

## Current Approaches and Emerging Directions in HER2-resistant Breast Cancer

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**ABSTRACT:** Human epidermal growth factor receptor-2 (HER2) is overexpressed in up to 30% of breast cancers; HER2 overexpression is indicative of poor prognosis. Trastuzumab, an anti-HER2 monoclonal antibody, has led to improved outcomes in patients with HER2-positive breast cancer, including improved overall survival in adjuvant and first-line settings. However, a large proportion of patients with breast cancer have intrinsic resistance to HER2-targeted therapies, and nearly all become resistant to therapy after initial response. Elucidation of underlying mechanisms contributing to HER2 resistance has led to development of novel therapeutic strategies, including those targeting HER2 and downstream pathways, heat shock protein 90, telomerase, and vascular endothelial growth factor inhibitors. Numerous clinical trials are ongoing or completed, including phase 3 data for the mammalian target of rapamycin inhibitor everolimus in patients with HER2-resistant breast cancer. This review considers the molecular mechanisms associated with HER2 resistance and evaluates the evidence for use of evolving strategies in patients with HER2-resistant breast cancer.

**KEYWORDS:** HER2-positive, human epidermal growth factor receptor-2, HER2 therapy resistance

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### Introduction

Up to 30% of invasive breast cancers overexpress human epidermal growth factor receptor-2 (HER2),<sup>1</sup> leading to stimulation of pathways involved in cell proliferation and survival, including the phosphatidylinositol 3-kinase/protein kinase B/mammalian target of rapamycin (PI3K/Akt/mTOR) and mitogen-activated protein kinase pathways.<sup>2</sup> HER2 overexpression is correlated with age older than 50 years, higher T stage, and histological grade,<sup>3</sup> and it is a marker of aggressive disease, including decreased recurrence-free survival, breast cancer-related survival, and overall survival (OS).<sup>3–7</sup>

Molecular profiling has led to the classification of breast cancers into distinct subtypes, including HER2-positive (HER2<sup>+</sup>) breast cancer. It is recommended that HER2 status be assessed for all invasive breast cancers, because it influences

prognosis and selection of therapy,<sup>8–10</sup> an immunohistochemistry staining result of 3 or more, a fluorescence in situ hybridization (FISH) result of more than six *HER2* gene copies per nucleus, or a FISH ratio greater than 2.2 is considered a positive HER2 result.<sup>11</sup> Prognostic and predictive biomarker approaches for assessing HER2 status and better optimizing therapies are also under investigation.<sup>12,13</sup>

The identification of HER2 in breast cancer pathogenesis has led to the development of therapies targeting this receptor. Trastuzumab is a monoclonal antibody that has demonstrated improved survival in the first-line setting in combination with chemotherapy in patients with HER2<sup>+</sup> advanced disease<sup>14,15</sup> and improved disease-free survival and OS in patients with HER2<sup>+</sup> early breast cancer when used in combination with or sequentially after adjuvant chemotherapy.<sup>16–18</sup>



Although the development of HER2-targeted therapy has transformed the treatment of patients with HER2<sup>+</sup> breast cancer, nearly 70% of patients with metastatic breast cancer have intrinsic resistance and nearly all become resistant to therapy after initial responsiveness.<sup>19,20</sup> In addition, despite HER2-targeted therapy, many patients develop central nervous system (CNS) progression, which is a population of patients with limited therapeutic options.<sup>21</sup> Development of novel treatment approaches for HER2<sup>+</sup> breast cancer is clinically significant, particularly in the context of strategies to overcome resistance to HER2-targeted therapy.

### Resistance to HER2-targeted Therapies

General mechanisms of resistance to HER2-targeted therapies occur at three levels.<sup>22</sup> The first includes mechanisms intrinsic to the target, such as molecular changes in the target receptor;<sup>23</sup> the expression of p95HER2, which is a truncated HER2 receptor;<sup>24,25</sup> and *HER2* gene amplification.<sup>26</sup> Resistance involving parallel signaling pathways bypassing HER2 inhibition, such as increased activation of HER3,<sup>27–31</sup> aberrant activation of pathways downstream of the receptor,<sup>32–34</sup> and compensatory crosstalk with other pathways, might also occur.<sup>23,35–38</sup> Resistance from defects in the apoptosis pathway in tumor cells or in extrinsic host factors participating in the action of the drugs is another potential mechanism of resistance to HER2-targeted therapy.<sup>22</sup>

### Current Treatment Options for HER2-resistant Breast Cancer

Lapatinib and trastuzumab emtansine (T-DM1) are licensed treatments for use in the setting of trastuzumab resistance.<sup>39,40</sup> Lapatinib is a dual HER2 and epidermal growth factor receptor (EGFR)/HER1-specific tyrosine kinase inhibitor that binds to the intracellular domain of HER2, allowing it to inhibit both full-length HER2 and truncated p95HER2.<sup>24,41</sup> Lapatinib monotherapy and lapatinib in combination with capecitabine were shown to provide the same clinical benefit, including progression-free survival (PFS), clinical benefit rate, and overall response rate (ORR), regardless of p95HER2 expression in breast tumors from the first- and second-line lapatinib clinical development program.<sup>25</sup>

