

Clinical Efforts to Combine Endocrine Agents with Targeted Therapies against Epidermal Growth Factor Receptor/Human Epidermal Growth Factor Receptor 2 and Mammalian Target of Rapamycin in Breast Cancer

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Abstract Enhancing the benefit of endocrine therapy by overcoming *de novo* or acquired resistance remains an important goal in systemic breast cancer therapy. Progress continues to be made in elucidating the molecular pathways by which estrogen receptor – positive breast cancer cells escape from endocrine therapy. The increasing recognition of the roles of epidermal growth factor receptor (EGFR) and human EGFR2 in cross-talk activation of estrogen receptor signaling has led to studies aimed at identifying whether small-molecule tyrosine kinase inhibitors targeted against these receptors give additive or synergistic effects when combined with endocrine agents. Activation of the phosphatidylinositol-3-OH kinase/Akt pathway has also been associated with resistance to either tamoxifen or estrogen deprivation, and preclinical studies have shown that the mammalian target of rapamycin antagonist temsirolimus can restore endocrine sensitivity in breast cancer cells. Randomized phase II trials of aromatase inhibitors combined with EGFR/human EGFR2 tyrosine kinase inhibitors or mammalian target of rapamycin antagonists have been completed in both the neoadjuvant and advanced breast cancer settings. Larger phase III trials with both approaches are now in progress and have been powered to detect whether either strategy can significantly prolong time to disease progression compared with endocrine therapy alone. The correlation of molecular and clinical results from these ongoing studies will be important to establish appropriate biological variables for selecting those patients who may benefit most from this combined approach.

Endocrine resistance in estrogen receptor (ER) – positive breast cancer is associated with enhanced expression of membrane growth factor pathways such as the type 1 peptide growth factor receptors human epidermal growth factor receptor (EGFR)-1 (HER1)/EGFR and HER2, together with activation of intracellular kinases and other proteins involved in signal transduction and cell survival. In ER-positive tumors resistant *de novo* to endocrine therapy, or which develop acquired resistance during prolonged therapy, overwhelming evidence now suggests that these pathways are used by breast cancer cells to bypass normal endocrine responsiveness (1, 2). As such, these represent attractive targets for pharmacologic intervention with small-molecule signal transduction inhibitors

that target aberrantly or excessively expressed proteins and kinases. Many drugs that target these or other downstream pathways are now in active development for breast cancer, including type 1 growth factor tyrosine kinase inhibitors, farnesyltransferase inhibitors, mitogen-activated protein kinase (MAPK)/extracellular signal-related kinase inhibitors, mammalian target of rapamycin (mTOR) antagonists, and various therapies that target pathways involved in tumor angiogenesis (Table 1).

The strategy for using signal transduction inhibitors in breast cancer is not restricted to treating endocrine-resistant tumors. Whereas experimental and clinical data suggest that signal transduction inhibitors given as monotherapy may have only minimal effect on tumor growth, especially in hormone-sensitive ER-positive breast cancer cells that lack activation and dependence on pathways targeted by these drugs, various strategies are being explored to combine signal transduction inhibitor therapies together with endocrine approaches (2). Preclinical models have suggested that a combined approach may prevent or delay the development of endocrine resistance and that this could significantly improve the therapeutic efficacy of currently available endocrine options. This article reviews clinical investigations of this strategy in breast cancer with the following classes of agents: (a) small-molecule tyrosine kinase inhibitors that target the EGFR; (b) dual inhibitors of EGFR and HER2; and (c) drugs that target mTOR function.

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Table 1. Current stage of development for breast cancer of targeted intracellular small-molecule inhibitors

