Beyond trastuzumab: what does it?

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Summary

Human epidermal growth factor receptor 2 (HER-2) gene amplification or protein overexpression occurs in 20% to 25% of breast tumors, often leading to an aggressive disease course and poor clinical outcomes. Successful targeting of HER-2-positive tumors in preclinical models with trastuzumab has translated to the clinic.
Overexpression of the HER-2 represents a biological subclass of breast cancer (BC) with distinct molecular alterations, clinical behavior, and response to systemic therapy. Trastuzumab is a monoclonal antibody directed against HER-2 which has revolutionized the management of both early and advanced BC. It may exert its anti-cancer effects through inhibition of intracellular signaling, up regulation of p27, impaired angiogenesis, induction of immune-mediated destruction, and blockade of cleavage of the extracellular domain of HER-2. In spite of its robust clinical activity, most women with metastatic HER-2 overexpressing BC eventually progress on trastuzumab therapy. Possible mechanisms of resistance include: altered receptor antibody interaction, PTEN loss and enhanced Akt signaling, p27 loss, signaling through other receptors. Preclinical experiments, clinical experience with the use of trastuzumab beyond progression, and a recent phase III clinical trial with Lapatinib, a dual EGFR/HER-2 tyrosine kinase inhibitor, demonstrate that the HER-2 signaling axis remains an important therapeutic target even after progression on trastuzumab. A variety of novel strategies are currently in development to exploit this pathway following the onset of resistance, such as receptor antibodies, sheddase inhibitors, signal transduction inhibitors, heat shock protein inhibitors, proteasome inhibitors, anti-angiogenic agents, and immunostimulatory therapies, either as single agents or in combination with trastuzumab. Rational clinical trial design, with attention to appropriate patient selection and prospective collection of biological material, is needed to ensure that the new generation of anti-HER-2 targeted therapies realizes its promise in the treatment of trastuzumab-resistant disease.

KEY WORDS: *trastuzumab beyond; metastatic breast cancer; HER-2; metastatic breast cancer.*

Introduction

HER-2 is amplified in 20 to 25% of BC and in these cases the encoded protein is present in abnormally high levels in the malignant cells (1, 2). Women with BC that overexpressing HER-2 have an aggressive form of disease with significantly shortened disease-free survival and overall survival (1-5). Laboratory studies indicate that amplification of HER-2 has a direct role in the pathogenesis of BC, (6-10) thereby providing investigators with an opportunity to target a therapeutic agent directly against the alteration. Several murine monoclonal antibodies against the extracellular domain of the HER-2 protein were found to inhibit the proliferation of human cancer cells that overexpressed HER-2, both *in vitro* and *in vivo* (11-13). To minimize immunogenicity, the antigen-binding region of one of the more effective antibodies was fused to the framework region of human IgG and tested against BC cells that overexpressed HER-2 *in vitro* and *in vivo* (14, 15). This antibody, called trastuzumab, inhibited tumor growth when used alone but had synergistic effects (13, 15-17) when used in combination with cisplatin and carboplatin, docetaxel, and ionizing radiation and additive effects when used with doxorubicin, cyclophosphamide, methotrexate, and paclitaxel (13, 18, 19). Phase 1 clinical trials showed that the antibody is safe and confined to the tumor. Subsequent phase 2 trials demonstrated that many women with HER-2-positive metastatic disease who had relapsed after chemotherapy had a response to trastuzumab (20, 21); as suggested by the preclinical data, the efficacy of trastuzumab when given with chemotherapy was superior to its effectiveness when used alone (21-24). In HER-2-positive metastatic breast cancer (MBC), trastuzumab provides significant clinical benefit as a monotherapy and in combination with numerous chemotherapies. In the phase III trial of first-line trastuzumab plus chemotherapy (25), overall response rate (ORR; 50%, $P < 0.001$), overall survival (25.1 months *vs* 20.3 months, $P = 0.046$) and time to disease progression improved significantly compared with chemotherapy alone (7.4 *vs* 4.6 months, $P < 0.001$), and second-line trastuzumab use after prior trastuzumab has

resulted in ORRs of up to 50%. Clinical success in the metastatic setting provided the rationale for assessing trastuzumab in early breast cancer (26-30). Four large trials of adjuvant trastuzumab demonstrated significant improvements in disease-free survival (33-52%) and overall survival (34-41%) despite tumor size, nodal or hormone-receptor status, and age. New approaches to maximize the clinical benefit of trastuzumab-based therapy are under investigation and include novel combinations with other targeted therapies such as bevacizumab, pertuzumab, lapatinib, neratinib and T-DM1.
This is an analysis of current knowledge on trastuzumab beyond progression in HER-2 positive advanced BC and our opinion on the future developments of new anti HER-2 drugs.

Methods

This review focused on the mechanisms of resistance to HER-2-targeted therapy and the strategies for overcoming this resistance. The reference list was update in February 2013. All identified phase III and II studies evaluating trastuzumab and other monoclonal antibodies and TKI in MBC are included. Data of this systematic review, obtained from the PubMed database using the following terms: “trastuzumab resistance”, “lapatinib”, “neratinib”, “T-DM1”, “HER-2 target therapy”, “HER-2 family TKIS” and “novel drug HER-2 pathway”. In addiction, abstracts from annual meetings of the American Society of Clinical Oncology (ASCO) and the San Antonio Breast Cancer Symposium 2012-2013 were retrieved for relevant abstracts using the same search terms.

Drug resistence: this is the problem

Drug resistence remains a major clinical problem that hinders the successful management of BC patients. Several mechanisms of resistance to HER-2-targeted therapy have been proposed. These include impediments to HER-2-trastuzumab binding, signaling through alternative pathways, up regulation of signaling pathways downstream of HER-2, and failure to elicit an appropriate immune response (31, 32). Specific factors implicated in resistance include the HER-2 copy number; HER-2 dimerization status; presence of truncated HER-2 (p95HER-2); Fc receptor status; loss of phosphatase and tensin homolog (PTEN) and p27Kip1 expression; activation of mutations of PI3KCA; amplification of the epidermal growth factor receptor (EGFR) gene or overexpression of the EGFR protein; overexpression of insulin-like growth factor receptor 1 (IGF1R), vascular endothelial growth factor receptor, and heat shock protein 90 (HSP90); activation of the cytoplasmic tyrosine kinase SRC; and mucin 4 glycopeptide expression (31-34).

Current strategies for overcoming resistance: maintaining HER-2-targeted therapy

Switching chemotherapeutic agent

In the clinical practice, various treatment strategies are used in attempts to overcome trastuzumab resistance (35). One strategy is to continue trastuzumab therapy beyond progression and combined it with an alternative chemotherapy regimen (36-40). Although the optimal duration of trastuzumab therapy remains controversial, data from preclinical studies indicating additive or synergistic effects of trastuzumab with several cytotoxic agents has led many clinicians to continue to administer trastuzumab beyond progression, in combination with second- or third-line chemotherapeutic agents (36-38). In one small, retrospective study, some patients experienced benefit with as many as four consecutive trastuzumab-containing regimens (37). Also, a randomized clinical trial of women with HER-2 -positive BC that progressed after previous trastuzumab therapy indicated that combination therapy with trastuzumab plus capecitabine provided significant benefit compared with capecitabine alone (39). Although this phenomenon may be explained by an additive action of trastuzumab and chemotherapy, further prospective randomized studies are needed to evaluate the clinical benefits of continuing trastuzumab beyond progression *vs* switching to a non-trastuzumab-containing regimen.