Lapatinib in combination with capecitabine is approved for patients with HER2<sup>+</sup> metastatic breast cancer that has progressed with trastuzumab, based on a phase-3, randomized study of 324 patients with HER2<sup>+</sup>, advanced or metastatic breast cancer who received previous treatment with an anthracycline, a taxane and trastuzumab (ClinicalTrials.gov identifier NCT00078572).<sup>39,42</sup> Patients were randomly assigned to receive either lapatinib plus capecitabine or capecitabine alone.<sup>42</sup> At a planned interim analysis, time to progression, the primary end point of the study, significantly favored the combination treatment arm (8.4 months with combination therapy versus 4.4 months with monotherapy; hazard ratio [HR] 0.49, 95% CI 0.34–0.71,  $P < 0.001$ ), and a non-significant trend

toward decreased CNS metastases with lapatinib therapy was observed. The significant time to progression results at the interim analysis prompted early termination of the study and subsequent crossover of the study arms. Although final exploratory analyses of OS (median OS, 75.0 vs 64.7 weeks; HR 0.87, 95% CI 0.71–1.08,  $P = 0.210$ ) showed a trend toward a survival advantage with lapatinib plus capecitabine, premature termination of enrollment and subsequent crossover resulted in insufficient power to detect significant differences.<sup>43</sup>

T-DM1 is an antibody–drug conjugate incorporating the HER2-targeted antitumor properties of trastuzumab with the cytotoxic activities of DM1, a microtubule agent that is a derivative of maytansine.<sup>44</sup> T-DM1 as a single agent is indicated for the treatment of patients with HER2<sup>+</sup> metastatic breast cancer in patients who previously received trastuzumab and a taxane.<sup>40</sup> Approval of T-DM1 is based on the EMILIA study (ClinicalTrials.gov identifier NCT00829166), which is a phase 3, randomized, open-label study of 991 patients with HER2<sup>+</sup> advanced breast cancer who received previous trastuzumab and taxane therapy.<sup>44</sup> Patients were randomly assigned to receive T-DM1 or lapatinib plus capecitabine, and the primary end points included PFS and OS, with two interim OS analyses completed. The median PFS was 9.6 months with T-DM1 versus 6.4 months with lapatinib plus capecitabine (HR 0.65, 95% CI 0.55–0.77,  $P < 0.001$ ), and the median OS at the second interim analysis crossed the stopping boundary for efficacy (30.9 vs 25.1 months; HR 0.68, 95% CI 0.55–0.85,  $P < 0.001$ ).

Results from the phase 3 TH3RESA study (ClinicalTrials.gov identifier NCT01419197) of T-DM1 in patients who received prior treatment with both trastuzumab and lapatinib for advanced disease have recently been reported, reaffirming the results from the EMILIA study in 602 patients with previously treated HER2<sup>+</sup> advanced breast cancer.<sup>45</sup> Patients with HER2<sup>+</sup> advanced breast cancer who had received at least two prior HER2-directed therapies for advanced breast cancer were randomized to receive T-DM1 or physician's choice of therapy, with optional crossover to T-DM1 in the control arm upon disease progression. The median PFS significantly favored T-DM1 compared with control (6.2 vs 3.3 months; HR 0.528, 95% CI 0.422–0.661,  $P < 0.0001$ ). Although the interim OS analysis favored T-DM1 (HR 0.552, 95% CI 0.369–0.826,  $P = 0.0034$ ), the efficacy stopping boundary was not crossed.

### Investigational Options for HER2<sup>+</sup> Therapy-resistant Breast Cancer

In addition to development of further novel HER-targeted therapies, several investigational treatments to manage resistance to HER2-targeted therapies are under development (Table 1). Maintaining HER2-targeted therapy, but switching chemotherapeutic agents, has been considered in the setting of HER2 resistance because some tumors that display resistance continue to depend on HER2-mediated signaling,<sup>46</sup> and there

