Inhibition of erbB Receptor (HER) Tyrosine Kinases as a Strategy to Abrogate Antiestrogen Resistance in Human Breast Cancer

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Abstract

It has been proposed that binding of ligand to the estrogen receptor (ER) releases its association with transcriptional corepressors, allowing the ER to recruit coactivators, which possess histone acetylase activity, and induce transcription of gene promoters containing estrogen response elements. It has also been proposed that the antiestrogen tamoxifen recruits transcriptional corepressors to the AF-2 region of the hormone-binding domain of the ER, thus blocking ER-mediated transcription. The ER cross-talks with a number of mitogenic signaling pathways and second messengers, like the epidermal growth factor receptor, the insulin-like growth factor-I receptor, mitogen-activated protein kinase, phosphatidylinositol-3 kinase/Akt, dopamine, and cyclic AMP. Some of these molecules may: (a) support ligand-independent ER transcription; (b) increase the association of ER with coactivators of transcription; and/or (c) reduce the antiestrogen-induced association of ER with corepressors. These events either alone or in combination may result in hormone independence and/or antiestrogen resistance. We have examined whether signaling by HER2/neu (erbB-2) receptor tyrosine kinase, which can induce antitumor resistance, can also disrupt the tamoxifen-induced interaction of ER with transcriptional corepressors. Notably, tamoxifen-induced association of ER with the transcriptional corepressors N-CoR or SMRT was reduced in HER2-overexpressing breast tumor cells but not in cells with low HER2 levels. Small molecule inhibitors of the HER2 kinase or MAP extracellular signal-regulated kinase 1/2 or dominant-negative MAP extracellular signal-regulated kinase 1/2 constructs restored the inhibitory effect of tamoxifen on both ER-mediated transcription and tumor cell proliferation. Treatment with both tamoxifen and the small molecule HER1/2 kinase inhibitor AG1478 reduced mitogen-activated protein kinase activity and markedly reduced growth of established MCF-7/HER2 xenografts in athymic nude mice. Similar results have been obtained with ZD1839 (‘Iressa’), an epidermal growth factor receptor (HER1) tyrosine kinase inhibitor. Taken together, these data suggest that exogenous inhibitors of the HER-signaling network and other mitogenic pathways can abrogate or delay the emergence of antiestrogen resistance, thus providing an evaluable therapeutic strategy in human breast carcinoma.

Introduction

The classical ERα belongs to the superfamily of nuclear steroid receptors that function as transcription factors on binding by their respective ligands (1). The ER contains 595 amino acids with a central DNA-binding domain and a COOH-terminal, hormone-binding domain. More recently, a smaller 350-amino acid ERβ was cloned (2). Binding of estradiol to ER induces conformational changes in the receptor and ER dimerization with subsequent high-affinity binding to DNA through their DNA-binding domain at specific EREs present in the promoter of estrogen-responsive genes. ER-induced gene transcription is mediated by two distinct transactivation domains: the hormone-independent AF-1 region in the NH2 terminus and the hormone-dependent AF-2 region in the COOH-terminal region of the receptor (3, 4). Studies with ER mutants have shown that, although AF-1 and AF-2 are required for maximal ER transcriptional activity in most cells, certain gene promoters, sometimes as a function of their cellular context, can be independently transactivated by AF-1 or AF-2 alone (5). This promoter and/or cell dependence of ER-mediated transcription appears to be modulated by the recruitment of coactivator and corepressor proteins, which serve as signaling intermediates between the DNA-bound receptor dimer and the basal transcriptional machinery (Refs. 1, 6, and references therein). These coactivators and corepressors of transcription possess intrinsic histone acetylase or histone deacetylase activities, respectively. Coactivators can acetylate histones, loosen their interaction with DNA and, therefore, expose motifs required for gene transcription. On the other hand, corepressor molecules can silence steroid-responsive genes by binding to the unliganded receptor, deacetylating histones, and potentially keeping the DNA in a conformation that occludes coactivator binding sites.

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1 The abbreviations used are: ER, estrogen receptor; ERE, estrogen response element; SERM, selective estrogen receptor modulator; EGF, epidermal growth factor receptor; EGF, epidermal growth factor; MAPK, mitogen-activated protein kinase; PI3K, phosphatidylinositol-3 kinase; MEKK, mitogen-activated protein kinase kinase kinase; MEK, mitogen-activated protein extracellular signal-regulated kinase; JNK, c-Jun-NH2-terminal kinase.

