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New strategies in HER2-overexpressing breast cancer: Many combinations of targeted drugs available

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Abstract

The anti-HER2 drugs trastuzumab and lapatinib are increasingly changing the natural history of early and metastatic HER2-overexpressing breast cancer. Many other agents targeted against the HER2 signaling network are in clinical development. These are or will soon be combined with the currently approved anti-HER2 therapies. We review herein recent data in support of the early use of combinations of agents targeted to the HER2 network as the most rational approach against this subtype of breast cancer. We propose the optimal combination or combinations of anti-HER2 agents delivered early in the natural history of HER2+ breast cancer should close to eliminate acquired drug resistance, shorten the duration of therapy, and potentially dispense with the need of concurrent chemotherapy.

Background

The antibody trastuzumab and the tyrosine kinase inhibitor (TKI) lapatinib are approved by the FDA for the treatment of HER2-overexpressing (HER2+) breast cancer (MBC). Trastuzumab binds to an epitope in the juxtamembrane region of the HER2 receptor. This binding induces uncoupling of ligand-independent HER2-HER3 heterodimers and inhibition of downstream signaling (1) as well as antibody-dependent, cell-mediated cytotoxicity (ADCC)(2). Several randomized adjuvant trials (NCCTG N9831, NSABP B-31, BCIRG 006, and HERA), have demonstrated that the addition of trastuzumab to standard chemotherapy reduces disease recurrence and the risk of death compared to chemotherapy alone in patients with surgically-resected tumors (3–5). In N-9831, a recent interim analysis showed that the benefit of concurrent trastuzumab and chemotherapy was more pronounced than that of chemotherapy followed by trastuzumab (6). Based on these data, the addition of trastuzumab to adjuvant chemotherapy has become standard of care in women with HER2+ early breast cancer.

The trastuzumab adjuvant trials focused on high-risk, lymph node positive HER2+ tumors. Thus, there is limited to no data on small tumors (< 1 cm) with negative nodes and patient outcome. However, two recent studies found a significantly higher rate of recurrence among T1abN0 HER2+ compared to HER2-negative tumors regardless of ER status (7, 8), suggesting adjuvant trastuzumab should be considered for these patients. However, the amount and type of chemotherapy to combine with the antibody in this setting is

undetermined. Most of the adjuvant trials used one year of trastuzumab. One study delivered only 9 weeks of the antibody, whereas the HERA trial included an arm where it was given for 2 years. In the first study, patients in the trastuzumab arm exhibited fewer overall recurrences and improved overall survival compared to patients treated with chemotherapy alone (9). Results in the 2-year arm in HERA are pending.

The dual EGFR/HER2 TKI lapatinib is active as first line monotherapy in patients with HER2+ MBC and in combination with capecitabine improves progression free survival compared to capecitabine alone (10, 11). In the latter registration trial, fewer brain metastases occurred in women in the combination than in the monotherapy arm, suggesting a potential difference between lapatinib and trastuzumab as it applies to recurrences in the CNS (11). In the registration study and in a second randomized trial of paclitaxel ± lapatinib in patients with MBC, the clinical benefit of lapatinib was limited to patients with HER2 overexpression by IHC and/or FISH (12).

On the Horizon

HER2 testing, discordance, and conversion

The clinical activity of anti-HER2 agents has been limited to patients with HER2+ tumors as defined by intense membrane staining with HER2 antibodies in the majority of tumor cells (3+ by immunohistochemistry – IHC) or ≥ 2 copies of the *HER2* gene determined by fluorescent *in situ* hybridization (FISH). In general, HER2 IHC and FISH correlate with each other (13–15). FISH appears superior to IHC to reproducibly assess tumors for HER2 overexpression at outside/local laboratories for entry into clinical trials (16). Intrinsic limitations of IHC are the variability in fixation methods and the impact of fixation of antigenicity of the HER2 protein. Conversely, the more stable DNA, whose loci are measured by FISH, is less susceptible to tissue fixation. For these reasons, excess copies of the *HER2* gene (so called HER2 positivity) defined by FISH has gained ground as the standard to define odds of tumor dependence on HER2 and, therefore, response to HER2 antagonists (17).

