Combinations of Endocrine and Biological Agents: Present Status of Therapeutic and Presurgical Investigations

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ABSTRACT
There is an increasing rationale to develop effective combinations of endocrine agents with novel therapeutics that target aberrant signal transduction pathways in estrogen receptor–positive breast cancer. Acquired resistance to endocrine therapy is associated with an increase in peptide growth factor signaling that results in crosstalk activation of estrogen receptor, and various signal transduction inhibitors (STI) can target these pathways to inhibit hormone-resistant growth. In experimental models of hormone-sensitive breast cancer, combinations of endocrine agents with STIs provide significantly greater growth inhibition than either alone, delaying the emergence of resistance. There are now several trials assessing the efficacy of combinations of tyrosine kinase inhibitors with various endocrine agents in the tamoxifen-resistant/second-line setting, together with five randomized phase II/III trials in the first-line setting. Similar work is ongoing with both farnesyltransferase inhibitors and mTOR antagonists where there are strong preclinical data to suggest additive or synergistic effects for either of these agents in combination with tamoxifen or estrogen deprivation therapies. More recently, presurgical studies with biological primary end points are being utilized as an alternative approach to investigate whether combined endocrine/STI therapy is a more effective strategy than endocrine therapy alone. This article reviews the rationale and current status of clinical trials in this area as well as the challenges that lie ahead for the development of these therapeutic combinations for breast cancer.

INTRODUCTION
Despite substantial improvements in the efficacy of endocrine therapy for breast cancer following the introduction of aromatase inhibitors (1), a major clinical issue remains initial (de novo) or subsequent (acquired) endocrine resistance (2). Recent research into the mechanisms of resistance has revealed that various growth factor pathways and oncogenes involved in the signal transduction cascade become activated and used by breast cancer cells to bypass normal endocrine responsiveness (3). As such, these pathways represent attractive targets for pharmacologic intervention with drugs that can inhibit the function of aberrantly or excessively expressed oncogene products. Figure 1 illustrates some of the key signal transduction pathways from cell surface–based growth factor receptors, intracellular kinase cascades, through the proteins that regulate both the cell cycle and transcription of genes involved in cell proliferation. In breast carcinomas that become resistant to endocrine therapy, estrogen receptor (ER) signaling still plays a crucial role in many cells, with evidence that various signaling pathways engage in crosstalk at several levels with the ER pathway (4). Several groups have shown that this interaction becomes the dominant pathway when breast cancer cells become hormone resistant, and that signal transduction inhibitors (STI) may inhibit the growth of these cells both in vitro and in vivo (5, 6).

ENHANCED SIGNAL TRANSDUCTION PATHWAYS AND ER ACTIVATION IN HORMONE-RESISTANT BREAST CANCER
Several intracellular kinases can become involved in the phosphorylation and activation of ER in hormone-resistant breast cancer (Fig. 1). The mitogen-activated protein kinase/extracellular signal-regulated kinase (MAPK/ERK) pathway, which may be activated by upstream growth factors such as human epidermal growth factor receptor 2 (HER2) and epidermal growth factor (EGF) receptor (EGFR); the phosphatidylinositol 3'-kinase (PI3K)/Akt pathway, which may be activated by insulin-like growth factor in addition to EGFR/HER2; pp90rsk; and the p38 MAPK pathway, activated by stress or various cytokines, can all phosphorylate and partially activate ER, in cells that coexpress ER and HER2/EGFR an enhanced agonist response to tamoxifen may occur with crosstalk activation of tamoxifen-ligated ER, resulting in tamoxifen-stimulated hormone-resistant growth (5).

Additional evidence has emerged that HER2 may itself become involved in activation of one of the major coactivators of ER-mediated gene transcription. Growth factor signaling may activate ER via phosphorylation and activation of the coactivator AIB1 (also called SRC-3), and evidence suggests that high levels of AIB1 may reduce the antagonist effects of tamoxifen (11, 12). Previous studies have shown that breast cancer cells that coexpress ER and HER2 are less responsive to tamoxifen (13), and recent clinical data suggested that coexpression of AIB1 with HER2 predicted for a worse outcome in patients treated with tamoxifen after surgery (12). In terms of mechanism, in vitro experiments have shown that in hormone-resistant, HER2-positive MCF-7 cells tamoxifen recruits coactivator complexes (ER, AIB1, CBP, and p300) to ER-regulated gene promoters, whereas in hormone-sensitive MCF-7 cells tamoxifen recruits...
Similar changes in intracellular signaling have been observed in clinical samples from breast cancer patients taken before tamoxifen and at the time of relapse on adjuvant tamoxifen several years later (14). In tumors with retained ER expression (the majority), there was enhanced expression of HER2 in some patients, with evidence that expression of the stress-activated kinase p38 MAPK was enhanced. Taken together, these data suggest that resistance to tamoxifen may be associated with increased activity of various upstream signaling pathways, which cause crosstalk activation of ER and tumor regrowth in the presence of tamoxifen.