**Table 1.** Summary of compounds under development for HER2-resistant breast cancer.

DEVELOPMENT PHASE	PREVIOUS TREATMENT	TRIAL NUMBER	REGIMEN	STATUS
<b>HER-targeted therapies</b>				
<b>PTZ</b>				
1/2	Previous TRA	NCT00875979	PTZ + T-DM1	Completed
2	0–1 prior metastatic Rx	NCT01276041	PTZ + TRA + chemo	Recruiting
2	1–3 prior TRA-based Rx	NCT00301899	PTZ + TRA	Completed
2	PD on TRA	NCT01912963	PTZ + TRA + ERI	Recruiting
<b>mTOR inhibitors</b>				
<b>EVE</b>				
1	PD or relapse on TRA	NCT00426556	EVE + TRA + PAC	Ongoing
2	Resistant to TRA and taxanes	NCT00426556	EVE + TRA + PAC	Ongoing
1	PD or relapse on TRA	NCT00426530	EVE + TRA + VIN	Completed
3	Resistant to TRA	NCT01007942	VIN + TRA ± EVE	Ongoing
<b>TEM</b>				
1/2	PD with TRA or LAP	NCT01111825	TEM + NER	Recruiting
<b>INK128</b>				
1	Various advanced tumors including HER2 <sup>+</sup> breast cancer failing standard of care therapy	NCT01351350	INK + PAC ± TRA	Ongoing
<b>PI3K inhibitors</b>				
<b>BKM120</b>				
1	Previously failed TRA	NCT01132664	BKM + TRA	Ongoing
1	Prior TRA and LAP allowed	NCT01300962	BKM + CAP ± TRA or LAP	Recruiting
1/2	TRA resistant	NCT01589861	BKM + LAP	Recruiting
<b>BYL719</b>				
1	PD on TRA and taxane Rx	NCT02038010	BYL719 + T-DM1	Recruiting
<b>GDC-0941</b>				
1	PD on TRA Rx	NCT00928330	GDC + T-DM1 or TRA	Completed
<b>XL147</b>				
1/2	PD on TRA Rx	NCT01042925	XL + TRA ± PAC	Completed
<b>Akt inhibitors</b>				
<b>MK2206</b>				
1	May have had PD on LAP	NCT01245205	MK + LAP	Recruiting
1	PD with TRA or LAP	NCT01705340	MK + TRA + LAP	Terminated
2	1 prior HER2-targeted Rx for MBC	NCT01277757	MK	Recruiting
<b>IGF1R inhibitors</b>				
<b>BMS-754807</b>				
1/2	Failed ≥1 TRA Rx	NCT00788333	BMS + TRA	Completed
<b>CIX (IMC-AI2)</b>				
2	PD with TRA regimens	NCT00684983	CAP + LAP ± CIX	Ongoing
<b>OSI-906</b>				
2	Previous TRA if HER2 <sup>+</sup>	NCT01205685	OSI + HT + ERL	Terminated
<b>Other tyrosine kinase inhibitors</b>				
<b>NER (HKI-272)</b>				
1	Prior HER2-targeted and taxane Rx	NCT01423123	NER + PAC + TRA	Ongoing
1/2	PD with ≥1 TRA regimen	NCT00398567	NER + TRA	Ongoing
1/2	Prior TRA (Part 2 only)	NCT00445458	NER + PAC	Ongoing
1/2	Prior taxane; PD with TRA (Part 2 only)	NCT00741260	NER + CAP	Ongoing

*(Continued)*



Table 1. (Continued)

DEVELOPMENT PHASE	PREVIOUS TREATMENT	TRIAL NUMBER	REGIMEN	STATUS
<b>NER (HKI-272)</b>				
1/2	≥1 prior TRA regimen (Part 2 only)	NCT00706030	NER + VIN	Ongoing
1/2	PD with TRA	NCT01111825	NER + TEM	Recruiting
2	Prior TRA and taxane	NCT00777101	NER vs LAP + CAP	Ongoing
2	PD with TRA (Arm A)	NCT00300781	NER	Ongoing
2	Prior TRA or LAP allowed	NCT01494662	NER	Recruiting
<b>AFA (BIBW2992)</b>				
2	If treated with TRA, must fail TRA	NCT01325428	AFA vs AFA + VIN	Ongoing
2	Failed prior TRA regimen	NCT00431067	AFA	Completed
2 (Lux-breast 2)	Failed prior TRA or LAP	NCT01271725	AFA vs AFA + PAC or VIN	Ongoing
2 (Lux-breast 3)	Prior TRA or LAP regimen	NCT01441596	AFA or AFA + VIN	Ongoing
3 (Lux-breast 1)	Failed 1 prior TRA	NCT01125566	AFA + VIN vs TRA + VIN	Ongoing
<b>Hsp90 inhibitors</b>				
<b>TAN</b>				
1/2	PD after TRA Rx	NCT00773344	TAN + TRA	Completed
<b>RET</b>				
2	2 prior HER2 regimens (TRA must have been given)	NCT00817362	RET + TRA	Terminated
<b>AUY922</b>				
1/2	TRA may have been used	NCT01361945	AUY + HT + LAP	Withdrawn
2	1–2 prior HER2 regimens (TRA must have been given)	NCT01271920	AUY + TRA	Ongoing
<b>GAN</b>				
2	Prior TRA if HER2 <sup>+</sup>	NCT01273896	GAN	Ongoing
<b>Telomerase inhibitors</b>				
<b>GRN (GRN163L)</b>				
1	Resistant to TRA	NCT01265927	GRN + TRA	Completed
<b>VEGF inhibitors</b>				
<b>PAZ</b>				
2	IBC; PD with TRA (if available)	NCT00558103	PAZ + LAP vs LAP	Completed
<b>MOT (AMG 706)</b>				
1/2	PD with TRA or LAP (for HER2 <sup>+</sup> tumors)	NCT01349088	MOT + IXA + CAP	Withdrawn