A reanalysis in a central laboratory of NSABP B-31 showed that 9.7% of patients enrolled on the basis of a test performed in a local laboratory had tumors that did not meet criteria for *HER2* amplification by FISH or IHC (18). Notably, these patients also benefited from trastuzumab. This suggests that the local laboratory was correct and/or there is discordance in the levels of HER2 expression between micrometastases, whose clinical recurrence defines the endpoint of adjuvant trials, and the primary tumor, where the HER2 alteration was measured. This possibility is further suggested by a study where 9/24 patients with breast cancer whose primary tumor was HER2-negative (HER2–) acquired *HER2* amplification in their circulating tumor cells (CTCs) during cancer progression (19). In another study, 10% of patients that recurred on adjuvant tamoxifen converted from HER2– to HER2+ in the relapsing tumor (20). Of note, however, the HER2 status of CTCs has yet to be linked to clinical outcome. Based on data like these, the NSABP is initiating a phase III trial in which patients with 1+ or 2+ HER2 by IHC and no *HER2* amplification by FISH will be randomized to adjuvant chemotherapy followed by one year of trastuzumab vs. placebo.

Several studies have shown changes in HER2 status in patients treated with trastuzumab. Pectasides *et al.* showed that 37% of patients with HER2+ primary tumors no longer exhibited *HER2* amplification in their metastatic lesions. Further, these patients exhibited a shorter TTP than the group that remained HER2+ (21). Hurley *et al.* reported that following treatment with neoadjuvant trastuzumab and chemotherapy, 43% of HER2+ tumors became HER2– as measured by FISH (22). Finally, Mittendorf *et al.* also reported that 32% of

HER2+ tumors treated with neoadjuvant chemotherapy and trastuzumab ‘converted’ to HER2– by FISH. Notably, at 37 months, relapse free survival (RFS) was statistically superior in patients whose residual tumors retained *HER2* amplification (23). These results have several implications. First, the change in HER2 status may reflect heterogeneity in HER2 expression in the primary tumor; the anti-oncogene therapy eliminates the HER2+ compartment and enriches for HER2– clones. Second, patients with HER2+ breast cancer who relapse after adjuvant or neoadjuvant anti-HER2 therapy should consider having their recurrent disease biopsied for reassessment of the HER2 status. Third, patients with ER–/HER2+ tumors at diagnosis that ‘convert’ to HER2– after treatment are at high risk of early recurrence. Further, there are no clear adjuvant (targeted) therapy standards for these patients who as a result, may exhibit a poor outcome.

Antibody-chemotherapy conjugates

Trastuzumab (T)-DM1 is an antibody-drug conjugate in which one molecule of trastuzumab is covalently bonded via a non-cleavable linker to three molecules of the microtubule polymerization inhibitor derivative of maytansine 1, DM1 (24). T-DM1 binds to HER2 with similar affinity as trastuzumab. It is postulated that after binding, the T-DM1/HER2 complex is internalized followed by degradation in the lysosome, release of DM1, and subsequent cell lysis. Although used at lower doses and frequency than trastuzumab, T-DM1 retains the ability to inhibit signaling and engaging immune effectors that mediate ADCC and is active against lapatinib-resistant xenografts (25). Phase I–II studies of T-DM1 demonstrated mild, reversible toxicity and a remarkable clinical response rate in excess of 25% in patients with heavily pretreated HER2+ MBC who had progressed after trastuzumab and lapatinib (26, 27). T-DM1 is being further evaluated in two large phase III randomized studies. The first trial compares T-DM1 vs. T-DM1 plus pertuzumab vs. the standard of trastuzumab plus a taxane in patients with HER2+ MBC previously untreated in the metastatic setting. The second trial compares T-DM1 vs. the standard of lapatinib and capecitabine in similar patients but who have previously received trastuzumab (Table 1).