Intracellular target	Drug	Phase of development in breast cancer
EGFR	Gefitinib (Iressa)	Phase II MBC; phase II/III RCT +/- Endo MBC, NeoAdj
	Erlotinib (Tarceva)	Phase II MBC; phase II + Chemo; phase II + 2C4
	EKB-569	Phase I/II
EGFR/HER2	Lapatinib (GW572016)	Phase II MBC; phase III RCT MBC +/- Endo
	AEE-788	Phase I
Pan-erbB	Canatarnib (CI-1033)	Phase II MBC
Farnesyltransferase	Tipifarnib (Zarnestra)	Phase II MBC; phase II RCT +/- Endo MBC
	Lonafarnib (Sarasar)	Phase II RCT; phase I + Chemo
	AZD3409	Phase I
mTOR	Everolimus (RAD-001)	Phase III RCT +/- Endo MBC, NeoAdj
	Temsirolimus (CCI-779)	Phase II MBC; phase III RCT +/- Endo MBC
VEGFR + PDGF	SU011248 (Sunitinib)	Phase II MBC
Raf	BAY-43-9006 (Sorafenib)	Phase I
MEK1/2	PD-0325901	Preclinical
	AZD6244	Phase I
NF- κ B	Bortezomib (Velcade)	Phase II + Chemo
Src	AZD0530	
PI3K	LY294002	Preclinical
IGFR-I	AG1024	Preclinical

Abbreviations: RCT, randomized controlled trial; MBC, metastatic breast cancer; NeoAdj, neoadjuvant setting; Endo, endocrine therapy; Chemo, chemotherapy; VEGFR, vascular endothelial cell growth factor receptor; PDGF, platelet-derived growth factor; MEK, MAPK/extracellular signal-related kinase kinase 1/2; NF- κ B, nuclear factor κ B; PI3K, phosphatidylinositol-3-OH kinase; IGFR-I, insulin-like growth factor receptor I.

Targeting EGFR Alone and in Combination with Endocrine Therapies

Role of EGFR in endocrine resistance. Enhanced expression of the EGFR, together with subsequent downstream activation of signaling pathways regulated by the MAPK/extracellular signal-related kinase, has been found in breast cancer cells that become resistant over time to endocrine therapy either with tamoxifen (3) or long-term estrogen deprivation (4). Treatment with gefitinib, which targets the internal tyrosine kinase domain of EGFR, has been used in preclinical models in an attempt to overcome this resistance by blocking this up-regulated signaling pathway (5). Of note, hormone-sensitive ER-positive cells in which EGFR was not expressed were unaffected by gefitinib.

Greater efficacy has been seen with gefitinib in various *in vitro* models of ER-positive breast cancer when it was combined with endocrine therapy. Combined tamoxifen and gefitinib provided near complete inhibition of phosphorylated MAPK/extracellular signal-related kinase 1/2 and Akt in hormone-sensitive MCF-7 breast cancer cells, together with greater G₀-G₁ cell cycle arrest and suppression of the cell-survival protein Bcl-2, than observed with tamoxifen alone (6). In particular, combined therapy prevented the acquired expression of EGFR/MAPK signaling and the subsequent resistance that occurred after 5 weeks in tamoxifen alone-treated cells. Recently, others have shown that gefitinib has additive effects when combined with the ER down-regulator fulvestrant in a panel of ER-positive breast cancer cells derived from patients, including hormone-refractory cells and those with varying expressions of EGFR, HER2, and HER3/4 (7).

Breast cancer cells with high expression of EGFR or HER2 were most sensitive to gefitinib, which induced a significant G₁-S cell cycle arrest, together with induction of apoptosis. In ER-positive KPL-3C cells, gefitinib increased the antitumor effect of fulvestrant in estrogen-supplemented cells, and combined therapy induced maximal growth retardation through additive effects on induction of the cyclin-dependent kinase inhibitor p21, together with enhanced suppression of Bcl-2.

ER-positive tumors that overexpress HER2 and become resistant to tamoxifen can be growth inhibited by gefitinib, which targets EGFR, due to disruption of heterodimerization between EGFR with HER2, which abrogates HER2-dependent growth. However, in models of established hormone-resistant HER2-positive breast cancer, the strategy of combined signal transduction inhibitors and endocrine therapy may be more effective than using signal transduction inhibitors alone (8). *In vivo*, gefitinib and tamoxifen provided maximal growth inhibition and significantly delayed the outgrowth of HER2-positive MCF-7 xenografts compared with gefitinib alone (9). Similar effects have been reported by this group for gefitinib combined with estrogen deprivation, which provided greater inhibition of growth and substantially delayed acquired resistance compared with estrogen deprivation alone (10).