**Abbreviations:** AFA, afatinib; CAP, capecitabine; CIX, cixutumumab; CT, chemotherapy; DOC, docetaxel; ERI, eribulin; ERL, erlotinib; EVE, everolimus; GAN, ganetespib; GRN, imetelstat; HT, hormone therapy; Hsp90, heat shock protein 90; IGF1R, insulin-like growth factor 1 receptor; IXA, ixabepilone; LAP, lapatinib; MOT, motesanib; NER, neratinib; PAC, paclitaxel; PAZ, pazopanib; PD, progressive disease; PTZ, pertuzumab; RET, retaspimycin; Rx, treatment; TAN, tanespimycin; T-DM1, trastuzumab emtansine; TEM, temsirolimus; TRA, trastuzumab; VEGF, vascular endothelial growth factor; VIN, vinorelbine.

are preliminary reports of the benefits of trastuzumab therapy beyond progression.<sup>47–53</sup> It is also suggested that the use of trastuzumab after diagnosis of CNS metastases may lead to improved overall survival when compared with patients who discontinue trastuzumab after diagnosis of metastases or who do not receive trastuzumab at all.<sup>13,54</sup>

Benefits of combination therapy with HER2 inhibitors have also been investigated. In a phase 3 study in patients with metastatic breast cancer resistant to trastuzumab therapy, trastuzumab in combination with lapatinib significantly improved PFS compared with lapatinib alone (median PFS, 12.0 vs 8.1 weeks; HR 0.73, 95% CI 0.57–0.93,  $P = 0.08$ ).<sup>55</sup>

The targeting of pathways downstream of HER2 has also been considered,<sup>23</sup> and other targets under assessment for HER2<sup>+</sup> treatment-refractory breast cancer include signal transduction molecules implicated in HER2 resistance, such as PI3K, Akt, mTOR, and insulin-like growth factor 1 receptor (IGF1R), as well as heat shock protein 90 (Hsp90), telomerase, and vascular endothelial growth factor (VEGF). Mediation of an interleukin (IL)-6 inflammatory loop, which has been implicated in HER2 overexpression and trastuzumab resistance, is also under consideration,<sup>56,57</sup> and preclinical reports of trastuzumab therapy in combination with antimalarial agents suggest improved activity in





trastuzumab-resistant breast cancers.<sup>58</sup> Availability of novel therapies that target different pathways and with unique mechanisms of action might improve outcomes in patients with trastuzumab-resistant disease.

**HER-targeted therapies.** The monoclonal antibody pertuzumab targets the HER2 receptor and prevents HER2 from coupling with other HER family members. Pertuzumab is approved for use in combination with trastuzumab and docetaxel in patients who have not received HER2-targeted therapy or chemotherapy for metastatic disease. The approval was based on the data from the CLEOPATRA study, which showed a significantly increased median PFS and OS, compared with trastuzumab in combination with docetaxel.<sup>59–62</sup>

Pertuzumab is under clinical investigation in patients with HER2-resistant breast cancer. In a phase 2, open-label, single-arm, two-stage study in 66 evaluable patients with advanced breast cancer who had no response to trastuzumab (ClinicalTrials.gov identifier NCT00301899), the ORR with pertuzumab therapy in combination with trastuzumab was 24% and the clinical benefit rate was 50% (8% had complete response, 17% had partial response, and 26% had stable disease for at least 6 months), with a median PFS of 5.5 months.<sup>63</sup> In an ongoing phase 2 study of pertuzumab in combination with trastuzumab and chemotherapy (ClinicalTrials.gov identifier NCT01276041), the preliminary 6-month PFS was 81% (95% CI 67–91; based on data from 18 January 2013) in evaluable patients (53 patients enrolled; 36 evaluable at 6 months).<sup>64</sup> A phase 2 study assessing pertuzumab in combination with eribulin and trastuzumab in patients with recurrent HER2<sup>+</sup> breast cancer is currently recruiting (ClinicalTrials.gov identifier NCT01912963).