Switching HER-2-targeted therapy

Another strategy for patients with trastuzumab-refractory disease is to switch to a different HER-2-targeted therapy. In a phase III study of women with HER-2-positive metastatic breast cancer that progressed on trastuzumab, combination therapy with capecitabine and lapatinib, substantially extended the time to progression by 4 months over capecitabine alone (8.4 months *vs* 4.4 months; $P < 0.001$) (41). Of note, multityrosine kinase inhibitors may be effective for women who overexpress p95HER-2. Expression of p95 HER-2 leads to *de novo* trastuzumab resistance (42-44) and, thus, poorer clinical outcomes (45), because the truncated protein lacks the extracellular domain required for trastuzumab binding. The binding of lapatinib to the intracellular domain of HER-2 (46) allows it to inhibit both full-length HER-2 and truncated p95HER-2 (44). Indeed, in a pooled analysis, lapatinib as monotherapy or in combination with capecitabine provided the same clinical benefit in patients who did and did not express p95HER-2 (47).
The novel antibody-drug conjugate T-DM1, may also provide some promise in patients who have developed resistance to trastuzumab alone. Recently, results from a randomised phase-II trial of T-DM1 *vs* trastuzumab/docetaxel in first-line, HER-2-positive MBC were presented by Perez et al. (48). In 137 patients with a median follow-up of 6 months, single-agent T-DM1 achieved an objective RR of 47.8%, as compared with 41.4% for the trastuzumab/docetaxel arm. A recent update of these data also demonstrated a significant increase in investigator-reported PFS with T-DM1 compared with the control arm (14.2 *vs* 9.2 months, respectively). Preliminary data from a single-arm phase-Ib/II trial evaluating the combination of pertuzumab and T-DM1 in patients with previously untreated (n=21) and relapsed (n=46) HER-2-positive MBC showed a RR of 57.1% in previously untreated patients [majority had received trastuzumab (86%), taxanes (71%) and anthracyclines (62%) in the adjuvant setting] and a RR of 34.8% in patients with relapsed disease (49). T-DM1 plus pertuzumab appeared

to be well-tolerated overall, although cardiotoxicity was observed with LVEF declines in two patients. The results of large global phase-III trials of T-DM1 (EMILIA; NCT00829166), including T-DM1 *vs* lapatinib plus capecitabine in 991 patients previously treated with a taxane and trastuzumab, showed a median PFS of 9.6 months with T-DM1 *vs* 6.4 months with lapatinib plus capecitabine [hazard ratio for progression or death from any cause, 0.65; 95% confidence interval (CI), 0.55 to 0.77; P<0.001], and median OS at the second interim analysis crossed the stopping boundary for efficacy (30.9 months *vs* 25.1 months; hazard ratio for death from any cause, 0.68; 95% CI, 0.55 to 0.85; P<0.001). The ORR was higher with T-DM1 (43.6%, *vs* 30.8% with lapatinib plus capecitabine; P<0.001); results for all additional secondary end points favored T-DM1 (50). It is believed that T-DM1 will play a role in the management of patients with advanced and early stage HER-2-positive breast cancer, but this awaits further study. Especially the ongoing phase III trials, MARIANNE (a three-arm trial evaluating T-DM1 *vs* T-DM1/pertuzumab *vs* trastuzumab/taxane in the first-line setting, NCT011 20184) (51) and THERESA (T-DM1 in comparison with treatment of physician's choice in patients with HER-2-positive MBC who have received two prior regimens of HER-2-directed therapy, NCT01419197) (52) will further give information the place of T-DM1 in the tretament algorithms for HER-2-positive disease. The trials of T-DM1 as a single agent and in combination with other chemotherapies have shown clinical activity and a favorable safety profile in patients with HER-2-positive metastatic breast cancer. There are ongoing studies with T-DM1 increasing tendency towards moving the study of these agents to earlier stages of the HER-2-positive breast cancer (Tab.1) (53).

Combining HER-2 inhibitors

Recent data suggest that dual HER-2 inhibition provides the clinical benefit in trastuzumab-resistant disease. In a phase III study of patients with metastatic breast cancer exposed to a median of three previous trastuzumab-containing regimens, treatment with trastuzumab plus lapatinib significantly improved median PFS compared with lapatinib alone (12.0 weeks *vs* 8.1 weeks; P = 0.008) without compromising safety (54). Importantly, data thus far only suggest a trend to overall survival improvement (51.6 *vs* 39.0 weeks; HR=0.75; *P*=0.106). Interim results of a phase-II trial evaluating the combination of lapatinib and trastuzumab in patients with HER-2-positive MBC [cohort 1 (*n*=40): no prior lapatinib, trastuzumab or chemotherapy for metastatic disease and >1 year since adjuvant trastuzumab, if received; cohort 2 (*n*=47): one to two prior lines of chemotherapy, including trastuzumab, or relapse within 1 year of adjuvant trastuzumab] were recently presented and showed objective RRs of 41.7% and 25% in cohorts 1 and 2, respectively (55). In phase II and III studies, the developmental humanized monoclonal antibody pertuzumab, shows promising efficacy when added to

Table 1 - Featured Ongoing Trials of T-DM1.

Trial Name	Phase	Patient Selection	Treatment	Endpoints
MARIANNE NCT01120184 (N=1095)	III	First-line with HER-2+ mBC or LABC	Randomize 1:1:1 to 3 arms T + taxane (open-label) vs T-DM1 + P (blinded) vs T-DM1 (blinded)	Primary: PFS + AEs Secondary: OS, ORR, DOR, TTF, CBR
THERESA NCT01419197 (N=600)	III	Previously treated with HER-2-targeted therapy (≥ 2 lines) HER-2+ mBC or LABC	Randomized, open-label T-DM1 *vs* TPC	Primary: PFS, OS Secondary: ORR, CBR, DOR, AEs
NCT00928330 (N=57)	I	T-pretreated pts with HER-2+ mBC or LABC	Three-arm GDC-0941 (PI3K inhibitor) + T-DM1 GDC-0941 + T GDC-0941 alone	Primary: Toxicity, AEs Secondary: PK, PFS, ORR, DOR
NCT 00934856 (N=50)	I	HER-2+ mBC or LABC	Nonrandomized, open-label T-DM1 + D +/- P	Primary: DLT, AEs Secondary: PFS, ORR, CBR, DOR, TTF, PK
NCT 00951665 (N=74)	Ib/IIa	HER-2+ mBC or LABC	Single-arm, open-label T-DM1 + Pac +/- P	Primary: AEs, PK, DLT Secondary: ORR, PFS, CBR, DOR
Approved, not yet active (N=500)	II	Early-stage, small HER-2+ BC	Non-randomized 3:1: T-DM1 *vs* Pac + T	Primary: Toxicity, DFS

T-DM1 = trastuzumab emtansine; PFS = progression-free survival; mBC = metastatic breast cancer; T = trastuzumab; AEs = adverse events; LABC = locally advanced breast cancer; P = pertuzumab; OS = overall survival; ORR = objective response rate; DOR = duration of response; TPC = treatment of physician's choice; CBR = clinical benefit rate; pts = patients; DLT = dose-limiting toxicity; D = docetaxel; Pac = paclitaxel; BC = breast cancer; DFS = disease-free survival; TTF = time to treatment failure; PK = pharmacokinetics.