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Molecular Basis for the ER Agonist/Antagonist Role of Tamoxifen

The antiestrogen tamoxifen antagonizes the transcription of genes that require the AF-2 region for ER-mediated transactivation. However, for gene promoters in which AF-2 function is dispensable and whose transcription is driven by the AF-1 domain, tamoxifen can function as an agonist (5, 7). This ability of tamoxifen to behave as an AF-2 antagonist and AF-1 agonist explains its antagonistic effect in breast epithelium, as well as its agonistic effect in other tissues, such as endometrium, bone, and liver. In addition to blocking AF-2-dependent gene transactivation, in some experimental systems, tamoxifen has been shown to repress ER-mediated transcription by recruiting corepressor proteins to the receptor (8, 9). In other experiments, overexpression of transcriptional coactivators, like SRC-1 or L7/SPA, results in enhancement of tamoxifen-stimulated ER reporter activity (8, 10). Because of their ability to either repress or activate the transcription of estrogen target genes, mixed antagonist/agonists like tamoxifen are now more appropriately referred to as SERMs.

The data summarized above imply that the net cellular response to SERMs may depend on the tissue repertoire, availability, and/or relative amounts of transcriptional coactivators and corepressors. Therefore, high levels of coactivators or low levels of corepressors may be permissive for the agonist activity of SERMs. These results suggest that the recruitment of coactivators or corepressors in different ratios to the ER-ERE complex could introduce a potential element of transcriptional control and diversity that may be more marked in some estrogen-responsive genes than others. Finally, these data have led to the evaluable hypothesis that de novo or acquired abundance of coactivators and/or the reduction or loss of transcriptional corepressors in breast tumor cells may result in hormone independence and antiestrogen resistance (11). This hypothesis, illustrated in Fig. 1, is supported by a recent experiment reported by Lavinsky et al. (9). On prolonged treatment with tamoxifen, ER-positive MCF-7 xenografts in athymic nude mice developed resistance to treatment and grew during continuous tamoxifen therapy. Tumor levels of N-CoR were reduced in the tamoxifen-resistant xenografts, suggesting that reduced levels of the corepressor may enable tamoxifen to stimulate ER-mediated transcription through unopposed AF-1 function (9). Interestingly, 4-hydroxytamoxifen failed to activate ER-dependent transcription in wild-type mouse embryonic fibroblasts but acted as a full agonist in N-CoR-null mouse embryonic fibroblasts. Introduction of an N-CoR expression vector was sufficient to reverse this effect (12), further implying that N-CoR is required for the ER-antagonistic effect of 4-hydroxytamoxifen and, second, that the ability of tamoxifen to function as an ER agonist may require functional inactivation of N-CoR.

Cross-Talk between ER and Peptide Growth Factor Receptor Signaling Pathways

A number of mitogenic signaling pathways have been shown to interact with ER transcriptional complexes and induce hormone-independent ER activation or, in some cases, limit or reverse tamoxifen-induced repression of ER transcription. Activators of protein kinase A or inhibitors of protein phosphatases are able to stimulate hormone-independent ER transcription, as well as that induced by tamoxifen-occupied ERs (13). Neurotransmitters like dopamine or SKF-38393 can induce ER reporter activity in the absence of added steroids (14). Exogenous EGF or overexpression of the EGFR-homologous HER2/neu (erbB2) proto-oncogene can reduce tamoxifen-induced association of ER with corepressors and thus limit the growth-inhibitory action of tamoxifen against breast carcinoma cells (9, 15). Arouica et al. (16) reported that insulin-like growth factor-I is also a potent activator of ERα and that this response could be blocked by pure antiestrogens, such as ICI 164,384. Activation of the Ras/MAPK signaling pathway, a frequent alteration in transformed cells, has been shown to phosphorylate Ser118 in the ER, leading to hormone-independent activation of the ER with loss of the inhibitory effect of tamoxifen on ER-mediated transcription (17, 18). Of note, EGF blocked the tamoxifen-induced association of wild-type ER with N-CoR but failed to block the association of a mutant S118A ER with N-CoR (9), implicating EGF-activated MAPK as the signal mediating the inhibition of tamoxifen action. Overexpression of PI3K and the PI3K-dependent serine/threonine kinase Akt activates ERα in the absence of added estrogen (19). Although PI3K increased the activity of both AF-1 and AF-2