**Therapies targeting downstream pathways—PI3K/Akt/mTOR inhibitors.** mTOR is a serine/threonine kinase that is downstream of PI3K/Akt<sup>65</sup> and integrates multiple signals from growth factors and hormones, thereby controlling cell growth, proliferation, and angiogenesis.<sup>66</sup> Overexpression of HER2 is associated with activation of the mTOR pathway, and hyperactivation of the pathway is associated with poor prognosis.<sup>67,68</sup> Multiple upstream components of the mTOR pathway become dysregulated in breast cancer and are believed to be involved in oncogenesis, suggesting that mTOR inhibition has the potential to interfere with tumor progression at several levels and that targeting multiple pathways with different agents might be more effective than monotherapy strategies.<sup>66,69</sup> Several clinical studies are investigating the potential of mTOR inhibitors to improve or overcome resistance to HER2-targeted therapy,<sup>66</sup> including phase 3 data for everolimus.<sup>45,70</sup>

The phase 1b dose-escalation study of everolimus in combination with trastuzumab and paclitaxel in 33 patients with metastatic HER2<sup>+</sup> breast cancer that progressed while on or after trastuzumab therapy reported an ORR of 44% and a disease control rate of 74%, with a median PFS of 34 weeks (95% CI 29.1–40.7) (J2101 study, ClinicalTrials.gov

identifier NCT00426556).<sup>71</sup> The phase 2 portion of the trial in 55 evaluable patients with resistance to trastuzumab and a taxane reported clinical benefit and objective response rates of 36% and 22%, respectively, and a median PFS of 5.5 months (95% CI 4.99–7.69).<sup>72</sup> The incidences of adverse events (AEs) were consistent with the safety profiles of the individual therapies. Grade 3/4 hematologic events included neutropenia (grade 3, 26%; grade 4, 4%), anemia (grade 3, 7%; grade 4, 0), and thrombocytopenia (grade 3, 6%; grade 4, 2%). Grade 3/4 non-hematological events included stomatitis (20%), diarrhea (6%), vomiting (6%), fatigue (6%), and pneumonia (6%). No grade 4 non-hematological events were reported.

A phase 1/1b study assessed dose-limiting toxicity (DLT) and overall tumor response of everolimus in combination with trastuzumab and vinorelbine in 50 patients with HER2<sup>+</sup> advanced breast cancer who experienced disease progression or relapse while previously taking trastuzumab with or without chemotherapy (J2102 study, ClinicalTrials.gov identifier NCT00426530).<sup>73</sup> Patients received either daily (5 mg/d) or weekly (20 or 30 mg/wk) everolimus therapy in combination with vinorelbine (25 mg/m<sup>2</sup> on day 1 and 8 every 3 weeks) and trastuzumab (2 mg/kg weekly), and, based on DLT findings, everolimus 5 mg/day was chosen as the optimal daily dose in combination with weekly trastuzumab and vinorelbine for further clinical development. Responses and disease stabilization in the 5 mg/day cohort were durable, and ORR was 20% and clinical benefit rate was 50%. Median PFS was 30.7 weeks in the 5 mg/day everolimus arm, 27.1 weeks in the weekly arm, and 30.7 weeks in the overall population. In the extension phase of the study, in which patients were allowed to continue everolimus and vinorelbine could be discontinued, two additional patients achieved complete response, one achieved partial response, and the overall PFS was 41 weeks.<sup>74</sup> In patients receiving everolimus 5 mg/day in combination with weekly trastuzumab and vinorelbine, neutropenia (grade 1/2, 10%; grade 3/4, 83%) was the most common hematological AE and stomatitis (grade 1/2, 70%; grade 3/4, 17%) was the most common non-hematological AE related to study treatment.

A post hoc analysis of these two phase 1/2 trials evaluated the efficacy of everolimus in patients pretreated with lapatinib.<sup>75</sup> Among 101 evaluable patients, the ORR was 21% and 29%, disease control rate was 88% and 81%, and mean PFS was 29.0 and 36.1 weeks in those pretreated with lapatinib and those not pretreated with lapatinib, respectively, suggesting that the effect of everolimus in combination with trastuzumab and chemotherapy in patients with HER2<sup>+</sup> metastatic breast cancer is independent of previous lapatinib therapy.<sup>75</sup>

BOLERO-3 is a phase 3, randomized, double-blind, placebo-controlled, multicenter, international clinical trial comparing the efficacy of everolimus in combination with vinorelbine and trastuzumab versus placebo in combination with vinorelbine and trastuzumab in 569 patients with HER2<sup>+</sup> advanced breast cancer resistant to trastuzumab and



who have previously been treated with a taxane.<sup>45,70</sup> At the time of analysis (data cutoff: 15 March 2013), the median duration of follow-up was 20 months, and 61 patients were continuing the study treatment. The most common reason for discontinuation was disease progression, and fewer patients discontinued treatment because of disease progression in the everolimus arm than in the control arm (68% vs 78%), whereas more patients in the everolimus arm discontinued treatment because of AEs than in the control arm (10% vs 5%).