trastuzumab in several different settings (56-59). As shown in cohort 3 of the BO17929 study, combination therapy with trastuzumab and pertuzumab provided the clinical benefit to patients who progressed after sequential trastuzumab and pertuzumab monotherapy (59). In the phase III Clinical Evaluation of Pertuzumab and Trastuzumab study of patients with HER-2-positive metastatic breast cancer, first-line combination therapy with trastuzumab plus pertuzumab and docetaxel prolonged median PFS by 6.1 months compared with trastuzumab and docetaxel alone (18.5 months *vs* 12.4 months; P < 0.001) (56). Ongoing trials are currently evaluating combinations of pertuzumab with trastuzumab and chemotherapy in HER-2-positive MBC, including a phase-II trial of trastuzumab/capecitabine±pertuzumab in the second-line setting (PHEREXA; NCT01026142) (60) and a large global phase-III trial of trastuzumab/docetaxel±pertuzumab in the first-line setting (CLEOPATRA; NCT 00567190) where the preclinical data are very encouraging; the combination of the anti-HER-2 monoclonal antibodies pertuzumab and trastuzumab with docetaxel as first-line therapy prolonged PFS in patients with HER-2-positive metastatic breast cancer. The increase of 6 months in PFS is striking. Overall survival data are event driven and are estimated to be released in 2013 (61, 62). Combinations of pertuzumab with T-DM1 (discussed before) are also under investigation in multiple phase-I–III studies (49).

HER family targeting drugs

Monoclonal Antibodies

Pertuzumab (Perjeta) is a monoclonal antibody, similar to trastuzumab. It is the latest drug to be approved for the treatment of HER-2 positive MBC and receveid its approval from FDA on June 2012. HER-2 is the preferred binding partner for dimerization for all the EGFR/ErbB receptor family members (EGFR/HER1, HER3 and HER4). By binding to and inhibiting HER-2, pertuzumab inhibits the potential dimerization of HER 2 with all other family receptors, resulting in the inhibition of growth (63-66). Preclinical studies in HER-2-positive breast cancer have demonstrated promising antitumors efficacy with associated down regulation of PI3K/Akt and MAPK signaling pathways both as a single-agent and synergistically with trastuzumab (67, 68).

T-DM1: the monoclonal antibody trastuzumab is conjugated to an anti-microtubule agent (emtansine). In addition to potently inhibiting microtubule assembly, T-DM 1 also appears to flag HER-2-positive cells for cytotoxic destruction by antibodies (69, 70). In a phase II study, intravenous T-DM 1 showed robust activity in 112 patients with heavily pretreated, trastuzumab resistant HER-2-positive breast cancer, recording an objective response rate of 25.9%, and median progression-free survival (PFS) time of 4.6 months. T-DM1 was well tolerated, with grade 3 or 4 hypokalemia (8.9%) and thrombocytopenia (8%) being the most common (72). In a second phase II study of 110 extensively pretreated patients, T-DM1 treatment provided an overall response rate of 33%, and a clinical benefit rate of 48%. The median PFS time was 6.9 months (71). These data suggest that an anti-HER-2 antibody may retain the capacity to interact with HER-2 in a clinically meaningful way, even after the development of resistance, and indicate that conjugated agents such as T-DM 1 may provide a new treatment alternative for those who have previously progressed on native trastuzumab.

HER-2 family TKIS

Lapatinib (Tyverb): similar to trastuzumab is also a drug that targets HER-2; however, it not an antibody. Chemically, it is an oral small molecule derivative of 4-anilinoquinazolin. Also it does not target HER-2 alone but is rather known to inhibit to tyrosine kinase activity of HER-2 as well as EGFR (73, 74). It was approved by FDA in 2007 for clinical use with capecitabine in combination therapy for MBC patients (75-77). In 2010, it was approved for treatment of postmenopausal women with hormone receptor positive, HER-2 over exespressing MBC (78). The mode of action of lapatinib involves targeting of C-terminus tyrosine kinase domain of target HER-2/EGFR where it binds to ATP binding site, resulting in the inhibition of phosphorylation and subsequent activation of downstream intracellular signalling pathway. Lapatinib has shown promise against trastuzumab-resistance cell (79, 80), which is of interest to clinicians dealing with the drug resistance associated with trastuzumab.

VEGFR TKIs

Sunitinib (Sutent; Pfizer) is an oral, multitargeted TKI against VEGFR, platelet-derived growth factor receptor and stem cell factor receptor (c-kit). In an open-label phase-II study of sunitinib monotherapy in patients with MBC previously treated with taxanes and anthracyclines, an overall RR of 11% was observed (81). However, no correlation between clinical response and ER or HER-2 status was found. At present, two early-phase-clinical trials are evaluating sunitinib/trastuzumab combinations in HER-2-positive breast cancer, including a phase-I trial of sunitinib plus trastuzumab and docetaxel in the first-line setting (82) and a phase-II trial of sunitinib plus trastuzumab in the second-line setting (83).

Pazopanib (Votrient; Glaxo Smith Kline) is an oral multitargeted TKI against VEGFR-1/2/3, platelet-derived growth factor receptor and c-kit. In a randomised phase-II study of pazopanib (400 mg per day) plus lapatinib (1000 mg per day) *vs* lapatinib alone (1500 mg per day) in HER-2-positive, locally advanced or MBC in the first-line setting, an interim analysis of 114 evaluable patients (total n ¼ 141) demonstrated modest efficacy with the dual TKI approach (84). Pazopanib plus lapatinib yielded a 12-week progressive disease rate of 15.9% *vs* 36.8% for lapatinib monotherapy (by investigator assessment). A secondary endpoint of 12-week RR also favoured the combination arm at 44.9% *vs* 27.8% (by investigator assessment; 36.2% *vs* 22.2% by independent assessment). Notably, four patients experienced declines in LVEF (three asymptomatic and one symptomatic) with the combined anti-HER-2/VEGF strategy.

Being tested HER-2 targeting drugs:

Neratinib (HKI-272; Pfizer, New York, NY, USA) is an irreversible, oral small-molecule TKI of EGFR/HER1, HER-2 and HER4 (85). Early clinical data for use of the multityrosine kinase inhibitor neratinib in trastuzumab-refractory disease are also promising. An open-label, phase-II multicenter trial of single-agent neratinib in advanced HER-2-positive breast cancer, which enrolled both trastuzumab-refractory (*n*=66) and trastuzumab-naive (*n*=70) patients, demonstrated modest clinical activity in both cohorts (42). Objective RRs of 24% and 56% were seen in the trastuzumab-refractory and trastuzumab-naive groups, respectively, with a median PFS of 22.3 and 39.6 weeks. Currently, studies of single-agent neratinib (neratinib *vs* lapatinib/capecitabine, NCT00777101) and neratinib combinations (with capecitabine, NCT00741260; trastuzumab, NCT00398567; paclitaxel, NCT00445458; vinorelbine, NCT00706030; and neratinib/paclitaxel *vs* trastuzumab/paclitaxel, NCT00915018) are under evaluation in HER-2-positive MBC (86-91).