Similar studies are now emerging to account for resistance to long-term estrogen deprivation (LTED), which in clinical terms relates to prolonged therapy with aromatase inhibitors. The laboratory data from several groups support the concept of a retained and hypersensitive ER as a means of eventual escape from estrogen deprivation (15, 16). In part, this is caused by an adaptive increase in ER expression and function, but there is also evidence for increased crosstalk between various growth factor receptor signaling pathways and ER analogous to the data described for tamoxifen resistance above. At the time of relapse, ER becomes activated and supersensitized to a number of different intracellular kinases, including MAPKs and the insulin-like growth factor/AKT pathway (15, 17, 18). Increased signaling from HER2/HER3, MAPK, and insulin-like growth factor receptors in cells that become resistant to LTED may activate residual enhanced levels of ER in a manner similar to that observed in cells with acquired tamoxifen resistance. Evidence to support this hypothesis is provided by increased levels of ER phosphorylated at Ser118, together with increased pp90RSK, which is one of the kinases involved in ER activation at Ser167 (10). It would seem that the ER remains an integral part of signaling even following failure of LTED. Thus, clinical and laboratory data support a concept that over time, breast cancer cells use alternative intracellular signaling pathways to enhance and activate ER signaling, and in particular that this allows cells to escape the growth-suppressive effects of their initial endocrine therapy.

Because enhanced expression of EGFR/HER2 and subsequent downstream MAPK activation has been found in breast cancer cells that become resistant over time to endocrine therapy with either tamoxifen or estrogen deprivation, treatment with various STI has been used in preclinical models in an attempt to treat this resistance by blocking up-regulated signaling pathways (19). In MCF-7 cells that developed resistance to tamoxifen, both gefitinib, which targets the internal tyrosine kinase domain of EGFR, and trastuzumab, which blocks the external domain of HER2, were effective at reducing downstream ERK1/2 MAPK signaling and inhibiting cell growth (6). Because EGFR and HER2 heterodimerize in the resistant cells, targeting either one of the receptors seemed to be an effective therapy. Of note, hormone-sensitive cells in which these receptors were not expressed were unaffected by either gefitinib or trastuzumab. Similar data have been reported by other groups in tamoxifen-resistant HER2-transfected MCF-7 cells with AG1478, a HER2 tyrosine kinase inhibitor, and with trastuzumab (20, 21). Likewise, in cells resistant to LTED, both growth- and ER-mediated gene transcription (which is enhanced 10-fold in cells that become resistant to LTED) can be abrogated by a number of different approaches to interrupt upstream signaling, including gefitinib, the MAPK kinase inhibitor UO126, and the ER down-regulator fulvestrant, which degrades residual estrogen receptor (15).

Other signaling and cell survival pathways are also activated in hormone-resistant breast cancer, in particular the PI3K/Akt pathway. Akt (or PKB) is a serine/threonine kinase that promotes cell survival and is activated in response to many

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**Fig. 1** Crosstalk between signal transduction pathways and ER signaling in endocrine-resistant breast cancer, with opportunities for targeted intervention. Estrogen (E2)-liganded ER activates E2-regulated genes in classical pathway (solid arrow), but following long-term tamoxifen therapy resistance can develop with bidirectional crosstalk (open arrows) between ER and growth factor receptors, with association of membrane bound ER with growth factor receptors, and/or IGFR or EGFR/HER2 activation of ER phosphorylation. Stars show various targeted therapies (AI, aromatase inhibitors; SERDs, selective estrogen receptor down-regulators; Modb, monoclonal antibodies; TKI, tyrosine kinase inhibitor; FTI, farnesyl transferase inhibitor; CCI, cell cycle inhibitor).
different growth factors including insulin, insulin-like growth factor-1, basic fibroblast growth factor, EGF, heregulin and vascular endothelial growth factor. Once activated, Akt exerts antiapoptotic effects through phosphorylation of substrates that directly regulate the apoptotic machinery (i.e., Bad and caspase 9). In addition, the mammalian target of rapamycin (mTOR) is a downstream effector of the PI3K/Akt signaling pathway that activates p70S6 kinase and 4E-binding protein-1, which in turn regulate translation through the G1-S phase of the cell cycle. Several breast cancer cell lines contain a constitutively activated PI3K/Akt pathway due to either upstream HER2 overexpression, loss of the regulatory PTEN suppressor gene, or overexpression of Akt itself; these factors may be associated with resistance to HER2- or EGFR-targeted therapies and to endocrine therapy with tamoxifen (22). Likewise, ER-positive breast cancer cells that develop resistance to tamoxifen acquire elevated levels of Akt- and MAPK-activated pp90rsk when compared with wild-type parental MCF-7 cells (23). Approaches to targeting these cell survival pathways have included either specific PI3K 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 PTEN suppressor gene) are especially sensitive to mTOR antagonism (24), and clinical trials in breast cancer with both temsirolimus and everolimus are in progress (see below).