Clinical trials with gefitinib. There have been three phase II monotherapy studies of gefitinib in patients with advanced breast cancer (11-13). The only trial to report a significant number of tumor responses was one that specifically included patients with ER-positive tamoxifen-resistant breast cancer (13), the setting in which preclinical models had shown the best evidence of activity for gefitinib. In this small phase II study, 6 of 9 (66%) patients with ER-positive endocrine-resistant

tumors had clinical benefit from gefitinib (1 complete response and 5 partial responses), compared with only 2 of 18 (11%) in those with ER-negative tumors. These investigators recently reported on their analysis of various biomarkers analyzed in the tumors before therapy with gefitinib, and again after a period of 4 to 8 weeks (14). Rather surprisingly, pretreatment levels of EGFR were lower in patients who had clinical benefit compared with those who progressed. Significant decreases in the tumor cell proliferation marker Ki-67 were seen in those who responded compared with those who progressed, together with an expected decrease in phosphorylated EGFR. An increase in pAkt was seen both at baseline and after treatment in those who progressed, supporting a possible role for this pathway in resistance to gefitinib.

The biological effects of gefitinib, either alone or in combination with the aromatase inhibitor anastrozole, have been recently examined in a double-blind, placebo-controlled randomized trial in 56 postmenopausal women with ER-positive, EGFR-positive, operable primary breast cancer (15). Women received gefitinib 250 mg daily with or without 1 mg anastrozole for 4 to 6 weeks before surgery, and changes in tumor cell proliferation (Ki-67) were assessed together with any reduction in tumor size or phosphorylation of MAPK, EGFR at Tyr⁸⁴⁵, or ER at Ser¹¹⁸. This trial confirmed the observations described above in advanced breast cancer that gefitinib given as monotherapy can reduce cell proliferation and inhibit downstream components of the EGFR pathway in those patients who had clinical benefit from therapy (Table 2). However, patients treated with combined therapy had a significantly greater reduction in Ki-67 ($P = 0.0054$) although effects on reduction of phosphorylated ER were similar in both groups. Clearly, a more detailed analysis of resistance pathways such as activated pAkt would be of interest. Likewise, a randomized biomarker comparison between combined gefitinib and anastrozole versus anastrozole alone will be of greater clinical significance in ascertaining whether any added benefit exists for this strategy; to this end, a preoperative study in 180 postmenopausal women with ER-positive breast cancer has completed recruitment.

Based on the preclinical rationale for added benefit, a number of phase II/III trials have been initiated with gefitinib in combination with tamoxifen, fulvestrant, or an aromatase inhibitor (Table 3). Some of these trials are in the second-line setting comparing gefitinib alone with the combination of gefitinib plus tamoxifen after progression on tamoxifen. Other trials are randomized phase II studies with only 100 to 200 patients, with the primary efficacy end point being tumor objective response rate with the hope of detecting greater antitumor activity for combined therapy than with endocrine therapy alone. The ultimate test will be the randomized phase III trials of endocrine therapy alone versus combined endocrine/gefitinib therapy. The primary aim of these studies is to investigate whether time to disease progression can be significantly prolonged by the addition of gefitinib to first-line endocrine therapy for advanced disease, and preliminary results are expected by late 2005 or early 2006.

Other clinical results with EGFR tyrosine kinase inhibitors. There are few clinical data with other EGFR-specific tyrosine kinase inhibitors in breast cancer, and a previous phase II monotherapy trial of erlotinib in breast cancer was relatively disappointing (16). However, biomarker changes following

treatment with 150 mg/d erlotinib have been analyzed in two separate studies in advanced and early breast cancer. In a small study of 15 unselected patients with advanced breast cancer, no significant changes were seen in Ki-67 and no tumor responses were reported (17). In the one tumor with detectable EGFR, changes in downstream biomarkers were detected but failed to correlate with any effect on cell proliferation. These negative data on tumor cell proliferation are very similar to those previously reported for gefitinib in unselected advanced breast cancer patients (ref. 12; Table 2). In contrast, more obvious biological changes have been described in unselected patients with primary early breast cancer treated with erlotinib for 7 to 14 days before surgery (18). A >75% decrease in Ki-67 was seen in 5 of 14 tumors and a >85% decrease in pMAPK in 8 of 14 tumors. Again, there was no clear correlation with EGFR protein, which was only detected in 4 of 14 tumors. In this small study, no obvious relationship was seen with pAkt but analyses of proteomic profiles that could predict for response or resistance to erlotinib are ongoing.