The primary efficacy analysis based on local radiological assessment resulted in an estimated 22% risk reduction in PFS (HR 0.78, 95% CI 0.65–0.95,  $P = 0.0067$ ), corresponding to a modest 1.22-month prolongation in median PFS, from 5.78 months in the placebo arm to 7.00 months in the everolimus arm.<sup>70</sup> The PFS benefit of everolimus was observed across patient subgroups defined by demographic characteristics, previous therapy, and disease characteristics, and patients with hormone receptor–negative tumors had better PFS outcomes with everolimus. Secondary end points included ORR, which was similar between treatment arms; OS data are not yet mature.

The non-hematological AE profile of everolimus in combination with trastuzumab and vinorelbine was consistent with the known safety profile of the individual drugs, and no new unexpected AEs were observed.<sup>45,70</sup> AEs typical of mTOR inhibitors occurred more frequently with everolimus and included stomatitis, non-infectious pneumonitis, rash, and hyperglycemia, whereas the incidence and grade of other non-hematological AEs were similar between treatment arms (everolimus plus trastuzumab and vinorelbine vs placebo plus trastuzumab and vinorelbine), with the exception of grade 3/4 stomatitis (13% vs 1%) and fatigue (12% vs 4%). The most common hematological AEs with everolimus plus trastuzumab and vinorelbine treatment were neutropenia (81%), anemia (49%), febrile neutropenia (17%), and thrombocytopenia (14%).

Other mTOR inhibitors under development in HER2-resistant breast cancer include temsirolimus and INK128, both with ongoing phase 1/2 studies. Investigational PI3K inhibitors include buparlisib (BKM120), BYL719, GDC-0941, and XL147; and investigational Akt inhibitors include MK2206, all under investigation in phase 1/2 clinical studies in patients with HER2<sup>+</sup> breast cancer who either experienced progression while taking trastuzumab or received prior trastuzumab therapy.

In a phase 1/2 study of the PI3K inhibitor buparlisib, in combination with trastuzumab in patients resistant to trastuzumab-containing therapy, the maximum tolerated dose (MTD) of buparlisib was reported to be 100 mg/day, with grade 3 AEs of asthenia, altered mood, rash,  $\gamma$ -glutamyl transpeptidase increase, hypokalemia, and hypersensitivity (in one patient each), and no grade 4 events were reported (ClinicalTrials.gov identifier NCT01132664).<sup>76</sup> In the 17 enrolled patients (18 were initially enrolled), two achieved partial

response and seven achieved disease stabilization. An additional phase 1/2 study evaluating safety and efficacy of buparlisib plus lapatinib in HER2<sup>+</sup> trastuzumab-resistant advanced breast cancer in patients with evidence of activation of the PI3K/Akt/mTOR pathway is currently recruiting (ClinicalTrials.gov identifier NCT01589861).<sup>77</sup>

**Therapies targeting downstream pathways—IGF1R inhibitors.** It is hypothesized that crosstalk between IGF1R and HER2 might occur in breast cancer cells, leading to receptor heterodimerization and causing tumor cells to escape trastuzumab toxicity.<sup>37</sup> In preclinical models, IGF1R overexpression led to trastuzumab resistance,<sup>36</sup> and the combination of an IGF1R inhibitor and trastuzumab resulted in greater cell death than did treatment with trastuzumab alone.<sup>37</sup> BMS-754807 and cixutumumab are IGF1R inhibitors undergoing phase 1/2 clinical investigation in patients with HER2-resistant breast cancer (ClinicalTrials.gov identifiers NCT00788333 and NCT00684983, respectively). A phase 2 study of linsitinib (OSI-906), a dual inhibitor of IGF1R and the insulin receptor,<sup>78</sup> in patients with metastatic breast cancer was terminated because of severe toxicity and lack of efficacy (ClinicalTrials.gov identifier NCT01205685).

**Therapies targeting downstream pathways—other tyrosine kinase inhibitors.** Neratinib (HKI-272) is an irreversible multi-tyrosine kinase inhibitor of EGFR/HER1, HER2, and HER4, which has undergone clinical investigation in treatment of patients with HER2-resistant disease.<sup>79</sup> Phase 1/2 study results in 72 patients who underwent previous HER2-targeted therapy and later were treated with neratinib in combination with capecitabine found that of the 22 patients evaluable for efficacy during the interim analysis, 11 achieved partial response (ORR of 50%) and two maintained stable disease for less than 24 weeks (ClinicalTrials.gov identifier NCT00741260).<sup>80</sup> In a phase 1 study of neratinib in combination with trastuzumab and paclitaxel in patients with metastatic HER2<sup>+</sup> breast cancer who had previously been treated with anti-HER agents and a taxane, the recommended phase 2 dose of neratinib in combination with trastuzumab and paclitaxel was determined to be 200 mg/day, and common grade 3/4 AEs were diarrhea (38%), dehydration (14%), electrolyte imbalance (19%), and fatigue (19%) (ClinicalTrials.gov identifier NCT01423123).<sup>81</sup> Objective responses (two patients with complete response; six patients with partial response) were achieved in 38% of patients, and the median time to disease progression was 3.7 months. In an ongoing phase 1/2 study of neratinib in combination with temsirolimus in patients who previously received trastuzumab therapy, six patients treated at the MTD (8 mg intravenous) were evaluable for response, four achieved partial response, and one had stable disease (ClinicalTrials.gov identifier NCT01111825).<sup>82</sup>