Combination of anti-HER2 therapies and abrogation of drug resistance

Dual HER2 blockade—Many *HER2*-amplified breast cancers do not respond to or eventually escape trastuzumab, suggesting both *de novo* and acquired mechanisms of resistance. A possible mechanism of *de novo* resistance is expression of the HER2 receptor as a kinase-active 95-kDa cytosolic fragment that lacks the trastuzumab binding epitope (28). Analysis of a cohort of patients with HER2+ MBC treated with trastuzumab and chemotherapy showed a very low response rate in tumors with cytosolic p95^{HER2} compared to those without (29). Lapatinib has been shown to inhibit the catalytic activity of p95^{HER2}. Therefore, patients with p95^{HER2}-positive breast cancers treated with lapatinib alone or in combination with capecitabine exhibited a similar PFS and overall response rate compared to p95^{HER2}-negative tumors (30), suggesting a clinical setting where a HER2 TKI might be advantageous.

Like lapatinib, the HER2/EGFR dual TKI neratinib (31) has shown clinical activity in patients with HER2+ MBC who have progressed on trastuzumab. These data suggest that trastuzumab-resistant tumors continue to be dependent on the HER2 tyrosine kinase. However, the response to each single agent TKI tends to be short-lived (10, 11). Further, these patients may still need trastuzumab beyond progression as suggested by a recent study where the combination of lapatinib and trastuzumab was superior to lapatinib alone at improving PFS, clinical response, and overall survival of patients with HER2+ MBC who had progressed on trastuzumab (32). Taken together, these data imply that even in advanced stages, HER2+ breast cancers remain dependent on HER2 and that single-agent trastuzumab and lapatinib are not adequate to inhibit the HER2 network completely. They also imply that

using combinations of HER2-targeted agents delivered early against HER2+ breast cancer is the rational approach to take. Along these lines, Adjuvant Lapatinib and/or Trastuzumab Treatment Optimization (ALTTO) is an ongoing large international adjuvant study comparing trastuzumab vs. lapatinib vs. dual HER2 blockade using both drugs. The activity of trastuzumab beyond progression is not limited to combinations with TKIs as it has also been shown in a study where the combination of trastuzumab plus capecitabine was clearly superior to capecitabine alone (33).

Inhibition of HER3 and HER2/HER3 dimers—The HER2 co-receptor HER3, is the key adaptor that once dimerized with and phosphorylated by HER2 engages and activates the phosphatidylinositol-3 kinase (PI3K)/Akt pathway (1, 34, 35). The association of HER2/HER3 dimers with PI3K is essential for the viability of HER2-dependent cells (36, 37). Indeed, HER2+ breast cancer cells are particularly sensitive to apoptosis induced by PI3K inhibitors (38), further underscoring the importance of HER3-mediated signaling in HER2-dependent cells. It is generally accepted that sustained inhibition of the output of HER2/HER3 to PI3K/Akt is required for the antitumor effect of HER2 inhibitors. Interestingly, HER2+ breast cancer cells upregulate HER3 expression upon inhibition of HER2 with lapatinib (39). This is problematic because several kinases such as MET, EGFR, Src, FGFR2 can phosphorylate HER3 (40–42), thus partially maintaining PI3K activity and limiting the antitumor effect of the HER2 inhibitor (Fig. 1).

Trastuzumab has been shown to block ligand-independent association between HER2 and HER3 whereas pertuzumab, an antibody that recognizes an epitope in heterodimerization domain II of HER2, blocks ligand-induced HER2-HER3 dimerization (43). Recent data using CLIA-certified dimer assays suggest levels HER2-containing homo and heterodimers (with EGFR and HER3) can be measured *in situ* and are highly variable among HER2+ tumors (44, 45). We hypothesize, for example, that a tumor with high levels of HER2/HER3 heterodimers would be relatively unresponsive to trastuzumab and thus may be a candidate for lapatinib or pertuzumab, each in combination with trastuzumab. These speculations and the question whether the addition of quantitative dimer assays to FISH and IHC for HER2 will refine the selection of the type of anti-HER2 therapy remain to be investigated.

