib ± cixutumumab | Trastuzumab-pretreated (154) | PFS | OS, TTTF, CTR, DR, AEs |
| **Met-blocking agents** | | | | | |
| NCT01138384 [56] | I/II | Safety and efficacy of foretinib in combination with lapatinib | Trastuzumab-pretreated (19) | MTD, toxicity | PK, preliminary efficacy |

**Table 6.5 Selected ongoing clinical trials with other emerging targeted agents in HER2-positive breast cancer.** AEs, adverse events; CBR, clinical benefit rate; CTR, confirmed tumor response; DCR, disease control rate; DR, duration of response; MTD, maximum tolerated dose; ORR, overall response rate; OS, overall survival; PFS, progression-free survival; PK, pharmacokinetics; TTP, time to progression; TTTF, time to treatment failure.

## Insulin growth factor inhibition

The insulin growth factor (IGF) signaling pathway represents an intracellular transduction system commonly deregulated in breast cancer [61], and has emerged as another rational therapeutic target in the setting of HER2-positive disease. Selective crosstalk of IGF-1R and HER2 has been documented in trastuzumab-resistant HER2-overexpressing breast cancer cells, which were resensitized to trastuzumab upon IGF-1R blockade [62]. A different study showed the formation of HER2/HER3/IGF-1R heterotrimeric complexes as a potential mechanism of resistance to trastuzumab; the formation of these complexes was disrupted by the knockdown of IGF-1 receptor (IGR-1R) and HER3 expression by short-hairpin RNA [9]. An interesting finding is that IGF-mediated trastuzumab resistance is associated with a decrease of

p27KipI protein levels through heightened ubiquitination involving the PI3K pathway [63].

Confirmatory results for the IGF-signaling pathway mediating resistance to trastuzumab were generated from a neoadjuvant trial assessing the combination of trastuzumab + vinorelbine in 48 patients with locally advanced HER2-positive breast cancer. Single and multigene biomarkers assessment studies were conducted, and positive IGF-1R membrane expression assessed by immunohistochemistry was associated with a lower response rate than negative expression (50% vs 97%; $P$=0.001) [64]. A variety of IGF-blocking agents are currently under clinical development, with some being assessed in the setting of HER2-positive breast cancer (Table 6.5).

### Met inhibition

Met is another signaling pathway contributing to the malignant progression of breast cancer, with overexpression of both the c-Met receptor and its corresponding ligand, hepatocyte growth factor (HGF), noted in one study in almost half of breast cancer cases [65]. Importantly, Met has been found to be often co-expressed with HER2 in HER2-overexpressing breast cancer cell lines and primary tumors, providing a growth advantage [10]. The same study showed that HGF mediates resistance to trastuzumab through abrogation of p27 induction; Met depletion by small-interfering RNA or pharmacologic inhibition sensitized HER2-overexpressing breast cancer cells to trastuzumab [10]. Moreover, preclinical evidence supports the involvement of Met in lapatinib resistance, which could be reversed with the addition of a dual Met/HER2 inhibitor [66]. Currently, several Met-blocking agents (anti-Met monoclonal antibodies such as onartuzumab; multitargeted TKIs: foretinib, fabozantinib; and small-molecule inhibitors such as BMS777607, PF-02341066, and ARQ197) are under clinical development in breast cancer, with one of them (foretinib) being assessed in the setting of HER2-positive disease (Table 6.5).

## Boosting immunological response to HER2 blockade

One of the main mechanisms by which trastuzumab exerts its antitumor activity is its immunologic action, with an abundance of evidence supporting a trastuzumab-triggered antibody-dependent-cellular-cytotoxicity (ADCC)

[67–69]. Moreover, adaptive immune responses involving CD8+ cytotoxic lymphocytes and myeloid differentiation have been implicated in trastuzumab-mediated antitumor activity [70,71].

Boosting the immunologic response to HER2 blockade has emerged as a potential strategy to improve clinical outcomes in this setting, with one exploratory approach being antibody modifications to enhance immune effector functions. Preclinical evidence showed that in a HER2-amplified breast cancer xenograft mouse model, afucosylated trastuzumab increased antitumor activity (mediated through heightened ADCC) compared with conventional trastuzumab, resulting in an increase in median PFS from 23.4 to 48 days [72]. Additional evidence for increased ADCC induced by afucosylated trastuzumab was found in an analysis of peripheral blood mononuclear cells from 30 volunteers, including 20 patients with breast cancer [73].