Phase 2 study results in 117 patients with advanced breast cancer who received no more than two prior trastuzumab regimens and who were randomly assigned to receive neratinib or lapatinib plus capecitabine have been reported.<sup>83</sup>



For neratinib and combination therapy, median PFS was 4.5 and 6.8 months (HR 1.3, 95% CI 1.0–1.8,  $P = 0.091$ ) and median OS was 19.4 and 19.0 months, respectively ( $P = 0.180$ ; and ORR was 29% and 41%,  $P = 0.067$ ), indicating that neratinib monotherapy was not as effective as lapatinib plus capecitabine (ClinicalTrials.gov identifier NCT00777101).

A series of studies assessing the effect of previous HER2-targeted therapy on neratinib activity are underway, although inconsistent effects have been reported. In a phase 1/2 study of neratinib in combination with paclitaxel, among the 99 evaluable patients included in the MTD assessment, ORR was 73% (95% CI 62.9–81.2) in the overall population, 71% among patients who experienced up to one previous chemotherapy regimen for metastatic disease and no previous lapatinib therapy, and 77% among those who experienced two or three previous chemotherapy regimens for metastatic disease and in whom prior lapatinib therapy was permitted (ClinicalTrials.gov identifier NCT00445458).<sup>84</sup> In another phase 1/2 study of neratinib in combination with vinorelbine, ORR was 41% in patients who had not received lapatinib previously and 8% in patients who had experienced prior lapatinib therapy (ClinicalTrials.gov identifier NCT00706030).<sup>85</sup> Phase 2 study results of neratinib monotherapy in 136 patients reported 16-week PFS rates of 59% in patients who had previous trastuzumab therapy and 78% in those who did not, with a median PFS of 22.3 and 39.6 weeks, respectively, and with an ORR of 24% and 56% (ClinicalTrials.gov identifier NCT00300781).<sup>86</sup> Additionally, a phase 2 trial of neratinib for patients with HER2<sup>+</sup> breast cancer and brain metastases is currently recruiting (ClinicalTrials.gov identifier NCT01494662).<sup>87</sup>

Afatinib (BIBW 2992) is an irreversible tyrosine kinase inhibitor undergoing clinical investigation in HER2-resistant disease. Phase 2 results of afatinib monotherapy in 41 patients with HER2<sup>+</sup> metastatic breast cancer that progressed after trastuzumab therapy have shown a median PFS of 15.1 weeks (95% CI 8.1–16.7) and a median OS of 61.0 weeks (95% CI 56.7–not evaluable), with 10% of patients achieving a partial response and 37% having stable disease, with an overall clinical benefit rate of 46% (ClinicalTrials.gov identifier NCT00431067).<sup>88</sup> There are a series of ongoing studies of afatinib in trastuzumab- or lapatinib-resistant breast cancer, which include LUX-Breast 1 (ClinicalTrials.gov identifier NCT01125566), a phase 3 study assessing afatinib in combination with vinorelbine versus trastuzumab plus vinorelbine in patients with metastatic breast cancer in whom one round of previous trastuzumab therapy was ineffective.<sup>89</sup> The study began June 2010, with an estimated enrollment of 508 patients and a planned completion date of June 2014. LUX-Breast 2 (ClinicalTrials.gov identifier NCT01271725) is a phase 2 study assessing afatinib monotherapy followed by afatinib in combination with paclitaxel or vinorelbine upon progression in patients with metastatic breast cancer who experienced progression while taking previous trastuzumab

or lapatinib therapy,<sup>90</sup> and LUX-Breast 3 (ClinicalTrials.gov identifier NCT01441596) is a phase 2 study assessing afatinib alone or in combination with vinorelbine versus investigator choice of treatment in patients with HER2<sup>+</sup> breast cancer and progressive brain metastases after trastuzumab- or lapatinib-based therapy.<sup>91</sup>