Thus, with both gefitinib and erlotinib in breast cancer, there is still much to understand. Clinical studies examining the drugs as monotherapy for unselected patients have suggested that they exert minimal effects, although biomarker changes have consistently been seen both in early breast cancer and in advanced breast cancer patients selected for resistance to endocrine therapy or expression of EGFR (Table 2). There is much greater expectation that a combined approach of an EGFR tyrosine kinase inhibitor with endocrine therapy will prove to be more promising, based on strong preclinical science outlined above. However, clinical response is likely to be modified by coexpression of other HER family members, together with activation of additional intracellular pathways such as the phosphatidylinositol-3-OH kinase/Akt cell survival pathway. Thus, alternative strategies to block both HER2 and EGFR, or to target downstream effectors of Akt, are being explored with or without concurrent endocrine therapies, as described below.

Approaches to Cotargeting of HER2 in Endocrine-Resistant Breast Cancer

Targeting HER2 together with ER and/or EGFR. Several groups have investigated targeting HER2 in tamoxifen-resistant MCF-7 cells using either the HER2 tyrosine kinase inhibitor AG1478 or the monoclonal antibody trastuzumab (8, 19). A synergistic effect has also been reported for trastuzumab combined with tamoxifen in ER-positive/HER2-positive BT-474 breast cancer cells with enhanced accumulation of cells in G₀-G₁ and reduction in S phase of the cell cycle compared with either therapy alone (20). Of interest, there was no evidence for any induction of apoptosis.

There is already widespread use of trastuzumab either as monotherapy or combined with chemotherapy in patients with metastatic breast cancer, often following progression on endocrine therapy for advanced disease. In spite of its availability, there are remarkably few clinical studies that have prospectively investigated the role of trastuzumab in modulating endocrine sensitivity. Several phase II studies are examining trastuzumab combined with aromatase inhibitors, and in a recent study of 32 patients (the majority of whom had received prior tamoxifen) with ER-positive, HER2-positive tumors who

Table 2. Biomarker studies in breast carcinomas and/or surrogate tissue following treatment with tyrosine kinase inhibitors that target EGFR/HER2

Drug	Clinical setting	Key pharmacodynamic findings	Author
Gefitinib	Unselected MBC	Tumor and skin <ul style="list-style-type: none"> •Reduction in pEGFR, Ki-67, pMAPK in skin •Reduction in pEGFR in tumor, but no change in Ki-67 or pMAPK 	Baselga et al. (12)
Gefitinib	Tamoxifen-resistant, ER ⁺ MBC	Tumor <ul style="list-style-type: none"> •Correlation of decrease in Ki-67 with clinical benefit •Lower pretreatment EGFR correlated with response •Increased pAkt correlated with lack of response 	Gee et al. (14)
Gefitinib ± AI	EGFR ⁺ primary EBC	Tumor with gefitinib alone <ul style="list-style-type: none"> •Significant inhibition of Ki-67 (mean 92% reduction) •Inhibition of downstream pEGFR/pMAPK •Reduced pER Ser¹¹⁸ and pMAPK Tumor with combined gefitinib + anastrozole <ul style="list-style-type: none"> •Significantly greater reduction in Ki67 than gefitinib alone (mean 98% reduction) •Greater reduction in pMAPK than gefitinib alone 	Polychronis et al. (15)
Erlotinib	Unselected MBC	Tumor <ul style="list-style-type: none"> •No significant change in Ki-67 •Reduction in pMAPK and pAkt in single EGFR⁺ tumor 	Tan et al. (17)
Erlotinib	Unselected primary EBC	Tumor <ul style="list-style-type: none"> •>75% decrease in Ki-67 in 5/14 tumors •>85% decrease in pMAPK in 8/14 tumors 	Guix et al. (18)
Lapatinib	HER2 ⁺ MBC	Clinical response correlated with <ul style="list-style-type: none"> •HER2 •Low level ER/PR •Posttreatment decline in serum HER2 ECD 	Blackwell et al. (29)
AEE788	Phase I	Skin + tumor <ul style="list-style-type: none"> •Dose-dependent inhibition of EGFR signaling •Inhibition of endothelial cell pMAPK and Ki-67 at higher doses 	Baselga et al. (32)