In trastuzumab-resistant xenografts and in patients with HER2+ breast cancer that have progressed on trastuzumab, only the combination of pertuzumab and trastuzumab but not each antibody alone exhibited clinical activity (46, 47). These data suggest that both HER2 antibodies might be required to completely inhibit HER2-HER3 dimerization *in situ*, potentially explaining their clinical activity in combination. To test this hypothesis, the phase III Cleopatra study (Table 1) is currently randomizing patients with HER2+ MBC to trastuzumab and docetaxel ± pertuzumab as first line therapy in the metastatic setting using PFS as a primary endpoint. Of note, in the recently reported NeoSphere trial in patients with HER2+ primary breast cancer (Table 1), the path CR rate was 45.8% vs. 29% ($p=0.01$) in patients treated with neoadjuvant docetaxel/trastuzumab/pertuzumab vs. docetaxel/trastuzumab, respectively (48). Currently, the HER3 monoclonal antibodies AMG-888 (49) and MM-121 (50) are completing phase I testing. We anticipate that like pertuzumab, they may also exert a synergistic effect in combination with trastuzumab or lapatinib in patients with HER2+ MBC.

PI3K and drug resistance—Amplification of PI3K signaling as a result co-expression of *PIK3CA*-activating mutations or loss of the lipid phosphatase PTEN in HER2+ breast cancer cells and primary tumors is associated with a lower response to trastuzumab and lapatinib (51–55). Several PI3K pathway antagonists are in clinical development and are the subject of recent reviews (56, 57). In preclinical studies, the addition of some of these inhibitors to trastuzumab or lapatinib has inhibited growth of HER2+ tumors resistant to

anti-HER2 therapy (53, 54). Interestingly, inhibitors of mTOR, a serine-threonine kinase downstream PI3K, have shown activity after progression on trastuzumab. Dalenc *et al.* recently reported a multicenter phase II study of 55 women with HER2+ MBC whose tumors were resistant to trastuzumab and taxanes. Patients were treated with the TOR inhibitor everolimus, paclitaxel, and trastuzumab, exhibiting an impressive partial response rate of 19% and an overall clinical benefit rate of 81% (58).

Neoadjuvant therapy as a platform for clinical research—Trastuzumab has been administered with chemotherapy in the neoadjuvant setting with rates of pathologic complete response (path CR) as high as 65% (22, 59–62). Achievement of path CR after neoadjuvant chemotherapy has been widely associated with improved long term outcome. Although not yet clear, recent data suggest similar conclusions may eventually also apply to patients treated with neoadjuvant anti-HER2 therapy. The neoadjuvant herceptin (NOAH) trial tested the efficacy of chemotherapy $\pm$ trastuzumab in patients with HER2+ locally advanced or inflammatory breast cancer. Path CR rate was 38% vs. 19% in the trastuzumab vs. the control group. There was a 71% vs. 56% three-year event-free survival in the trastuzumab vs. control arms in all subgroups tested. Overall survival was not different between both arms but this finding is qualified by the fact that a significant proportion of patients ‘crossed-over’ to adjuvant trastuzumab (62). Nonetheless, NOAH is the first trial in patients with HER2+ tumors where path CR mirrors longer term event-free survival, suggesting use of the neoadjuvant therapy space as a platform for clinical investigation which we will discuss below.