Afatinib: (BIBW 2992; Boehringer Ingelheim, Ingelheim, Germany), an anilino-quinazoline-derived irreversible, oral small-molecule ErbB family TKI (EGFR/HER1, HER-2 and HER4), has also demonstrated activity in early-phase trials of advanced solid tumours and trastuzumab-refractory HER-2-positive breast cancer (92-94). An open-label, single-arm phase-II study of afatinib in 41 patients with HER-2-positive MBC following trastuzumab failure demonstrated partial responses in 4 patients and stable disease in 8 patients (maintained for at least four cycles) (92). A global phase-III trial of afatinib in HER-2-positive MBC was initiated (LUX-Breast 1; NCT01125566), which is evaluating vinorelbine/afatinib *vs* vinorelbine/trastuzumab in patients with prior trastuzumab therapy (95).

Future strategies for overcoming HER-2 resistance
There are several agents targeted against other pathways and molecules implicated in HER-2 resistance that are in various stages of clinical development. Aside from PI3K/Akt/mTOR inhibitors, other agents currently being investigated in clinical trials of HER-2 -resistant breast cancer include inhibitors of IGFR1, HSP90, and telomerase.

IGF-1R Inhibitors: the rationale for assessing IGF1R inhibition in HER-2-resistant breast cancer is the hypothesis that cross-talk between IGF1R and HER-2 may occur in breast cancer cells, leading to receptor heterodimerization, which may, in turn, allow cells to escape trastuzumab cytotoxicity (96). In preclinical studies, overexpression of IGF1R led to trastuzumab resistance (97), and the addition of an IGF1R inhibitor to trastuzumab resulted in greater cell death than treatment with trastuzumab alone. Currently, there are no clinical data on the efficacy and safety of IGF1R inhibition in HER-2 -resistant breast cancer. There are, however, ongoing trials of the IGF1R inhibitors BMS-754 807, cixutumumab, and OSI-9 06.

HSP90 Inhibitors: a novel therapeutic approach involves targeting the hsp90 molecular chaperone, whose function includes regulating the stability and maturation of various oncoproteins including HER (98). Preclinical data have shown that HER-2 is chaperoned by HSP90, suggesting that inhibition of HSP90 should be evaluated in HER-2-positive breast cancer patients. Tanespimycin (17-AAG, KOS-953; Bristol-Myers Squibb, New York, NY, USA), a first-generation geldanamycin derivative, has demonstrated robust antitumor activity in preclinical models of HER-2-positive breast cancer (99, 100). A phase-I study of tanespimycin plus trastuzumab was encouraging, and antitumor activity was observed in patients with HER-2-positive MBC (101). In a subsequent single-arm phase-II trial of tanespimycin (IV weekly) plus trastuzumab in patients with HER-2-positive MBC and disease progression following trastuzumab, an overall RR of 22% and CBR of 59% were reported (102). Tanespimycin was well-tolerated overall, with diarrhoea, fatigue, nausea and headache as the most common toxicities. Although further clinical development of tanespimycin has been halted, other hsp90 inhibitors, including retaspimycin (IPI-504; Infinity Pharmaceuticals, Cambridge, MA, USA) and AUY922 (Novartis, Cambridge, MA, USA) are currently under evaluation in early-phase clinical trials as single agents or in combination with trastuzumab (103-105).
Telomerase expression is essential for cellular proliferation, and telomerase overexpression has been linked to tumorigenesis. Inhibition of telomerase ultimately results in apoptosis or cell senescence, making it a potential target for anticancer therapy. In a trastuzumab-resistant cell line, the telomerase inhibitor GRN163L was able to restore trastuzumab sensitivity, suggesting that telomerase inhibition may be a possible strategy for overcoming HER-2 resistance in breast cancer. Although no clinical data currently exist, a trial of GRN163L in women with trastuzumab- resistant breast cancer is ongoing (106).

Discussion

Trastuzumab has provided significant clinical benefit in patients with HER-2-positive metastatic breast cancer, but *de novo* and acquired resistance is the major problem to be overcome and we needed to develop a novel treatment strategies or new combination drugs. All experimental drugs demonstrated modest clinical activity in this setting and highlights the importance of ongoing HER-2 blockade in trastuzumab-refractory states.
An alternative very interesting approach is the use of novel antibody-drug conjugates such as trastuzumab-emtansine, which recently demonstrated activity in MBC. T-DM1 is considered a “Miracle Drug” for advanced BC: almost half of the women in the trials (45 percent) had a positive response, which was defined essentially as a halt to tumor growth. Furthermore this was a population that had run out of options and whose tumors had not responded to previous drugs.
HER-2-positive patients with metastatic disease have another very powerful therapy that offers a real hope for prolonged disease control with less toxicity. We are still learning how to properly use this agent and the setting in which it might best be used.
Ongoing studies are anticipated to determine the efficacy of T-DM1 in combination with other HER-2-targeted agents, in combination with chemotherapy and in early-stage disease.
Especially the two ongoing phase III trials, MARIANNE, and THERESA, will give us further information about the

place of T-DM1 in the treatment algorithms for HER-2-positive disease.
There are multiple effective therapies for patients with HER-2-positive breast cancer and it is quite likely that this new drug will be available in the near future.
Soon as it will be possible the use in clinical practice of the new drugs, the choice of anti-HER-2 targeted therapy in metastatic BC could be as follows:

- In first-line setting the association of trastuzumab plus pertuzumab and chemotherapy;
- In second-line the use of T-DM1;
- In third-line the association of lapatinib and capecitabine;
- In fourth-line also the use of trastuzumab plus chemotherapy.

The challenge now is how to define the better sequence of such treatments and just how much is "enough" for each patient. HER-2-positive BC research is a rapidly evolving field, and from now there is more treatment choices that have permissed a better outcome to the patients.