Taken together, these experimental data indicate that activation of various signaling pathways may account for endocrine resistance in breast cancer. This has prompted a number of phase II clinical studies in advanced breast cancer that have investigated different STI approaches, usually in pretreated patients, including those with hormone-resistant disease.

CLINICAL STUDIES OF STIs AS MONOTHERAPY IN THE SECOND-LINE TAMOXIFEN-RESISTANT SETTING

To date the main emphasis in the clinical development of STIs for breast cancer has been with small molecule receptor tyrosine kinase inhibitors (TKI), farnesyl transferase inhibitors (FTI), and drugs that target regulation of the cell cycle such as the mTOR antagonists. However, proof of principle in breast cancer for therapies that target growth factor pathways has already been provided with trastuzumab (Herceptin), the monoclonal antibody to the external domain of HER2. Clinical use of trastuzumab has been exclusively in HER2-positive breast cancer, most of which tends to be ER negative, and in practice trastuzumab in metastatic breast cancer is often given in combination with taxane-based or other chemotherapy. However, significant activity for trastuzumab has been seen as first-line monotherapy, with up to 48% clinical benefit rate in HER2/HER3-positive tumors, including a 34% objective response rate in tumors reported positive by fluorescence in situ hybridization (25). In this trial of 114 patients, 57% had received prior endocrine therapy either in the adjuvant or advanced breast cancer setting, many of whom would have developed endocrine resistance at the time of entry in the trial of trastuzumab as first-line therapy. With some evidence that HER2 may become up-regulated in tumors that were originally ER positive/HER2 negative, there is now a greater rationale to study trastuzumab in the endocrine-resistant setting. Few, if any, clinical studies have addressed this specific ER-positive population with trastuzumab monotherapy, as assumptions tend to be based on the HER2-negative status of the primary tumor. At least two clinical trials, however, are examining the role of trastuzumab in combination with aromatase inhibitors in the hope that combined inhibition of ER and HER2 signaling may be a more effective treatment for patients with tumors that coexpress both receptors (see below).

At least four inhibitors of EGFR tyrosine kinase are in clinical development for breast cancer, including gefitinib (Iressa), which is an orally active low molecular weight synthetic anilinoquinoxaline and a potent selective inhibitor of EGFR-TK. In various breast cancer cell lines that express EGFR and/or HER2, gefitinib given as a single agent induced a dose-dependent antiproliferative effect, which delayed growth (26). Experiments have shown that gefitinib may inhibit the growth of acquired endocrine-resistant MCF-7 breast cancer cells in vitro (6, 15, 27) and in vivo (5). There have been three phase II monotherapy studies of gefitinib in patients with advanced breast cancer (28-30). Overall, the data are relatively disappointing with low clinical response rates and short times to disease progression (Table 1). The only trial to report a significant number of responses included patients with ER-positive tamoxifen-resistant breast cancer (30), the setting in which preclinical models had shown the best evidence of activity for gefitinib (6). Pharmacodynamic studies have been done in one of these trials, confirming that EGFR tyrosine kinase signaling is inhibited in both skin and tumor biopsies by doses of gefitinib delivered orally (29). However, there was discordance in the effect of gefitinib on downstream intracellular signaling in treated tumor biopsies, with lack of inhibition of Ki-67 (a marker of cell proliferation) in tumor but not in matched skin biopsies. This suggested that activation (in breast cancer as opposed to normal skin cells) of other intracellular pathways downstream of EGFR may determine the clinical response to gefitinib. More research is required to establish tumor phenotypes in responding versus nonresponding patients (31). For example, overexpression of the PI3K/Akt pathway may possibly play an important role in resistance to tyrosine-kinase inhibitor therapy.

Fewer clinical data exist regarding the other three EGFR TKIs in breast cancer. A phase II monotherapy trial of the selective EGFR-TKI erlotinib (OSI-774) in breast cancer was

<table>
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<th>Agent</th>
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<th>Number of patients</th>
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<th>CBR (%)</th>
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<td>7</td>
<td>24</td>
<td>(PF at 16 wk)</td>
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Abbreviations: ORR, objective response; CBR, clinical benefit rate; TTP, time to disease progression.
relatively disappointing (32). Lapatinib (GW572016) is a dual inhibitor of EGFR and HER2 (33), and in the phase I study where diarrhea and skin rash were the main toxicities, clinical activity was reported in two trastuzumab-resistant breast cancer patients (34). A phase II trial of lapatinib is ongoing in patients with advanced breast cancer who had progressed on prior trastuzumab-containing regimens and were heavily pretreated with either chemotherapy or hormonal therapy. 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 (35). Finally, canertinib (CI-1033) is a selective and irreversible pan-erbB inhibitor that targets HER1, HER2, HER3, and HER4. Clinical activity has been shown in solid tumors in phase I studies with an acceptable side effect profile (36), and phase II studies of different doses/schedules are under way in metastatic breast cancer.