Abbreviations: EBC, early breast cancer; AI, aromatase inhibitor; ECD, extracellular domain; PR, progesterone receptor; Ki-67, cell proliferation marker.

were treated at progression on tamoxifen with combined trastuzumab and letrozole, clinical benefit (objective response and stable disease) was observed in 52% patients (21). The true benefit of adding trastuzumab to aromatase inhibitors in this setting is being tested in ongoing randomized phase III trials that are expected to report later this year.

Cooperative activation of different type I growth factor receptors (EGFR, HER2, HER3, or HER4) may limit the efficacy of targeting just one single receptor, and thus several strategies to achieve concurrent blockade of both HER2 and EGFR have been explored both experimentally and in the clinic. In an experimental model of ER-positive, HER2-positive, tamoxifen-resistant breast cancer, near complete disappearance of xenografts has been observed by combining endocrine therapy (estrogen deprivation or tamoxifen) with gefitinib, trastuzumab, and the monoclonal antibody pertuzumab (2C4), which inhibits HER2 heterodimerization with all other HER family members (22). Combined therapy of tamoxifen with gefitinib, trastuzumab, and pertuzumab was able to totally block cross-talk with ER and seemed to produce the most dramatic antitumor effects in the xenograft model, with complete resolution of 18 of 20 (90%) tumors and no regrowth after

129 days. However, one phase I/II trial clinical study combining gefitinib and trastuzumab did not confirm the pre-clinical findings, with an unexpectedly low response rate and possible antagonism (23). It remains unclear whether combining small-molecule tyrosine kinase inhibitors and monoclonal antibodies is the best pharmacologic approach.

Alternative strategies to cotargeting of EGFR and HER2 have been to develop potent dual inhibitors of both EGFR and HER2 such as lapatinib (GW572016), which has been shown to inhibit autophosphorylation of both receptors (24). In the phase I study, diarrhea and skin rash were the main toxicities (25), and encouraging clinical activity was reported in trastuzumab-resistant breast cancer patients (26). In a laboratory model, lapatinib has been shown to cooperate with tamoxifen to provide more rapid and profound cell cycle arrest than either therapy alone in ER-positive hormone-resistant cells (27). The two drugs together caused a greater reduction in cyclin D1 in various tamoxifen-resistant breast cancer cell lines, together with a significantly greater increase in the kinase inhibitor p27, which enhanced cyclin E/cyclin-dependent kinase 2 inhibition. Lapatinib was able to restore tamoxifen sensitivity in either EGFR- or HER2-expressing cells whereas

in vivo combined lapatinib and tamoxifen caused maximal regression of HER2-overexpressing, tamoxifen-resistant MCF-7 xenografts (27). In addition to inhibiting mitogenic signaling and cell cycle progression, lapatinib inhibited estrogen-stimulated ER transcriptional activity and cooperated to further reduce ER-dependent gene transcription. These data are consistent with some of those described above for gefitinib and tamoxifen, and strongly support exploring combined endocrine therapy with lapatinib in breast cancer patients.

Clinical trials with lapatinib in breast cancer. A phase II trial of lapatinib has been completed in heavily pretreated patients with advanced breast cancer whose tumors progressed on prior trastuzumab-containing regimens. A recent interim analysis in the first 41 patients confirmed clinical activity for lapatinib in breast cancer, with partial responses in 7% of patients and/or stable disease in 24% of patients after 16 weeks of therapy (28). Biomarker analysis of tissue and serum from patients in this study has suggested that response correlated with HER2, low levels of ER and progesterone receptor, and that a significant decline in HER2 extracellular domain shed into serum after 4 to 8 weeks may predict response to therapy (ref. 29; Table 2). More recently, an encouraging tumor objective response rate of 35% was reported for lapatinib given as first-line therapy in the absence of prior trastuzumab therapy in a study of 40 patients with metastatic disease positive for HER2 by fluorescence *in situ* hybridization (30). These data consistently suggest that lapatinib is an active drug in breast cancer.