References

1. Slamon DJ, Clark GM, Wong SG, Levin WJ, Ullrich A, McGuire WL. Human breast cancer: correlation of relapse and survival with amplification of the HER2-2/neu oncogene. Science 1987; 235:177-182.
2. Slamon DJ, Godolphin W, Jones LA, et al. Studies of the HER-2/neu proto-oncogene in human breast and ovarian cancer. Science 1989; 244:707-712.
3. Seshadri R, Firgaira FA, Horsfall DJ, McCaul K, Setlur V, Kitchen P. Clinical significance of HER-2/neu oncogene amplification in primary breast cancer. J Clin Oncol 1993; 11:1936-1942.
4. Press MF, Pike MC, Chazin VR, et al. HER-2/neu expression in node-negative breast cancer: direct tissue quantitation by computerized image analysis and association of overexpression with increased risk of recurrent disease. Cancer Res 1993; 53:4960-4970.
5. Ravdin PM, Chamness GC. The c-erbB-2 proto-oncogene as a prognostic and predictive marker in breast cancer: a paradigm for the development of other macromolecular markers - a review. Gene 1995; 159:19-27.
6. Hudziak RM, Schlessinger J, Ulrich A. Increased expression of the putative growth factor receptor p185HER2 causes transformation and tumorigenesis of NIH 3T3 cells. Proc Natl Acad Sci USA 1987; 84:7159-7163.
7. Di Fiore PP, Pierce JH, Krasu MH, Segatto O, King CR, Aaronson SA. ErbB-2 is a potent oncogene when overexpressed in NIH/3T3 cells. Science 1987; 237:178-182.
8. Guy CT, Webster MA, Schaller M, Parsons TJ, Cardiff RD, Muller WJ. Expression of the neu protooncogene in the mammary epithelium of transgenic mice induces metastatic disease. Proc Natl Acad Sci USA 1992; 89:10578-10582.
9. Chazin VR, Kaleko M, Miller AD, Slamon DJ. Transformation mediated by the human HER-2 gene independent of the epidermal growth factor receptor. Oncogene 1992; 7:1859-1866.
10. Pietras RJ, Arboleda J, Reese DM, et al. HER-2 tyrosine kinase pathway targets estrogen receptor and promotes hormone-independent growth in human breast cancer cells. Oncogene 1995; 10:2435-2446.
11. Hudziak RM, Lewis GD, Winget M, Fendly BM, Shepard HM, Ullrich A. p185HER2 Monoclonal antibody has antiproliferative effects in vitro and sensitizes human breast tumor cells to tumor necrosis factor. Mol Cell Biol1989; 9:1165-1172.
12. Shepard HM, Lewis GD, Sarup JC, et al. Monoclonal antibody therapy of human cancer: taking the HER2 protooncogene to the clinic. J Clin Immunol 1991; 11:117-127.
13. Pietras RJ, Fendly BM, Chazin VR, Pegram MD, Howell SB, Slamon DJ. Antibody to HER-2/neu receptor blocks DNA repair after cisplatin in human breast and ovarian cancer cells. Oncogene 1994; 9:1829-1838.
14. Carter P, Presta L, Gorman CM, et al. Humanization of an anti-p185HER2 antibody for human cancer therapy. Proc Natl Acad Sci U S A 1992; 89:4285-4289.
15. Pietras RJ, Pegram MD, Finn RS, Maneval DA, Slamon DJ. Remission of human breast cancer xenografts on therapy with humanized monoclonal antibody to HER-2 receptor and DNA-reactive drugs. Oncogene 1998; 17:2235-2249.
16. Pegram M, Hsu S, Lewis G, et al. Inhibitory effects of combinations of HER-2/neu antibody and chemotherapeutic agents used for treatment of human breast cancers. Oncogene 1999; 18:2241-2251.
17. Konecny G, Pegram MD, Beryt M, et al. Therapeutic advantage of chemotherapy drugs in combination with Herceptin against human breast cancer cells with HER-2/neu overexpression. Breast Cancer Res Treat 1999; 57:114-114.
18. Pietras RJ, Poen JC, Gallardo D, Wongvipat PN, Lee HJ, Slamon DJ. Monoclonal antibody to HER-2/neu receptor modulates repair of radiation-induced DNA damage and enhances radiosensitivity of human breast cancer cells overexpressing this oncogene. Cancer Res 1999; 59:1347-1355.
19. Baselga J, Norton L, Albanell J, Kim YM, Mendelsohn J. Recombinant humanized anti-HER2 antibody (Herceptin) enhances the antitumor activity of paclitaxel and doxorubicin against HER-2/neu overexpressing human breast cancer xenografts. Cancer Res 1998; 58:2825-2831 [Erratum, Cancer Res 1999; 59:2020.].
20. Baselga J, Tripathy D, Mendelsohn J, et al. Phase II study of weekly intravenous recombinant humanized anti-p185HER2 monoclonal antibody in patients with HER2/neu-overexpressing metastatic breast cancer. J Clin Oncol 1996; 14:737-744.
21. Cobleigh MA, Vogel CL, Tripathy D, et al. Multinational study of the efficacy and safety of humanized anti-HER-2 monoclonal antibody in women who have HER2-overexpressing metastatic breast cancer that has progressed after chemotherapy for metastatic disease. J Clin Oncol 1999; 17:2639-2648.
22. Pegram MD, Lipton A, Hayes DF, et al. Phase II study of receptor-enhanced chemosensitivity using

recombinant humanized anti-p185HER2/neu monoclonal antibody plus cisplatin in patients with HER-2/neu-overexpressing metastatic breast cancer refractory to chemotherapy treatment. J Clin Oncol 1998; 16:2659-2671.
23. Smith G, Henderson JC. New treatments for breast cancer. Semin Oncol 1996; 23:506-528.
24. Fossati R, Confalonieri C, Torri V, et al. Cytotoxic and hormonal treatment for metastatic breast cancer: a systematic review of published randomized trials involving 31,510 women. J Clin Oncol 1998; 16:3439-3460.
25. Slamon DJ, Leyland-Jones B, Shak S, et al. Use of chemotherapy plus a monoclonal antibody against HER2 for metastatic breast cancer that overexpresses HER2. N Engl J Med 2001; 344:783-792.
26. Ross JS, Slodkowska EA, Symmans WF, et al. The HER-2 receptor and breast cancer: ten years of targeted anti-HER-2 therapy and personalized medicine. Oncologist 2009; 14:320-368.
27. Aebi S, Davidson T, Gruber G, et al. Primary breast cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol 2010; 21(Suppl 5):v9-v14.
28. Cardoso F, Senkus-Konefka E, Fallowfield L, et al. Locally recurrent or metastatic breast cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol 2010; 21(Suppl 5):v15-v19.
29. National Comprehensive Cancer Network. NCCN Clinical Practice Guidelines in Oncology™: Breast Cancer. Fort Washington, PA; 2011.
30. Dahabreh IJ, Linardou H, Siannis F, et al. Trastuzumab in the adjuvant treatment of early-stage breast cancer: a systematic review and meta-analysis of randomized controlled trials. Oncologist 2008; 13:620-630.
31. Hubalek M, Brunner C, Mattha K, et al. Resistance to HER2-targeted therapy: mechanisms of trastuzumab resistance and possible strategies to overcome unresponsiveness to treatment. Wien Klin Wochenschr 2010; 160:506-512.
32. Pohlmann PR, Mayer IA, Mernaugh R. Resistance to Trastuzumab in Breast Cancer. Clin Cancer Res 2009; 15:7479-7491.
33. Ross JS, Slodkowska EA, Symmans WF, et al. The HER-2 receptor and breast cancer: ten years of targeted anti-HER-2 therapy and personalized medicine. Oncologist 2009; 14:320-368.
34. Muthuswamy SK. Trastuzumab resistance: all roads lead to SRC. Nat Med 2011; 17:416-418.
35. Hubalek M, Brunner C, Attha K, et al. Resistance to HER2-targeted therapy: mechanisms of trastuzumab resistance and possible strategies to overcome unresponsiveness to treatment. Wien Klin Wochenschr 2010; 160: 506-512.
36. Gelmon KA, Mackey J, Verma S, et al. Use of trastuzumab beyond disease progression: observations from a retrospective review of case histories. Clin Breast Cancer 2004; 5: 52-58.
37. Garcia-Saenz JA, Martin M, Puente, J et al. Trastuzumab associated with successive cytotoxic therapies beyond disease progression in metastatic breast cancer. Clin Breast Cancer 2005; 6:325-329.
38. Fabi A, Metro G, Ferretti G, et al. Do HER-2 positive metastatic breast cancer patients benefit from the use of trastuzumab beyond disease progression? A mono-institutional experience and systematic review of observational studies. Breast 2008; 17:499-505.
39. von Minckwitz G, du Bois A, Schmidt M, et al. Trastuzumab beyond progression in human epidermal growth factor receptor 2-positive advanced breast cancer: a german breast group 26/breast international group 03–05 study. J Clin Oncol 2009; 27:1999-2006.
40. Mannocci A, De Feo E, de Waure C, et al. Use of trastuzumab in HER2-positive metastatic breast cancer beyond disease progression: a systematic review of published studies. Tumori 2010; 96: 385-391.
41. Geyer CE, Forster J, Lindquist D, et al. Lapatinib plus capecitabine for HER2-positive advanced breast cancer. N Engl J Med 2006; 355: 2733-2743.
42. Burstein HJ, Sun Y, Dirix LY, et al. Neratinib, an irreversible ErbB receptor tyrosine kinase inhibitor, in patients with advanced ErbB2-positive breast cancer. J Clin Oncol 2010; 28: 1301-1307.
43. Chandarlapaty S, Scaltriti M, Angelini P, et al. Inhibitors of HSP90 block p95-HER2 signaling in Trastuzumab-resistant tumors and suppress their growth. Oncogene 2010; 29:325-334.
44. Scaltriti M, Rojo F, Ocana A, et al. Expression of p95HER2, a truncated form of the HER2 receptor, and response to anti-HER2 therapies in breast cancer. J Natl Cancer Inst 2007; 99:628-638.
45. Sperinde J, Jin X, Banerjee J, et al. Quantitation of p95HER2 in paraffin sections by using a p95-specific antibody and correlation with outcome in a cohort of trastuzumab-treated breast cancer patients. Clin Cancer Res 2010; 16:4226-4235.
46. Wood ER, Truesdale AT, McDonald OB, et al. A unique structure for epidermal growth factor receptor bound to GW572016 (Lapatinib): relationships among protein conformation, inhibitor off-rate, and receptor activity in tumor cells. Cancer Res 2004; 64: 6652-6659.
47. Scaltriti M, Chandarlapaty S, Prudkin L, et al. Clinical benefit of lapatinib-based therapy in patients with human epidermal growth factor receptor 2-positive breast tumors coexpressing the truncated p95HER2 receptor. Clin Cancer Res 2010; 16:2688-2695.
48. Perez EA, Dirix L, Kocsis J, Gianni L, Lu J, Vinholes J, Ng V, Linehan C, Agresta S, Hurvitz S. Efficacy and safety of trastuzumab-DM1 vs trastuzumab plus docetaxel in HER2-positive metastatic breast cancer patients with no prior chemotherapy for metastatic disease: preliminary results of a randomized, multicenter, open-label phase 2 study (TDM4450G) Ann Oncol 2010; 21viii2Abstract LBA3.
49. Dieras V, Harbeck N, Albain K, Burris H, Awada A, Crivellari D, Andre F, Choi YJ, Huang J, Miller KD. 2010. A phase Ib/II trial of trastuzumab-DM1 with pertuzumab for patients with HER2-positive, locally advanced or metastatic breast cancer: interim efficacy and safety results Presented at: 33rd Annual San Antonio Breast Cancer Symposium December 8-12, 2010; San Antonio, TX. Abstract P3-14-01.
50. Verma S, et al. Trastuzumab Emtansine for HER2-Positive Advanced Breast Cancer. N Engl J Med 367;19 nejm.org november 8, 2012.