As for other STIs, monotherapy activity for the FTI tipifarnib has been reported in advanced breast cancer: 76 patients were treated with tipifarnib either as a continuous dose of 300 or 400 mg twice daily (\( n = 41 \)), or an intermittent dose of 300 mg twice daily for 21 days followed by 7 days off-therapy (\( n = 35 \); ref. 37). In the continuous treatment arm, there were four partial responses (10%) lasting between 4 and 12 months and six patients with stable disease (15%) for at least 6 months. In the intermittent treatment arm, there were five partial responses (14%) and three patients with stable disease (9%). The main toxicities were neutropenia, thrombocytopenia, neurotoxicity, and fatigue. These results were independent of ras status, estrogen/progesterone receptor or HER2/EGFR receptor status, and 40% of patients had received only prior adjuvant and/or metastatic endocrine therapy at entry into the trial. Thus, whereas FTIs may have modest antitumor activity as a single agent therapy, stabilization of disease in those resistant to endocrine therapy may represent a meaningful response.

Finally, single agent activity has been documented for the mTOR antagonist temsirolimus (CCI-779) in a preliminary i.v. dose-finding phase II trial in patients with locally advanced or metastatic breast cancer (38). The main toxicities included alteration in transaminases, mucositis, rash, and mild nausea. Further clinical trials with orally active formulations of temsirolimus in combination with aromatase inhibitors are ongoing (see below).

**PRECLINICAL RATIONALE FOR COMBINATIONS OF ENDOCRINE THERAPY AND STIs**

The evidence above strongly suggests that enhanced signal transduction pathways may be one of the key adaptive changes accounting for endocrine-resistant growth in breast cancer, and that STIs could treat resistant tumor growth. In contrast, several reports have implied that in hormone-sensitive breast cancer, STIs as monotherapy may have only a minimal effect on tumor growth, and indeed in ER-positive tumors could be less effective than endocrine therapy (39). This could relate to ER signaling being the dominant determinant of growth in hormone-sensitive cells, with an absence of the adaptive changes in signal transduction pathways described above. However, in vitro data from Gee et al. (39) suggest that in hormone-sensitive cells, combined treatment with tamoxifen and the TKI gefitinib may provide greater antiproliferative and proapoptotic effects than tamoxifen alone. Analysis of downstream signaling showed that the combination of tamoxifen and gefitinib provided maximal and near complete inhibition of phosphorylated ERK1/ERK2 MAPK and Akt, together with greater G0-G1 arrest and suppression of the cell-survival protein bcl-2 than that observed with just tamoxifen. In particular, combined therapy prevented the acquired up-regulation of EGFR/MAPK signaling that occurred after 5 weeks in tamoxifen alone–treated cells. The authors concluded that combined endocrine/TKI therapy could prove a more effective strategy than either therapy alone, and in particular could delay the emergence of acquired resistance related to EGFR/ER crosstalk activation.

Other STIs that have only a minimal effect on hormone-sensitive breast cancer may also be more effective when combined with endocrine therapy. The FTI tipifarnib inhibits the growth of a number of human breast cancer cell lines in vitro, most of which contain normal wild-type ras genes (40). In vivo tipifarnib produced a modest cytostatic effect on hormone-sensitive MCF-7 xenograft growth, with evidence of induction of apoptosis and enhanced expression of the cell cycle inhibitory protein p21 (41). In contrast, when tipifarnib was combined with tamoxifen or estrogen deprivation therapy, combined treatment induced significantly greater tumor regression compared with either endocrine therapy alone (42). The mechanism for any interaction of FTIs with endocrine therapy remains unclear. In the xenograft experiments, analysis of the excised tumors showed significantly lower cell proliferation (Ki-67 scores) without any enhanced apoptosis (42). Three other groups have since reported a similar interaction for FTIs with tamoxifen or aromatase inhibitors and have suggested either a synergistic (43) or an additive antitumor effect (44). One recent study implied an additive effect on G0-G1 cell-cycle arrest, and that the FTI-277 when combined with tamoxifen maintained higher levels of the cyclin-dependent kinase inhibitor p21 (waf/cip1), resulting in an additive effect on inactivation of cyclin E/Cdk2 complexes and decreased phosphorylation of pRb (45). These emerging data have led initiation of several clinical trials in breast cancer to investigate the combination of an FTI with endocrine therapy (see below).