Another promising strategy to boost immunologic response to HER2 blockade is the co-administration of agents that impede the programmed death protein 1 (PD-1) receptor pathway. The PD-1 receptor is an inhibitory T-cell receptor utilized by cancer cells as part of their wide repertoire of immune suppression mechanisms, and is activated upon the binding of programmed cell death ligand 1/2, which can be expressed by both cancer and stromal cells [74,75]. Results from a recent study noted a synergy between trastuzumab and an anti-PD-1 monoclonal antibody in a HER2-overexpressing transgenic breast cancer mouse model, providing the rationale and evidence for subsequent clinical testing [71]. Of note, there is currently an ongoing trial sponsored by the International Breast Cancer Study Group (IBCSG), supported by BIG, that is evaluating the addition of MK-3475, an anti-PD1 monoclonal antibody, to trastuzumab, for patients with metastatic HER2-positive disease that experience trastuzumab resistance (NCT02129556) [76].

Lastly, efforts to target HER2 overexpression for therapeutic vaccination, which induces long-lasting antitumor activity by the immune system, are underway. The US National Cancer Institute has listed HER2 as a candidate vaccine antigen [77], with multiple HER2-targeting vaccines under clinical development (Table 6.6) [78–81]. A joint analysis of two clinical trials assessed a vaccine generated from E75, an HER2-derived peptide, which was administered with granulocyte macrophage colony-stimulating factor in 186 conventionally treated patients with breast cancer (both HER2-positive and HER2-negative) at high risk

for recurrence. There was a statistically significant prophylactic effect of vaccination at 20 months of follow-up (recurrence rate of 5.6% in vaccinated patients vs 14.2% in the nonvaccinated controls; $P$=0.04), but it lost its significance at 26 months of median follow-up [82]. A Phase I study also assessed a HER2 multipeptide vaccine in ten patients with metastatic HER2-negative breast cancer. Five patients had stable disease and one had a PR, providing proof of evidence that patients with bulky disease are immunocompetent and thus susceptible to respond to vaccination [83]. Whether such strategies will result in objective tumor responses in the metastatic setting is yet to be documented.

| Trial | Phase | Description | Patients (n) | Primary endpoint(s) | Secondary endpoint(s) |
|---|---|---|---|---|---|
| NCT01355393 [78] | I/II | Safety and efficacy of a HER-2/neu peptide vaccine in combination with rintatolimod ± sargramostim | Optimally treated, Stage II-IV (98) | IR, safety | DFS, OS |
| NCT00791037 [79] | I/II | Safety of adoptive T cell therapy following in vivo priming with a HER2 ICD peptide-based vaccine | Trastuzumab-pretreated (20) | Safety | IR, development of CD4+ and CD8+ epitope spreading, response of skeletal disease |
| NCT01632332 [80] | I | Safety and immunogenicity of a multi-epitope HER2 peptide vaccine | Optimally treated, Stage II-III (24) | Safety, IR | DFS |
| NCT01526473 [81] | I | Safety and antitumor activity of AVX901 | Trastuzumab-pretreated (12) | Safety | IR |

**Table 6.6 Selected ongoing clinical trials with HER2 vaccines in HER2-positive breast cancer.** DFS, disease-free survival; ICD: intracellular domain; IR, immune response; OS, overall survival.

## Vascular endothelial growth factor inhibition

Vascular endothelial growth factor (VEGF), a potent inducer of angiogenesis, is another therapeutic target for HER2-positive breast cancer. In vitro experiments showed that HER2 overexpression in breast cancer cell lines is associated with increased VEGF mRNA expression [84], with a subsequent study finding

that this HER2-mediated VEGF upregulation involves two different promoter regions, the core promoter through SP1 binding sites and the hypoxia responsive element [85]. In mice, transfection of human breast cancer cells with constitutively active HER2 kinase resulted in tumors of increased microvessel density, and was linked to increased VEGF protein synthesis [86]. In a clinical setting, HER2 and VEGF expression were found to be positively correlated in two breast cancer studies, the first a cohort of 611 consecutive unselected patients ($P<0.001$) [87] and the second a trial of 107 patients ($P<0.01$) [88].

Based on this evidence, clinical trials have assessed the antitumor activity of bevacizumab, an anti-VEGF humanized monoclonal antibody, in HER2-positive breast cancer. In the neoadjuvant setting, a Phase II single-arm trial studied bevacizumab in combination with trastuzumab, nab-paclitaxel, and carboplatin in 28 patients with locally advanced HER2-positive breast cancer. Objective response rates reached 86% and pCR was achieved in 54%; however, bevacizumab-related complications such as wound-healing delays and left ventricular ejection fraction decreases were noted postoperatively [89]. Bevacizumab was assessed in combination with fluorouracil, epirubicin, and cyclophosphamide (cycles 1–4) and with docetaxel and trastuzumab (cycles 5–8) preoperatively in a Phase II study of primary inflammatory HER2-positive breast cancer (BEVERLY-2). Out of 52 patients, 33 had a centrally confirmed pCR (63.5%); the common AEs were asthenia (69%), nausea (69%), alopecia (65%), and mucosal inflammation (63%) [90].