51. A Study of Trastuzumab Emtansine (T-DM1) Plus Pertuzumab/Pertuzumab Placebo Versus Trastuzumab [Herceptin] Plus a Taxane in Patients With Metastatic Breast Cancer (MARIANNE) (ClinicalTrials.gov Identifier: NCT01120184).
52. A Study of Trastuzumab Emtansine in Comparison With Treatment of Physician's Choice in Patients With HER2-Positive Breast Cancer Who Have Received at Least Two Prior Regimens of HER2-Directed Therapy (TH3RESA) (ClinicalTrials.gov Identifier: NCT01419197).
53. Diéras V, Harbeck N, Albain K, et al. A phase Ib/II trial of trastuzumab-DM1 with pertuzumab for patients with HER2-positive, locally advanced or metastatic breast cancer: interim efficacy and safety results. Cancer Res. 2011;70(24 supplement):P3-14-01.
54. Blackwell KL, Burstein HJ, Storniolo AM, et al. Randomized study of lapatinib alone or in combination with trastuzumab in women with ErbB2-positive, trastuzumabrefractory metastatic breast cancer. J Clin Oncol 2010; 28:1124-1130-
55. Lin NU, Mayer IA, Najita JS, Hobday TJ, Falkson CI, Dees EC, Rimawi MF, Nanda R, Gelman RS, Josephs K, Richardson A, Flores L, Van den Abbeele AD, Yap JT, Arteaga CL, Wolff AC, Krop IE, Winer EP. 2011. TBCRC 003: Phase II trial of trastuzumab (T) and lapatinib (L) in patients (pts) with HER2+ metastatic breast cancer (MBC) J Clin Oncol 29 Abstract 527.
56. Baselga J, Cortes J, Kim SB, et al. Pertuzumab plus trastuzumab plus docetaxel for metastatic breast cancer. N Engl J Med 2012; 366: 109-119.
57. Schneeweiss A, Chia S, Hickish T, et al. Neoadjuvant pertuzumab and trastuzumab concurrent or sequential with an anthracycline-containing or concurrent with an anthracycline-free standard regimen: a randomized phase II study (TRYPHAENA). Presented at the 34th Annual San Antonio Breast Cancer Symposium, December 6-10, 2011, San Antonio, TX.
58. Gianni L, Pienkowski T, Im YH, et al. Efficacy and safety of neoadjuvant pertuzumab and trastuzumab in women with locally advanced, inflammatory, or early HER2-positive breast cancer (NeoSphere): a randomised multicentre, openlabel, phase 2 trial. Lancet Oncol 2012; 13:25-32.
59. Cortes J, Fumoleau P, Bianchi GV, et al. Pertuzumab monotherapy after trastuzumab-based treatment and subsequent reintroduction of trastuzumab: activity and tolerability in patients with advanced human epidermal growth factor receptor 2-positive breast cancer. J Clin Oncol 2012; 30:1594-1600.
60. A Study of a Combination of Trastuzumab and Capecitabine With or Without Pertuzumab in Patients With HER2-positive Metastatic Breast Cancer (PHEREXA) (ClinicalTrials.gov Identifier: NCT01026142).
61. A Study to Evaluate Pertuzumab + Trastuzumab + Docetaxel vs Placebo + Trastuzumab + Docetaxel in Previously Untreated HER2-positive Metastatic Breast Cancer (CLEOPATRA) (ClinicalTrials.gov Identifier: NCT00567190).
62. Baselga J, Cortés J, Kim SB, et al. Pertuzumab Plus Trastuzumab Plus Docetaxel for Metastatic Breast Cancer *N Engl J Med*. 2011 Dec 7.
63. Agus DB, Akita RW, Fox WD, Lewis GD, Higgins B, Pisacane PI, Lofgren JA, Tindell C, Evans DP, Maiese K, Scher HI, Sliwkowski MX. Targeting ligand-activated ErbB2 signaling inhibits breast and prostate tumor growth. Cancer Cell 2002; 2:127-137.
64. Nahta R, Hung MC, Esteva FJ. The HER-2-targeting antibodies trastuzumab and pertuzumab synergistically inhibit the survival of breast cancer cells. Cancer Res 2004;64: 2343-2346.
65. Franklin MC, Carey KD, Vajdos FF, Leahy DJ, de Vos AM, Sliwkowski MX. Insights into ErbB signaling from the structure of the ErbB2-pertuzumab complex. Cancer Cell 2004; 5:317-328.
66. Scheuer W, Friess T, Burtscher H, Bossenmaier B, Endl J, Hasmann M. Strongly enhanced antitumor activity of trastuzumab and pertuzumab combination treatment on HER2-positive human xenograft tumor models. Cancer Res 2009; 69:9330-9336.
67. Portera CC, Walshe JM, Rosing DR, Denduluri N, Berman AW, Vatas U, Velarde M, Chow CK, Steinberg SM, Nguyen D, Yang SX, Swain SM. Cardiac toxicity and efficacy of trastuzumab combined with pertuzumab in patients with [corrected] human epidermal growth factor receptor 2-positive metastatic breast cancer. Clin Cancer Res 2008; 14: 2710-2716.
68. Baselga J, et al. Phase II trial of pertuzumab and trastuzumab in patients with human epidermal growth factor receptor 2-positive metastatic breast cancer that progressed during prior trastuzumab therapy. J Clin Oncol 2010 Mar 1; 28(7):1138-44.
69. Lewis Phillips GD, Li G, Dugger DL, Crocker LM, Parsons KL, Mai E, Blättler WA, Lambert JM, Chari RV, Lutz RJ, Wong WL, Jacobson FS, Koeppen H, Schwall RH, Kenkare-Mitra SR, Spencer SD, Sliwkowski MX. Targeting HER2-positive breast cancer with trastuzumab-DM1, an antibody-cytotoxic drug conjugate. Cancer Res 2008 Nov 15; 68(22):9280-9290.
70. Junttila TT, Li G, Parsons K, Phillips GL, Sliwkowski MX. Trastuzumab-DM1 (T-DM1) retains all the mechanisms of action of trastuzumab and efficiently inhibits growth of lapatinib insensitive breast cancer. Breast Cancer Res Treat 2011 Jul; 128(2):347-56.
71. Krop I, Lo Russo P, Miller KD, Modi S, Yardley D, Rodriguez G, Agresta S, Zheng M, Amler L, Rugo H. A phase II study of trastuzumab-DM1 (T-DM1), a novel HER2 antibody-drug conjugate, in patients with HER2+ metastatic breast cancer who were previously treated with an anthracycline, a taxane, capecitabine, lapatinib, and trastuzumab Presented at: the Annual CTRC-AACR San Antonio Breast Cancer SymposiumDecember 9-13, 2009; San Antonio, TX. Abstract 5090.
72. Burris HA 3rd, Rugo HS, Vukelja SJ, Vogel CL, Borson RA, Limentani S, Tan-Chiu E, Krop IE, Michaelson RA, Girish S, Amler L, Zheng M, Chu YW, Klencke B, O'Shaughnessy JA. Phase II study of the antibody drug conjugate trastuzumab-DM1 for the treatment of human epidermal growth factor receptor 2 (HER2)-positive breast cancer after prior HER2-directed therapy. J Clin Oncol 2011 Feb 1; 29(4):398-405.