A similar rationale has emerged to support the combination of mTOR antagonists with estrogen deprivation therapy in preclinical models of ER-positive hormone-sensitive breast cancer (46). The estrogen-dependent growth of both wild-type MCF7 and aromatase-expressing (MCF7/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 MCF7/Aro cells, the combination of letrozole and everolimus produced maximal growth inhibition, with clear evidence for additive/synergistic effects for the combination over either therapy alone (46). As such, randomized clinical trials of everolimus and letrozole are now planned in both the advanced breast cancer and presurgical neoadjuvant settings (see below).
For hormone-resistant breast cancer, in particular ER-positive cells that overexpress HER2, the strategy of combined STIs and endocrine therapy may also be more effective than using STIs alone. Evidence has come from three independent groups who have investigated ER/HER2–positive breast cancer cells that are known to be tamoxifen resistant. Arteaga’s group first reported that signal transduction blockade using a HER2 tyrosine kinase inhibitor (AG1478) or a MAPK inhibitor (U0126) may abrogate antiestrogen resistance (20). In particular, they provided compelling evidence that combined treatment with tamoxifen and either STI was significantly more effective than either therapy alone, not only at inhibiting estrogen-mediated gene transcription and tumor colony survival in vitro, but also at delaying tumor xenograft growth in vivo (20). More recently Osborne’s group reported that whereas hormone-resistant MCF-7 cells with up-regulated HER2 signaling are sensitive to the TKI gefitinib, combined therapy of gefitinib and tamoxifen provided maximal growth inhibition and significantly delayed the time to progression of the disease (5). Using an in vivo model of MCF-7/HER2 overexpressing xenografts, similar effects were seen when gefitinib combined with estrogen deprivation, which provided greater inhibition of growth and substantially delayed acquired resistance compared with estrogen deprivation alone (47). Finally, a synergistic effect has recently been reported for trastuzumab when combined with tamoxifen in ER/HER2–positive BT-474 breast cancer cells (48). In particular, there was synergistic growth inhibition in vitro for combined trastuzumab/tamoxifen, with enhanced accumulation of cells in G0–G1 and reduction in S phase of the cell cycle compared with either therapy alone. Of interest, there was no evidence for any induction of apoptosis, data that are consistent with the interaction of FTIs with tamoxifen.

CURRENT CLINICAL TRIALS OF ENDOCRINE THERAPY WITH OR WITHOUT STIs IN BREAST CANCER

Based on the evidence outlined above, a number of small phase I/II trials were initiated with either trastuzumab, TKIs, or FTIs in combination with tamoxifen, fulvestrant, or an aromatase inhibitor (Table 2). Some of these trials are in the post-tamoxifen setting, with at least two trials enrolling patients whose tumor is progressing on tamoxifen, then adding either the FTI tipifarnib or the TKI lapatinib to tamoxifen to see if clinical responses can be observed and resistance reversed. Another trial will compare gefitinib alone to the combination of gefitinib plus tamoxifen after progression on tamoxifen. Three other phase II trials in this setting are studying the combination of aromatase inhibitors with STIs, including trastuzumab, gefitinib, and temsirolimus, whereas two trials will study fulvestrant in combination with either tipifarnib or lapatinib.

In all of these phase II studies clinical response is the primary end point, with stable disease for at least 6 months being an additional end point in some studies. Whether these are appropriate end points for such trials is discussed below. It is unlikely that any overlapping toxicities will be seen for combinations of STIs with endocrine therapy, and at least two trials are ensuring no pharmacokinetic interactions exist whereby tamoxifen- or aromatase inhibitor–induced hepatic enzymes could enhance clearance of STIs and lower serum concentrations. For example, pharmacokinetic and pharmacodynamic end points have been assessed using a sequential design in 11 patients treated initially with the FTI tipifarnib (either 200 or 300 mg twice daily for 21 of 28 days) and after 1 week tamoxifen was added (49). There was no significant change in the pharmacokinetic profile for tipifarnib, and moreover, the pharmacodynamic end point (inhibition of farnesyl transferase in peripheral blood mononuclear cells) was enhanced from 30% enzyme suppression to 41% by the combination. In addition, such trials may help determine the optimal schedule for the combination. For example, a small three-arm randomized study has compared two different schedules of oral temsirolimus (10 mg continuous or 30 mg for 5 of 14 days) combined with letrozole. Initial higher doses of temsirolimus were used but were poorly tolerated (grade 2/3 stomatitis) when combined with long-term endocrine therapy (50). Preliminary results have suggested similar response rates for the combination compared with letrozole alone. However, clinical efficacy end points in small nonrandomized phase II studies must be viewed cautiously, and the primary role of such studies should be to provide safety and supportive biological data for the combination in advance of definitive randomized trials.