A phase I pharmacokinetic study has investigated the combination of lapatinib with letrozole in 39 patients heavily pretreated with endocrine therapy (31). Of 18 breast cancer patients in the study, 3 patients had prolonged stable disease for >6, >10, and >11 months, respectively; all had ER-positive,

HER2-positive tumors and had failed prior antiestrogen therapy with tamoxifen and either fulvestrant (1 patient) and/or other aromatase inhibitors (2 patients). The pivotal trial for lapatinib will be the large randomized phase III trial in >760 patients with ER-positive advanced breast cancer who will receive letrozole with or without lapatinib as first-line therapy. This study is powered to detect a 30% improvement in median time to disease progression from 10 to 13 months (hazard ratio, 0.769). The recently published preclinical data showing the ability of lapatinib to reverse hormone resistance and to cooperate with endocrine therapy in providing maximal growth arrest strongly underpin the design of this study (27). Secondary end points in the trial will include objective response rate and clinical benefit rate, which should allow the effect of the combination on delaying disease progression to be detected. Other phase III clinical trials (Cancer and Acute Leukemia Group B 40302) are looking at the combination of lapatinib with fulvestrant, with trastuzumab based on encouraging preclinical and phase I data, or with tamoxifen in tamoxifen-resistant disease (Table 3).

Other approaches to target EGFR and HER2. Other small-molecule tyrosine kinase inhibitors that target EGFR and HER2 together are in development. AEE-788 is an orally active, reversible, small-molecule multi-targeted kinase inhibitor with an IC₅₀ of <100 nmol/L against EGFR, HER2, and vascular endothelial cell growth factor receptor 2. In a phase I pharmacokinetic and pharmacodynamic study in 69 patients, studies of pre- and posttreatment skin and tumor biopsies confirmed dose-dependent targeting and inhibition of tumor and skin EGFR signaling pathways, together with inhibition of endothelial cell pMAPK and Ki-67 (32). Our group have recently shown that combinations of AEE-788 with tamoxifen

Table 3. Current phase II/III clinical trials of EGFR and EGFR/HER2 tyrosine kinase inhibitors in combination with endocrine therapy in breast cancer

Trial sponsor	Trial design	Trial phase/clinical setting	No. patients	Primary end point
Second-line Rx				
Gefitinib				
NCI-CTEP	Gefitinib ± tamoxifen	Phase II RCT/MBC	46	CBR/PK
CTRC, San Antonio	Gefitinib + anastrozole	Phase II/MBC	78	ORR/PK
Lapatinib				
NCI-CTEP	Lapatinib + tamoxifen	Phase II/MBC	50	ORR/CBR
CALGB 40302	Fulvestrant ± lapatinib	Phase II RCT/MBC	60	ORR/CBR
First-line Rx				
Gefitinib				
AstraZeneca 0225	Tamoxifen +/- gefitinib	Phase II RCT/MBC	274	TTP
AstraZeneca 0713	Anastrozole +/- gefitinib	Phase II RCT/MBC	174	TTP
AstraZeneca 0223	Anastrozole +/- gefitinib	Phase II RCT/NeoAdj	180	Ki-67
EORTC 10021	Anastrozole +/- gefitinib	Phase II RCT/MBC	108	ORR/CBR
ECOG 4101	Anastrozole + gefitinib vs fulvestrant + gefitinib	Phase II RCT/MBC	106	ORR
Lapatinib				
GSK EGFR30008	Letrozole +/- lapatinib	Phase III RCT/MBC	760	TTP

Abbreviations: NCI-CTEP, National Cancer Institute Cancer Therapy Evaluation Program; CTCR, Cancer Therapy and Research Center; EORTC, European Organization for Research and Treatment of Cancer; ECOG, Eastern Cooperative Oncology Group; GSK, GlaxoSmithKline; ORR, objective response rate; CBR, clinical benefit rate; TTP, time to progression; PK, pharmacokinetics; CALGB 40302, Cancer and Acute Leukemia Group B 40302.