73. Rusnak DW, Lackey K, Affleck K, Wood ER, Alligood KJ, Rhodes N, Keith BR, Murray DM, Knight WB, Mullin RJ, Gilmer TM. The effects of the novel, reversible epidermal growth factor receptor/ErbB-2 tyrosine kinase inhibitor, GW2016, on the growth of human normal and tumor-derived cell lines in vitro and in vivo. Mol Cancer Ther 2001 Dec; 1(2):85-94.
74. Konecny GE, Pegram MD, Venkatesan N, Finn R, Yang G, Rahmeh M, Untch M, Rusnak DW, Spehar G, Mullin RJ, Keith BR, Gilmer TM, Berger M, Podratz KC, Slamon DJ. Activity of the dual kinase inhibitor lapatinib (GW572016) against HER-2-overexpressing and trastuzumab-treated breast cancer cells. Cancer Res 2006 Feb 1; 66(3):1630-9.
75. Geyer CE, Forster J, Lindquist D, Chan S, Romieu CG, Pienkowski T, Jagiello-Gruszfeld A, Crown J, Chan A, Kaufman B, Skarlos D, Campone M, Davidson N, Berger M, Oliva C, Rubin SD, Stein S, Cameron D. Lapatinib plus capecitabine for HER2-positive advanced breast cancer. N Engl J Med 2006; 355:2733-2743.
76. Cameron D, Casey M, Press M, Lindquist D, Pienkowski T, Romieu CG, Chan S, Jagiello-Gruszfeld A, Kaufman B, Crown J, Chan A, Campone M, Viens P, Davidson N, Gorbounova V, Raats JI, Skarlos D, Newstat B, Roychowdhury D, Paoletti P, Oliva C, Rubin S, Stein S, Geyer CE. A phase III randomized comparison of lapatinib plus capecitabine versus capecitabine alone in women with advanced breast cancer that has progressed on trastuzumab: updated efficacy and biomarker analyses. Breast Cancer Res Treat 2008 Dec; 112(3):533-43.
77. Cameron D, Casey M, Oliva C, Newstat B, Imwalle B, Geyer CE. Lapatinib plus capecitabine in women with HER-2-positive advanced breast cancer: final survival analysis of a phase III randomized trial. Oncologist. 2010; 15(9):924-934.
78. Finn RS, Press M, Dering J, Florance A, Platek G, Arbushites M, Koehler M, Johnston S. Progression-free survival (PFS) of patients with HER2-negative estrogen-resepctor (ER)-low metastatic breast cancer (MBC) with the addition of lapatinib to letrozole: biomarker results of EGF30008 J Clin Oncol 2009a.
79. Blackwell KL, Burstein HJ, Storniolo AM, Rugo H, Sledge G, Koehler M, Ellis C, Casey M, Vukelja S, Bischoff J, Baselga J, O'Shaughnessy J. Randomized study of Lapatinib alone or in combination with trastuzumab in women with ErbB2-positive, trastuzumab-refractory metastatic breast cancer. J Clin Oncol 2010 Mar 1; 28(7):1124-1130.
80. Lin NU, Mayer IA, Najita JS, Hobday TJ, Falkson CI, Dees EC, Rimawi MF, Nanda R, Gelman RS, Josephs K, Richardson A, Flores L, Van den Abbeele AD, Yap JT, Arteaga CL, Wolff AC, Krop IE, Winer EP. 2011. TBCRC 003: Phase II trial of trastuzumab (T) and lapatinib (L) in patients (pts) with HER2+ metastatic breast cancer (MBC) J Clin Oncol 29Abstract 527.
81. Burstein HJ, Elias AD, Rugo HS, Cobleigh MA, Wolff AC, Eisenberg PD, Lehman M, Adams BJ, Bello CL, DePrimo SE, Baum CM, Miller KD. Phase II study of sunitinib malate, an oral multitargeted tyrosine kinase inhibitor, in patients with metastatic breast cancer previously treated with an anthracycline and a taxane. J Clin Oncol 2008 Apr 10; 26(11):1810-6. doi: 10.1200/JCO.2007.14.5375.
82. Study Of SU011248 In Combination With Docetaxel And Trastuzumab In Patients With Advanced Breast Cancer HER-2 Positive (Clinical Trials.gov I dentifier: NCT00372424).
83. Open Label Study Of SU011248 In Combination With Trastuzumab For Patients With Metastatic Breast Cancer (ClinicalTrials.gov Identifier: NCT00243503).
84. Slamon D, Gomez HL, Kabbinavar FF, Amit O, Richie M, Pandite L, Goodman V. Randomized study of pazopanib + lapatinib vs lapatinib alone in patients with HER2-positive advanced or metastatic breast cancer. J Clin Oncol 2008; 26: 45s. Abstract 1016 treated with an anthracycline and a taxane. J Clin Oncol 26: 1810-1816.
85. Rabindran SK, Discafani CM, Rosfjord EC, Baxter M, Floyd MB, Golas J, Hallett WA, Johnson BD, Nilakantan R, Overbeek E, Reich MF, Shen R, Shi X, Tsou HR, Wang YF, Wissner A. (2004) Antitumor activity of HKI-272, an orally active, irreversible inhibitor of the HER-2 tyrosine kinase. Cancer Res 64: 3958-3965.
86. Study Evaluating Neratinib Versus Lapatinib Plus Capecitabine For ErbB2 Positive Advanced Breast Cancer (ClinicalTrials.gov Identifier: NCT00777101).
87. Study Evaluating The Combination Of Neratinib And Capecitabine In Solid Tumors And Breast Cancer (ClinicalTrials.gov Identifier: NCT00741260).
88. A Phase 1/2 Study Of HKI-272 (Neratinib) in Combination With Trastuzumab (Herceptin) In Subjects With Advanced Breast Cancer (ClinicalTrials.gov Identifier: NCT00398567).
89. A Phase 1/2 Study of HKI-272 (Neratinib) in Combination With Paclitaxel (Taxol) in Subjects With Solid Tumors and Breast Cancer (Clinical Trials.gov Identifier: NCT00445458).
90. Study Evaluating Neratinib (HKI-272) In Combination With Vinorelbine In Subjects With Solid Tumors And Metastatic Breast Cancer (Clinical Trials.gov Identifier: NCT00706030).
91. Study Evaluating Neratinib Plus Paclitaxel VS Trastuzumab Plus Paclitaxel In ErbB-2 Positive Advanced Breast Cancer (NEFERTT) (Clinical Trials.gov Identifier: NCT00915018).
92. Hickish T, Wheatley D, Lin N, Carey LA, Houston S, Mendelson DS, Solca F, Uttenreuther-Fischer M, Winer E. Use of BIBW 2992, a novel irreversible EGFR/HER1 and HER2 tyrosine kinase inhibitor to treat patients with HER2-positive metastatic breast cancer after failure of treatment with trastuzumab. Cancer Res 2009; 69: 2191-2194. Abstract 5060.
93. Yap TA, Vidal L, Adam J, Stephens P, Spicer J, Shaw H, Ang J, Temple G, Bell S, Shahidi M, Uttenreuther-Fischer M, Stopfer P, Futreal A, Calvert H, de Bono JS, Plummer R. Phase I trial of the irreversible EGFR and HER2 kinase inhibitor BIBW 2992 in patients with advanced solid tumors. J Clin Oncol 2010; 28:3965-3972.
94. Yamamoto N, Katakami N, Atagi S, Hida T, Goto K, Horai T, Inoue A, Ichinose Y, Kobayashi K, Takeda K, Kiura K, Saka H, Tamura T, Okamoto I, Nogami N, Moringa R, Nishio K, Seki Y, Lorence R, Shahidi M. (2011) A phase II trial of afatinib (BIBW 2992) in patients (pts) with advanced non small cell lung