Table 2

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<tr>
<th>Trial sponsor</th>
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<th>Trial phase</th>
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Abbreviations: RCT, randomized controlled trial; ORR, objective response rate; CBR, clinical benefit rate; PK, pharmacokinetics; PD, pharmacodynamics.
The ultimate clinical test for the hypothesis that STIs enhance the efficacy of endocrine therapy is the randomized controlled clinical trial. Many of these trials are in the first-line setting, where clinical and experimental data have shown that STIs alone may have limited activity. Therefore, the primary end point for these trials is to investigate whether time to disease progression (TTP) can be significantly prolonged by the addition of an STI to endocrine therapy, thus delaying the emergence of resistance as shown in various preclinical models described above. Table 3 lists the 12 current randomized, controlled clinical trials of endocrine therapy with or without STIs in advanced breast cancer. The majority are placebo-controlled, double-blind studies, except the trastuzumab trials, which utilize an open design because of the i.v. delivery of the STI. Many are randomized phase II studies with only 100 to 200 patients, and in several studies, the primary efficacy end point is objective response rate. In the first-line ER-positive setting such studies are asking the combination to provide greater initial antitumor activity than endocrine therapy alone, hopefully enhancing the response in tumors with de novo endocrine resistance. Given the mechanism of action of these drugs in combination (i.e., enhanced G0-G1 arrest without enhanced apoptosis), an enhanced clinical benefit rate may be a better end point for these trials, with more stable disease, which ultimately will contribute to prolonged TTP.

Two of the endocrine/TKI trials illustrate some important considerations in the design of so-called first-line studies (Figs. 2 and 3). First, it will be important to stratify for prior endocrine therapy (usually tamoxifen) in the adjuvant setting and in particular the interval since completion of such therapy. This is important as it may have implications for the presence or absence of activated signaling pathways in the relapsed tumor, which could determine the efficacy of the STI. Second, biological studies are required to help predict for those patients more likely to benefit from combined endocrine/STI therapy. Both the gefitinib/tamoxifen and lapatinib/letrozole trials will undertake such studies (Figs. 2 and 3).

### Table 3: Current/planned randomized phase II/III clinical trials of endocrine therapy ± STIs in locally advanced/metastatic breast cancer

<table>
<thead>
<tr>
<th>Trial sponsor/no.</th>
<th>Trial design</th>
<th>Trial phase</th>
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Abbreviations: TTP, time to disease progression; PFS, progression-free survival; ORR, objective response rate; CBR, clinical benefit rate.

*Open label study.
Suppression of the proliferation marker Ki-67 after 2 and 12 weeks was significantly greater with anastrozole than with tamoxifen ($P = 0.004$ and $P < 0.001$) but similar between tamoxifen and the combination ($P = 0.600$ and $P = 0.912$). In this larger study, there was no significant correlation overall between a fall in Ki-67 and clinical tumor response. However, the 2-week change in Ki-67 in the neoadjuvant IMPACT trial closely paralleled the relative recurrence-free survival after a median 31 months in the adjuvant ATAC trial that compared these same agents in the adjuvant setting (56). For the first time, therefore, change in an intermediate biomarker in an endocrine therapy trial may predict for the subsequent long-term outcome and provide a quicker and more reliable outcome measure than clinical response criteria alone.

This approach is now being tested for combined endocrine/STI therapy in two separate presurgical studies. Figure 5 shows the design of a multicenter double-blind, randomized, controlled trial that will assess whether the addition of the TKI gefitinib to anastrozole will improve its efficacy. A novel aspect of this trial is that for the first time the primary end point will be change in cell proliferation as assessed by Ki-67. In addition, the design will look at whether the change in Ki-67 after just 2 weeks of therapy can predict long-term benefit of therapy for the combination (arm A) versus endocrine therapy alone (arm C), and in arm B whether the addition of gefitinib after an initial 2 weeks of therapy with anastrozole can further lower Ki-67 in those tumors that do not respond to initial aromatase inhibitor therapy. Secondary end points will include clinical response, tolerability, and pharmacokinetics, as well as proteomics and gene expression profiles that may predict for response to therapy. Proposals are also underway for a similar neoadjuvant study comparing letrozole with the combination of letrozole and the mTOR antagonist everolimus (RAD-001), based on preclinical evidence for an additive/synergistic interaction for the combination (46).