**Hsp90 inhibitors.** Hsp90 is a chaperone protein that promotes protein folding and stabilization and prevents rapid protein degradation. Pre-clinical data show that HER2 is chaperoned by Hsp90.<sup>92</sup> In a phase 2 study of 31 patients with trastuzumab-refractory HER2<sup>+</sup> breast cancer, combination therapy with an Hsp90 inhibitor (tanespimycin; development of which was discontinued because of a corporate decision) and trastuzumab provided a clinical benefit rate of 59%, and a median PFS and OS of 6 and 17 months, respectively (ClinicalTrials.gov identifier NCT00773344).<sup>92</sup> Phase 1/2 clinical investigation of other Hsp90 inhibitors in patients with HER2-resistant breast cancer includes AUY922 and ganetespib. Phase 2 clinical investigation of retaspimycin was terminated because of lack of efficacy at an interim analysis (ClinicalTrials.gov identifier NCT00817362).<sup>93</sup>

**Telomerase inhibitors.** Telomerase expression is necessary for cellular proliferation, with telomerase overexpression correlated with tumorigenesis, and with telomerase inhibition resulting in apoptosis or cell senescence.<sup>94</sup> In a trastuzumab-resistant cell line, the telomerase inhibitor imetelstat (GRN163L) restored trastuzumab sensitivity. A phase 1 study of imetelstat in patients with trastuzumab-resistant breast cancer has been completed, and, in an initial report of 10 patients with trastuzumab-refractory HER2<sup>+</sup> metastatic breast cancer enrolled in the study, no objective responses were reported; however, two patients achieved stable disease (ClinicalTrials.gov identifier NCT01265927).<sup>95</sup>

**VEGF inhibitors.** HER2 induction of VEGF expression is believed to play a role in the pathogenesis of HER2-amplified breast cancer.<sup>96</sup> In human breast cancer xenografts transplanted into mice, trastuzumab-resistant clones showed elevated VEGF expression, and sensitivity to trastuzumab was restored upon treatment with bevacizumab, which is a monoclonal antibody against VEGF,<sup>97</sup> and with bevacizumab in combination with trastuzumab plus pertuzumab.<sup>98</sup> Although bevacizumab has been investigated as first-line therapy in patients with HER2<sup>+</sup> metastatic breast cancer,<sup>99</sup> planned or ongoing clinical studies of bevacizumab in patients with HER2-resistant breast cancer have not been reported.

Phase 2 results for pazopanib, an oral therapy approved for use in advanced renal cell carcinoma and advanced soft tissue sarcoma,<sup>100</sup> in combination with lapatinib versus lapatinib monotherapy have been reported in patients with HER2<sup>+</sup> inflammatory breast cancer, including those who experienced relapse after trastuzumab therapy (if the therapy was considered readily available) (ClinicalTrials.gov identifier NCT00558103).<sup>101</sup> ORR for patients treated





with lapatinib 1500 mg, lapatinib 1000 mg plus pazopanib 400 mg, and pazopanib 800 mg was 47%, 58%, and 31%, respectively, and median PFS was 16.0, 16.0, and 11.4 weeks, respectively. There was no consistent effect of prior trastuzumab therapy on response rate in the lapatinib-containing treatment arms.

**Vaccines.** HER2 is a tumor-specific antigen and an ideal immunotherapeutic target, and vaccines based on HER2-derived peptides, including E75, GP2, and AE37, are being developed.<sup>79</sup> A phase 1 study assessed HER2 immunotherapy in combination with lapatinib in 12 patients with metastatic breast cancer resistant to trastuzumab. However, the effect of lapatinib on the immune responses induced by vaccination was inconclusive, with vaccination triggering variable levels of anti-HER2 antibodies in all patients and an HER2-specific T-cell response in one patient.<sup>102</sup>

Clinical investigation of vaccines in patients who have undergone or are currently receiving trastuzumab therapy is underway (ClinicalTrials.gov identifiers NCT01632332, NCT00343109). A phase 2 study of a vaccine in trastuzumab-resistant disease has been terminated because of a change in the development plan (ClinicalTrials.gov identifier NCT00522457).

## Conclusions

HER2 overexpression is associated with poor prognosis in patients with breast cancer. Targeting HER2 with therapies has markedly improved outcomes in patients with HER2<sup>+</sup> breast cancer. However, because resistance to HER2-targeted therapies is common, the development of strategies to overcome resistance is important, because it might lead to improved outcomes for patients with HER2<sup>+</sup> breast cancer. Identifying the underlying mechanisms contributing to resistance has facilitated the development of novel therapeutic strategies to overcome resistance. These strategies include novel HER2-targeted therapies; therapies targeting downstream pathways; and use of Hsp90, telomerase, and VEGF inhibitors. It is hoped that ongoing and completed clinical trials, including phase 3 data for the mTOR inhibitor everolimus, will lead to additional treatment strategies to manage patients with HER2-resistant disease.

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## Author Contributions

AB conceived the concept, analyzed the data, was involved in writing the first draft and contributed to the writing of the manuscript, agreed with manuscript results and conclusions, developed the structure and arguments for the paper, made critical revisions and approved the final manuscript. Under the direct supervision of AB, Tricia Newell, PhD, and Matthew Grzywacz, PhD of ApotheCom assisted in the writing of the

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