Neo-ALTTO is a 450 patient study where HER2+ tumors >2 cm were randomized to trastuzumab, lapatinib, or the combination for 6 weeks, time at which paclitaxel is added to each of the arms for additional 12 weeks. After surgery, all 3 arms will receive adjuvant chemotherapy with FEC followed by the respective HER2 inhibitor either alone or in combination for 34 weeks. About half of the patients enrolled had ER+ tumors. There was increased but manageable toxicity in the lapatinib arms (diarrhea, transaminitis). Path CR, defined as no invasive cancer in the breast or only DCIS in the breast specimen, was significantly higher in the combination arm (51.3%) vs. 29.5% and 24.7% in the trastuzumab and lapatinib arms, respectively. In all 3 arms, the path CR rate was lower in the ER+ vs. the ER– tumors (63). Whether path CR correlates with disease-free and overall survival is pending further follow-up.

Other combinations—The data summarized above suggest that in addition to the combination of trastuzumab and lapatinib, many other rational combinations are or will soon be available for clinical testing. Examples of two-drug combinations that will inhibit the HER2 network and its output to PI3K/Akt more comprehensively are: trastuzumab plus pertuzumab, trastuzumab or lapatinib plus a HER3 neutralizing antibody, trastuzumab or lapatinib plus a PI3K or an AKT inhibitor, a PI3K pathway inhibitor plus a HER3 antibody, T-DM1 plus pertuzumab or a PI3K inhibitor, a combination of HER2 and IGF-IR pathway antagonists, among others. Some of these are shown in Table 1. We speculate many of these or all will be well tolerated and effective against HER2+ MBC but that gain will likely be only incremental. With the establishment of current anti-HER2 agents as adjuvant therapy for HER2+ early disease and the increasing limitation in patient resources, it will be difficult to move these combinations to the adjuvant setting to test their true anti-(micro)metastatic potency using survival as an endpoint.

However, the increasing use of pre-operative therapy should provide a clinical research platform where these combinations can be compared and triaged using path CR as a clinical endpoint predictive of long term outcome. Another benefit of a pre-operative platform is that residual tumor tissue is available at the time of surgery. These ‘drug resistant’ residual

cancers may well reflect the molecular profile of drug-resistant micrometastases and can be interrogated with open ended molecular approaches to identify biomarkers and/or effectors of resistance to anti-HER2 therapies. It would not be too surprising if the clinical activity of these combinations turns out to be equivalent. However, toxicity and cost may turn out to be important differentiating factors. If so, just like options for endocrine therapy in ER+ breast cancer, this scenario would provide a plethora of treatment choices for patients with HER2-overexpressing breast cancer who in the end will be the winners.

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Figure 1.

Upregulation of HER3 and resistance to anti-HER2 therapies. The kinase dead HER3 receptor, but not EGFR or HER2, can couple directly to p85, the regulatory subunit to PI3K. HER2/HER3 heterodimers are the most potent signaling complexes of the HER receptor network. Upon inhibition of HER2 and PI3K (as with lapatinib in figure), tumor cells upregulate expression of the HER3 protein. Other tyrosine kinases such as MET, FGFR2, Src, and EGFR present in HER2+ cancer cells can then phosphorylate tyrosines in the C-terminus of HER3 which, in turn, couples to p85 to partially maintain PI3K active and thus limiting the anti-tumor effect of HER2 inhibitors. Alternatively, the cell can use other receptor networks, i.e., IGF-IR to activate the PI3K/Akt survival pathway.

Table 1
Planned and ongoing high impact randomized phase III trials in patients with HER2-overexpressing breast cancer.