**CHALLENGES FOR CLINICAL DEVELOPMENT OF STIs**

There is much enthusiasm surrounding these novel STIs and their role in the management of breast cancer. However, considerable thought is needed in order to maximize their potential and ensure that disappointments seen for the combination of these agents with chemotherapy in other solid tumors are not repeated. Central to their development will be a clear understanding of the molecular biology of these pathways, in particular the differences between hormone-sensitive and hormone-resistant disease. Preclinical models both in vitro and in vivo are important to better understand the benefit and utility of combined endocrine/STI therapy. For clinical trials, appropriate patient selection will be important, and parallel biological studies are now an obligate requirement for development of these drugs. Concern remains about whether the appropriate clinical end points are always chosen in advanced breast cancer trials, and phase II studies should be used to determine the optimum dose and schedule for a given STI, to ensure that there are no pharmacokinetic or safety concerns, and possibly to examine intermediate pharmacodynamic end points. Randomized studies remain central to determine the added benefit for combined endocrine/STI therapy and must be appropriately powered for relevant end points in order to encourage further testing.
tumors are not truly the development of resistance. Months, and here an STI in combination could prevent or delay were some low Ki-67 levels that then came back up again by 3 of patients in the IMPACT study were showing a fall in Ki-67 upfront is going to be better than an AI alone. The majority which at relapse. I do have concerns as to whether an AI with an addressing the science of which combination to give first and what is better than current standard therapy, rather than always treatment of choice, so the regulatory authorities wish to know are comparing it against. Today, AIs are becoming the frontline the FDA with the registration package, you must say what you are implying that AIs work so well that they may not need a tyrosine kinase inhibitor (TKI) as the partner drug, but I have seen data presented by Mitch Dowsett in the IMPACT study for the cohort of patients who overexpress HER2. In those patients, the proliferation rate in the surgical specimen, as measured by Ki-67 after 12 weeks of neoadjuvant anastrozole, was already going way up relative to the pre-therapy Ki-67. In the study by Ellis et al., for the patients who were ErbB2 gene amplified by fluorescence in situ hybridization, Ki-67 was up after 4 months of neoadjuvant letrozole relative to the pretherapy measurement. We have data with MCF-7 cells overexpressing aromatase and HER2. These cells are clearly resistant to letrozole in transcription and short-term growth assays. All these data would imply that HER2 overexpression can result in early escape from AIs and that there is a rationale for AIs in combination with TKIs.

**OPEN DISCUSSION**

**Dr. Carlos Arteaga:** You are implying that AIs work so well that they may not need a tyrosine kinase inhibitor (TKI) as the partner drug, but I have seen data presented by Mitch Dowsett in the IMPACT study for the cohort of patients who overexpress HER2. In those patients, the proliferation rate in the surgical specimen, as measured by Ki-67 after 12 weeks of neoadjuvant anastrozole, was already going way up relative to the pre-therapy Ki-67. In the study by Ellis et al., for the patients who were ErbB2 gene amplified by fluorescence in situ hybridization, Ki-67 was up after 4 months of neoadjuvant letrozole relative to the pretherapy measurement. We have data with MCF-7 cells overexpressing aromatase and HER2. These cells are clearly resistant to letrozole in transcription and short-term growth assays. All these data would imply that HER2 overexpression can result in early escape from AIs and that there is a rationale for AIs in combination with TKIs.

**Dr. Stephen Johnston:** The reality is that when you go to the FDA with the registration package, you must say what you are comparing it against. Today, AIs are becoming the frontline treatment of choice, so the regulatory authorities wish to know what is better than current standard therapy, rather than always addressing the science of which combination to give first and which at relapse. I do have concerns as to whether an AI with an STI upfront is going to be better than an AI alone. The majority of patients in the IMPACT study were showing a fall in Ki-67 with AI. You are right that in the HER2-positive group, there were some low Ki-67 levels that then came back up again by 3 months, and here an STI in combination could prevent or delay the development of resistance.

**Dr. Arteaga:** Maybe these ER-positive, HER2-positive tumors are not truly de novo resistant, but they escape the AI sooner compared to ER-positive, low-HER2 tumors. Molecularly, this is very plausible. In that setting, an initial clinical response to AIs may not be too different between HER2-positive and low-HER2 tumors, but time to progression may well differ because of the ability of the HER2-positive tumors to escape the AI sooner.

**Dr. Kent Osborne:** Other than in the HER2-overexpressing subset, there is not a shred of preclinical or clinical data to show that adding an EGFR inhibitor like gefitinib or an HER2 inhibitor like trastuzumab will do anything to augment estrogen deprivation treatment either in vivo or in vitro. That is because estrogen deprivation already blocks the crosstalk, since you are removing the ligand for the membrane receptor. Now, in HER2 it is different because it provides such a powerful growth factor stimulus. Increasing HER2 activity is down-regulating the ER and getting rid of it, leading to the development of a hormone-independent tumor, a process that can be markedly delayed by adding a growth factor inhibitor. In all these metastatic studies, we are using one pathway inhibitor—gefitinib seems to be the favorite—and there are many redundant pathways that can achieve the same end. I am concerned that in the metastatic disease setting, these combinations may not show much efficacy. That may not mean that we ought to get rid of that paradigm, but it is simply going to show us that the agent by itself doesn’t work in metastatic disease. That is why we need to change our approach for studying these new agents from the way we used to study chemotherapy, which was in the far advanced metastatic setting.