cancer previously treated with erlotinib or gefitinib. Poster presented at: the Annual Meeting of the American Society of Clinical Oncology, June 4-8, 2011; Chicago, IL. Abstract 7524.
95. LUX-Breast 1: BIBW 2992 (Afatinib) in HER2-positive Metastatic Breast Cancer Patients After One Prior Herceptin Treatment (ClinicalTrials.gov Identifier: NCT01125566).
96. Nahta R, Yuan LX, Zhang B, et al. Insulin-like growth factor-I receptor/human epidermal growth factor receptor 2 heterodimerization contributes to trastuzumab resistance of breast cancer cells. Cancer Res 2005; 65:11118-11128.
97. Lu Y, Zi X, Zhao Y, et al. Insulin-like growth factor-I receptor signaling and resistance to trastuzumab (Herceptin). J Natl Cancer Inst 2001; 93:1852-1857.
98. Trepel J, Mollapour M, Giaccone G, Neckers L. Targeting the dynamic Hsp90 complex in cancer. Nat Rev Cancer 2010; 10:537-549.
99. Munster PN, Srethapakdi M, Moasser MM, Rosen N. Inhibition of heat shock protein 90 function by ansamycins causes the morphological and functional differentiation of breast cancer cells. Cancer Res 2001; 61:2945-2952.
100. Munster PN, Marchion DC, Basso AD, Rosen N. Degradation of HER2 by ansamycins induces growth arrest and apoptosis in cells with HER2 overexpression via a HER3, phosphatidylinositol 30-kinase-AKTdependent pathway. Cancer Res 2002; 62:3132-3137.
101. Modi S, Stopeck AT, Gordon MS,Mendelson D, Solit DB, Bagatell R, MaW, Wheler J, Rosen N, Norton L, Cropp GF, Johnson RG, Hannah AL, Hudis CA. Combination of trastuzumab and tanespimycin (17-AAG, KOS-953) is safe and active in trastuzumab-refractory HER-2 overexpressing breast cancer: a phase I dose-escalation study. J Clin Oncol 2007; 25: 5410-5417.
102. Modi S, Stopeck AT, Linden HM, Solit DB, Chandarlapaty S, Rosen N, D'Andrea G, Dickler MN, Moynahan ME, Sugarman S, Ma W, Patil S, Norton L, Hannah AL, Hudis C. Hsp90 inhibition is effective in breast cancer: A phase 2 trial of tanespimycin (17AAG) plus trastuzumab in patients with HER2-positive metastatic breast cancer progressing on trastuzumab. Clin Cancer Res 2011; 17:5132-5139.
103. Efficacy and Safety of IPI-504 With Trastuzumab Pretreated, Locally Advanced or Metastatic HER2 Positive Breast Cancer (ClinicalTrials.gov Identifier: NCT00817362).
104. Phase I-II Study to Determine the Maximum Tolerated Dose (MTD) of AUY922 in Advanced Solid Malignancies, and Efficacy in HER2+ or ER+ Locally Advanced or Metastatic Breast Cancer Patients (ClinicalTrials.gov Identifier: NCT00526045).
105. Combination of AUY922 With Trastuzumab in HER2+ Advanced Breast Cancer Patients Previously Treated With Trastuzumab (ClinicalTrials.gov Identifier: NCT01271920).
106. Goldblatt EM, Erickson PA, Gentry ER, et al. Lipid-conjugated telomerase template antagonists sensitize resistant HER2-positive breast cancer cells to trastuzumab. Breast Cancer Res Treat 2009; 118:21-32.