or letrozole in EGFR- and/or HER2-overexpressing breast cancer cells provide superior antiproliferative effects to either therapy alone (33). The addition of AEE-788 did not affect ER-mediated transcription and it has been suggested that any interaction is by an additive selective mechanism. Other small-molecule tyrosine kinase inhibitors that target both EGFR and HER2 include EKB-569 and the irreversible pan-erbB tyrosine kinase inhibitor CI-1033. A phase I trial of CI-1033 in 32 patients found dose-limiting toxicities of diarrhea, skin rash, and anorexia with a maximum tolerated dose of 450 mg in a 14/21 day cycle (34), and recruitment to a phase II study as monotherapy in advanced breast cancer has been completed.

Combined Endocrine Therapy and Targeting of Akt/mTOR

Role of Akt in endocrine resistance. The phosphatidylinositol-3-OH kinase/Akt pathway is a cell survival pathway that is also activated in hormone-resistant breast cancer. Akt (or protein kinase B) is a serine/threonine kinase that promotes cell survival and is activated in response to many different growth factors, including insulin, insulin-like growth factor I, basic fibroblast growth factor, EGF, heregulin, and vascular endothelial cell growth factor. Akt activates various downstream effectors, including antiapoptotic pathways, through phosphorylation of substrates that directly regulate the apoptotic machinery (i.e., Bad, caspase 9). In addition, mTOR is a downstream effector of the phosphatidylinositol-3-OH kinase/Akt signaling pathway that activates p70S6 kinase and 4E-binding protein-1, which in turn regulate transition through the G₁-S phase of the cell cycle. Approaches to targeting these cell survival pathways have included either specific phosphatidylinositol-3-OH kinase inhibitors, such as LY294002, or rapamycin analogues, such as temsirolimus (CCI-779) or everolimus (RAD-001), that target mTOR. Breast cancer cell lines with activated Akt (e.g., via loss of the regulatory *PTEN* tumor suppressor gene) have been shown to be especially sensitive to mTOR antagonism (35).

More recently, a rationale has emerged to support the combination of mTOR antagonists with either tamoxifen or an aromatase inhibitor in preclinical models of ER-positive hormone-sensitive and hormone-resistant breast cancer (36, 37). The estrogen-dependent growth of both wild-type MCF-7 and aromatase-expressing (MCF-7/Aro) breast cancer cells was inhibited in a dose-dependent manner by the mTOR antagonist everolimus (RAD-001), suggesting that mTOR signaling is required for the estrogen-dependent proliferation of these cells. In subsequent experiments with the MCF-7/Aro cells, the combination of letrozole and everolimus produced maximal growth inhibition with clear evidence for additive/synergistic effects (36). Evidence has emerged that activation of Akt/protein kinase B and the downstream mTOR pathway can cause resistance to tamoxifen (38, 39). MCF-7 cells expressing a constitutively active Akt were able to proliferate under reduced estrogen conditions and were resistant to the growth inhibitory effects of tamoxifen, both *in vitro* and *in vivo*, in xenograft models (37). However, cotreatment with temsirolimus inhibited mTOR activity and restored sensitivity to tamoxifen, primarily through induction of apoptosis, thus suggesting that Akt-induced tamoxifen resistance may in part be mediated by signaling through the mTOR pathway.

Clinical trials with mTOR antagonists. Single-agent activity with partial responses in 11% patients and stable disease in an additional 33% of patients has been reported for temsirolimus in 109 heavily pretreated patients with locally advanced or metastatic breast cancer (40). Based on the encouraging preclinical data for possible synergistic effects when mTOR antagonists were combined with endocrine therapy (36, 37), clinical trials examining combined therapy have been initiated. A three-arm randomized phase II study compared two different schedules of oral temsirolimus (10 mg daily or 30 mg for 5 days every 2 weeks) combined with letrozole to letrozole alone. Initial higher doses of temsirolimus were used but were poorly tolerated (grade 2/3 stomatitis) when combined with long-term endocrine therapy (41). Updated efficacy results have shown a nonsignificant enhanced clinical benefit rate of 80% for the combination compared with 69% for the letrozole alone (42). A large-scale phase III clinical trial with the oral formulation of temsirolimus (30 mg for 5 days every 2 weeks) in combination with letrozole compared with letrozole alone has started, and aims to recruit >1,200 postmenopausal patients with ER-positive, locally advanced or metastatic breast cancer suitable for first-line hormonal therapy with an aromatase inhibitor. The primary end point of this study will be to look for a 25% improvement in progression-free survival. Similar randomized phase III studies assessing letrozole with or without everolimus are planned both in the neoadjuvant and advanced breast cancer settings.