Another interesting study conducted in the neoadjuvant setting of HER2-positive breast cancer was the AVATAXHER trial [91]. This was a Phase II study that enrolled 142 evaluable patients with early-stage HER2-positive breast cancer that received initially two cycles of preoperative docetaxel plus trastuzumab. Before each of the first two cycles, an FDG-PET assessment was performed, with the change in the standardized uptake value (SUV) being used to predict pCR in each patient. Patients that were evaluated as responders continued to receive standard of care, whereas patients deemed to be non-responders (n=73) were randomized (2:1) to receive another four cycles of docetaxel/trastuzumab with (Group A, n=48) or without adding bevacizumab (Group B, n=25). pCR cases were achieved in 37 PET responders (53.6%, 95% CI, 41.2–65.7), 21 of those in group A (43.8%, 29.5–58.8), and six of those in group B (24.0%, 9.4–45.1), indicating that early PET evaluation can predict pCR in this setting, with

predicted non-responders benefiting from the addition of bevacizumab. Of note, the frequency of grade 3–4 AEs were similar in all three groups. The most common grade 3–4 AEs were neutropenia (4 in PET responders, 5 in group A, and 3 in group B), febrile neutropenia (1, 3, and 1, respectively), and myalgia (4, 0, and 1, respectively) [91].

Bevacizumab was combined with lapatinib in a Phase II trial of 52 patients with HER2-positive metastatic breast cancer, most of whom had received prior chemotherapy (96%) and/or trastuzumab (90%) in the metastatic setting. Median PFS reached 24.7 weeks, the CBR was 30.8%, and the most common AEs were diarrhea, rash, and fatigue [92]. A Phase II single-arm study assessed the combination of bevacizumab + trastuzumab in 50 patients with locally recurrent or metastatic HER2-positive breast cancer. Bevacizumab + trastuzumab therapy led to an ORR of 48%, median time to progression of 9.2 months, and a median OS of 43.8 months [93]. Another Phase II single-arm trial assessed the combination of bevacizumab with trastuzumab + capecitabine as first-line treatment in 88 patients with HER2-positive metastatic breast cancer. Antitumor activity was noted, with an ORR of 73% and a median PFS of 14.4 months; the main grade ≥3 AEs were hand-foot syndrome (22%), diarrhea (9%), and hypertension (7%) [94].

Lastly, the Phase III AVEREL trial randomized 424 patients with HER2-positive locally recurrent and/or metastatic breast cancer to receive docetaxel + trastuzumab with or without bevacizumab as first-line treatment [95]. The study did not meet its primary endpoint, with the investigator-assessed PFS showing a prolongation in the bevacizumab arm without reaching statistical significance (HR=0.82; 95% CI, 0.65–1.02; $P$=0.078). Additionally, there was no difference in time to treatment failure (9.8 vs 7.7 months; HR=0.94; 95% CI, 0.76–1.15; $P$=0.539) and investigator-assessed ORR (74.3% vs 69.9%; $P$=0.349) between the bevacizumab and nonbevacizumab treatment arms [95]. Interestingly, patients with high baseline VEGF-A levels derived a greater benefit from bevacizumab than those with low levels (HR=0.70 vs 0.83). Median PFS in patients with high baseline plasma VEGF-A was 16.6 months in those given bevacizumab and 8.5 months in those not given bevacizumab [95]. These findings should be viewed as hypothesis-generating, with the VEGF-A level as a potential biomarker to predict response to bevacizumab in this patient population.

## Conclusions

HER2-positive breast cancer oncology represents one of the most dynamically evolving fields. Trastuzumab has proven efficacy in all settings of HER2-positive disease, opening the way for the clinical development of an abundance of HER2-targeted agents, as exemplified by the success stories of lapatinib and, more recently, pertuzumab and trastuzumab-DM1. However, resistance to HER2 blockade is a clinical reality, mandating the development of new targeted agents. Second-generation TKIs, namely afatinib and neratinib, have shown antitumor activity in Phase I/II trials and are moving further in Phase III clinical evaluation. Moreover, molecular elucidation of HER2 signaling, along with emerging knowledge of alternate oncogenic signaling pathways mediating resistance to HER2 inhibition, have led to several other classes of targeted compounds currently under clinical investigation for HER2-positive breast cancer. Results from ongoing clinical trials are eagerly awaited, with predictive biomarkers guiding treatment selection among this abundance of riches still to be identified.

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