Trial	Design	Population	# of patients	Treatment	Trial endpoints	Data expected
TEACH	Phase III, adjuvant	Stage I–IIIC, s/p neoadjuvant chemotx with an anthracycline, a taxane, and CMF	3000	Placebo vs. Lapatinib 1500 mg/day for 12 months	DFS, RFS, DRFS, OS, rate of CNS recurrence, QoL	2012
ALTO, BIG2-06/N063D	Phase III, adjuvant	Stage I–IIIC, s/p neoadjuvant anthracycline based chemotx (4 cycles)	8000	Standard chemotx with a) H, b) L, c) H x 12 weeks → L after 6-week washout, d) H+L (all regimens for up to 1 year)	DFS, OS, TTDR, TTR, CNS recurrence rate	2013
ExteNET	Phase III, adjuvant	Stage II–IIIC after adjuvant H for 1 year	120	Placebo vs. Neratinib	DFS	2017
NEPERTT	Phase III, MBC	HER2+, no prior therapy for MBC	1200	P + H vs. P + Neratinib	PFS, OS	2012
Neo-ALTTO	Phase III, neoadjuvant	HER2+ >2 cm	450	a) L x 6 wks → L+P x 12 wks vs. b) H x 6 wks → H+P x 12 wks vs. c) L+H x 6 wks → H+L+P x 12 wks; surgery; FECX3 → a) L vs. b) H vs. c) H+L to complete 1 year	pCR rate, ORR, DFS, OS	2011
NSABP B-41	Phase III, neoadjuvant	HER2+ >2 cm	522	ACx4 → a) P+H x 12 wks vs. b) P+L x 12 wks vs. c) P+H+L x 12 wks	pCR rate, ORR, RFS, OS	2010
CALGB 40601	Phase III, neoadjuvant	HER2+ Stage II–III		H+P vs. L+P vs. H+L+P → surgery → adjuvant chemotx	pCR rate, ORR, DFS, OS	2011
Gepar-Quinto	Phase III, neoadjuvant	HER2+ requiring neoadjuvant chemotx	2547 (whole trial)	EC → D → H vs. EC → D → L	pCR rate	2010
BETH, NSABP B-44	Phase III, adjuvant	HER2+, LN+ or high-risk LN	3500	TCH → H (up to 1 y) vs. TCH → H + bevacizumab (up to 1 y)	DFS, OS, RFS	2012
BO22589	Phase III, MBC	HER2+, no prior therapy for MBC	1092	H + taxane vs. T-DM1 vs. T-DM1 + pertuzumab	PFS	2012
EMILIA	Phase III, MBC	HER2+ LABC or MBC	580	T-DM1 vs. Capecitabine + Lapatinib	PFS, OS, ORR	2013
CLEOPATRA	Phase III, MBC	HER2+, no prior therapy for MBC	800	Docetaxel + H + placebo vs. Docetaxel + H + pertuzumab	PFS	2011
PHEREXA	Phase II, MBC	HER2+ MBC, 2 nd line after Herceptin	450	Capecitabine + H vs. Capecitabine + H + pertuzumab	PFS, TTP, TTF, ORR, CBR	2015
BOLERO-1	Phase III, MBC	HER2+, no prior therapy for MBC	717	P + H vs. P + H + everolimus	PFS, OS, ORR, CBR, TTR	2012
BOLERO3	Phase III, MBC	HER2+, resistant to H	572	Vinorelbine + H vs. Vinorelbine + H + everolimus	PFS, OS, ORR, CBR	2012

H=Herceptin; L=Lapatinib; P=Paclitaxel; D=docetaxel; TC= docetaxel, carboplatin; TCH= docetaxel, carboplatin; TCH → H = docetaxel, carboplatin, hereceptin; DFS=disease-free survival; RFS=relapse-free survival; DRFS=distant relapse-free survival; OS=overall survival; QoL=quality of life; TTR=time to recurrence; TTDR=time to distant recurrence; PFS=pathological complete response; ORR=overall

response rate; PFS=progression-free survival; TTP=time to progression; TTF=time to treatment failure; CBR=clinical benefit rate; ALTTO=adjuvant lapatinib & trastuzumab treatment optimization; AC=adriamycin/taxol; LABC=locally advanced breast cancer; MBC=metastatic breast cancer; T-DM1=trastuzumab-derivative of maytansine; CMF=cyclophosphamide, methotrexate, 5-fluorouracil