Optimism for the Future

There is much genuine enthusiasm surrounding targeted therapies in breast cancer and in particular their potential to further enhance the efficacy of current endocrine treatment for breast cancer. However, considerable thought is needed to maximize their potential. Central to their development will be a clear understanding of the molecular pathways, and preclinical models are important to better understand the benefit and utility of combined endocrine/signal transduction inhibitor therapy. For clinical trials, appropriate patient selection is crucial and translational studies are a requirement for development of these drugs. In particular, it is important in early clinical trials to prove that the target for a particular new drug is being hit and that inhibition of the target has the desired anticancer effect in the tumor. Ultimately, the randomized trial design remains central to proving that these therapies improve or enhance the efficacy of current treatments, and presurgical neoadjuvant/biomarker studies in particular are providing useful to gain relevant clinical and biological information about these novel combination strategies.

Open Discussion

Dr. Carlos Arteaga: There is a perception that lapatinib, as a dual EGFR/HER2 inhibitor, should be better than trastuzumab. However, there is not a lot of data on that in lung cancer, and it has been used in enough lung cancers to at least see a dramatic response in a few mutant tumors. The rash is not that prominent with this agent, so it may be a better HER2 kinase inhibitor than an EGFR inhibitor.

Dr. Johnston: Lapatinib is certainly a very good HER2-targeted therapy. The issue really is that the number of patients who have EGFR and HER2 overexpressed together is minimal. We don't really know how much EGFR is expressed and how best to measure it. Gefitinib will inhibit HER2-dependent growth because EGFR partners with HER2 and HER3. You may not need a lot of EGFR in a tumor that is driven by HER2 for inhibiting EGFR to make a difference. But the converse is not necessarily the case, which is the point that you are making. I don't think there is a lot of evidence that targeting HER2 will actually switch off HER1-driven tumors.

Dr. Arteaga: Anna Maria Storniolo from Indianapolis showed some data on the combination of trastuzumab plus lapatinib at American Society of Clinical Oncology. There was a >20% response rate in trastuzumab failures. Some of those patients were women who had failed trastuzumab/gefitinib in our Eastern Cooperative Oncology Group 1100 trial [Breast Cancer Res Treat 2004;88(Suppl 1):A-25].

Dr. Eric Winer: The problem here is that the small number of patients with HER2-positive metastatic disease makes it difficult to do a purely randomized trial. I was intrigued by the data about serum HER2 extracellular domain levels and response to lapatinib. My first reaction was to think that maybe it was a surrogate for time since trastuzumab. What do you think is going on there?

Dr. Johnston: I genuinely don't know. I also find those data about serum extracellular domain fascinating. You are right: there could well be a carryover effect because all these patients were selected as progressing on trastuzumab, so the data are perhaps not as clean as they could be. But there is still the fact that there were very significant shifts with 4 to 8 weeks of lapatinib alone, and it was highly selective in predicting who was going to respond and who was not. That must be telling us something biologically about what the drug is doing at the site of action. The subsequent studies are going to be investigating serum extracellular domain as an assay, so it may actually end up being quite helpful in terms of predicting response.

Dr. Mitch Dowsett: You showed data indicating that the ER-positive and progesterone receptor-positive tumors were less likely to respond. Given that it was just a univariate analysis, is that only telling us that progesterone receptor positivity/negativity correlated with HER2?

Dr. Johnston: Probably, because these patients were all trastuzumab failures, which suggests that an ER-negative/HER2-positive population was selected. I don't know that there are that many ER-positive/HER2-positive patients in there. But in the univariate analysis, there was a negative effect on likelihood of response. In other words, ER-negative patients were more likely to respond than ER positive.

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