**Dr. Johnston:** But that is the patient population where there is often the need to do something different. My concern, similarly, is that a company involved with developing such a drug might say “oh, this drug doesn’t work in breast cancer” and will stop. With gefitinib we can go into the neoadjuvant setting because there are safety data from thousands of patients treated for lung cancer. But that is not the case with some of the farnesyl transferase inhibitors (FTIs); as you know, there was an issue with peripheral neuropathy with a continuous dosing of tipifarnib that was solved by giving it in intermittent schedules. IRBs are not going to be happy to approve studies in potentially curable patients that are using agents with the liability of unusual toxicities. So the drugs that have gotten into the neoadjuvant study model have often got a host of safety data behind them.  

**Fig. 4 Endocrine Rx ± STI Neoadjuvant Trial Design. Schema of randomized neoadjuvant clinical trials in ER+ postmenopausal primary breast cancer to determine the additional benefit of STIs to aromatase inhibitors (AI) on various clinical, radiological, and molecular endpoints.**

**Fig. 5 Phase II Neoadjuvant Anastrozole ± Gefitinib (ZD1839IL/0223) Trial Design. Design of a presurgical, randomized, placebo-controlled trial of anastrozole with or without gefitinib in postmenopausal women with operable ER-positive primary breast cancer.**
Dr. Osborne: The thing that is different in this setting compared to chemotherapy of the past is that these drugs have been developed to hit a specific target, and many of them are hitting a very similar pathway, so we have learned from the first of these agents what the toxicities are likely to be. We can predict what the side effects of MAP kinase or Akt inhibitors are going to be, so we don’t need to have 1000 patients treated for years. For example, do you need to have a 10-year toxicity profile for toremifene? No. You know what the side effects are going to be from tamoxifen. So I think it is different than investigating totally new drugs like chemotherapy drugs of the past.

Dr. Richard Santen: I have the sense that we don’t know for sure what these drugs are doing and we need to sample tissue in these ongoing studies to test emerging hypotheses. For example, the most recent data suggest that FTIs block mTOR, which appears to stimulate apoptosis very potently. So I think it is important in the FTI studies to look at the tissue to see if mTOR is being blocked.

Dr. Johnston: When we did the FTI monotherapy study in 76 patients, we built into that design biopsying advanced disease where we could, which tended to be skin biopsies of local or regional recurrence. We actually only got paired biopsies in about 14 patients, and then the meaningful data become very slim when the clinical benefit rate is only around 25%. This is why biopsying in advanced disease is fraught with problems. Designing a trial with biopsy as an integral end point, in other words making sure you recruit patients with regional metastases, makes doing that trial very difficult. It selects for a subgroup of patients with the more slow-growing, indolent skin and chest wall metastases. So I come back to the point about the biology being better assessed in early stage breast cancer, either in the 3-month neoadjuvant model or what we call the incidental model, which is 2 weeks of presurgical treatment and then examining the changes in the excised tumors. As soon as you have safety data for an oral agent, you can move into that study design to get the biological data quicker.

Dr. Osborne: The animal models are a perfect segue into testing the biological concepts that have been shown in vitro, which would probably be the place to go in terms of studying the FTIs and mTOR. The mTOR/apoptosis mechanism is something that could be demonstrated in an animal model.

Dr. Arteaga: But one could do the same in patients treated presurgically. The benefit from these studies is that they can allow you to exclude patients who are unlikely to benefit from a novel drug. For example, we have a small study with the EGFR inhibitor erlotinib in which women with newly diagnosed operable breast cancer are treated with the inhibitor for 7 to 14 days immediately before tumorectomy. In some of them, tumor cell proliferation as measured by Ki-67 is dramatically inhibited. Interestingly, in the overwhelming majority of them, phospho-MAPK goes away, implying that the erbB pathway is a major input to MAPK, and that erlotinib is working biochemically, an important control for molecular target validation. In the few numbers that we have, inhibition of proliferation appears limited to the ER-positive tumors. If our results hold with an increasing number of patients, exclusion of patients with ER-negative tumors from EGFR inhibitor trials is going to enrich phase II studies to obtain a more informative result.

Dr. Osborne: You hit on a statement that I think should be recorded in our consensus. Most of the agents we are discussing that need to go forward are applicable only to ER-positive disease. Other than the few ER-negative patients who over-express HER2, I don’t think we have a clue as to what is driving ER-negative tumors. I think we need to make an effort in identifying unique pathways like that.

Dr. Johnston: That really comes back to the principle of targeting new therapy to the appropriate patient population, whereas a lot of these so-called signaling agents have gone straight into unselected patient populations without a rationale.

Dr. Aman Buzdar: I think all of us would like to evaluate these agents in early stage disease, but from the human protection point of view, we need to have reasonable safety data before they can be given to patients with early stage disease for even a short period of time. I am not aware of a single drug in oncology in the past 30 years that does not show any activity in metastatic setting and then does wonders in early stage disease.

REFERENCES


Combinations of Endocrine and Biological Agents: Present Status of Therapeutic and Presurgical Investigations

Stephen RD Johnston


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