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Fig. 1 A molecular hypothesis to support sensitivity or resistance to SERMs. In tamoxifen-sensitive tumor cells (A), corepressors predominate over coactivators in tamoxifen-occupied ERs. In this setting, the tamoxifen-mediated inhibitory effect on AF-2 is dominant, resulting in a blockade of hormone-induced gene transcription. With progression to SERM resistance (B), the ER coactivator:corepressor ratio is reversed, resulting in a loss of tamoxifen-mediated inhibition of AF-2 and/or unmasking of its agonistic effect on AF-1 function (modified from Takimoto et al., Ref. 11).
regions, Akt enhanced the activity of only the hormone-independent AF-1 domain. Moreover, Akt overexpression protected MCF-7 breast cancer cells from tamoxifen-induced apoptosis (19).

Other nonreceptor kinases downstream of neurotransmitter or (HER) erbB receptors have been implicated in dysregulation of ER function. Activation of the Src nonreceptor tyrosine kinase, which is elevated in the majority of human breast cancers, markedly enhances the transcriptional activity of the unliganded ER and the tamoxifen-ER complex. This effect appeared in part mediated by the ability of Src to increase the AF-1-coactivating function of CREB-binding protein and the glucocorticoid receptor-interacting protein (20). Expression of wild-type MEKK1 and an active Rac1 increased the activity of the unliganded ER. Moreover, expression of constitutively active MEKK1 increased the agonistic activity of 4-hydroxytamoxifen to a level comparable to that of 17β-estradiol and fully blocked its antagonistic activity (21). Finally, phosphorylation of the SMRT corepressor by MEKK1 and MEK1 inhibited the ability of SMRT to tether to its transcription partners. These kinases also induced a redistribution of SMRT from the nucleus to a perinuclear/cytosolic compartment, further contributing to loss of its corepressor function (22). These results suggest that different elements of non-ER mitogenic signaling pathways can modulate ER function in some cases by modifying steady-state levels and/or the posttranslational state of coactivators/corepressors of ER transcription. In general, the identification of the specific steroid-responsive genes whose expression is altered as a result of the cross-talk between ER and other signal transduction systems requires further investigation. Nonetheless, these data suggest that, in the context of parallel oncogenic signaling pathways, the estradiol- or SREB-bound ER-ERE-coactivator-corepressor complex can behave as a platform for multiple inputs leading to transcriptional diversity and the selection of hormone-independent phenotypes.

**HER (erbB) Tyrosine Kinase Receptors and Antiestrogen Resistance**

The HER2/neu (erbB2) transmembrane receptor tyrosine kinase has been causally associated with hormone independence and antiestrogen resistance of human breast cancer cells. This kinase is the protein product of the HER2 proto-oncogene and a member of the EGF receptor (HER1, erbB1) family of transmembrane tyrosine kinases, which also includes HER3 (erbB3) and HER4 (erbB4). On binding of ligands to the EGFR, HER3, or HER4, HER2 is recruited as the preferred partner of these ligand-bound receptors into active, phosphorylated heterodimeric complexes, which activate several signaling pathways involved in the proliferation and survival of tumor cells (Ref. 23, 24, and references therein). Cytoplasmic signal transducers induced by the HER network include PLC-γ1, Ras-Raf-MEK-MAPKs, PI3K and Akt, Src, the stress activated protein kinases, PAK-JNK-JNK, and the signal transducers and activators of transcription (23, 24). Of note, aberrant activation of these molecules has been shown to dysregulate ER transcription (above). HER2 can transform normal mammary epithelial cells and is overexpressed in a cohort of breast tumors, where it is associated with a more virulent behavior and poor patient outcome (24).

Multiple lines of experimental evidence suggest that overexpression of HER2 confers antiestrogen resistance to breast tumor cells. MCF-7 human breast cancer cells transfected with either a full-length HER2 cDNA or with ectopic heregulin-β1, the HER3 ligand that activates HER2, lose sensitivity to tamoxifen or estrogen dependence (15, 25–27). Several clinical studies have shown that metastatic tumors with high HER2 expression and/or high circulating levels of the HER2 ectodomain exhibit a statistically lower clinical response rate and/or a shorter duration of response to antiestrogen therapy (28–36), further associating higher tumor levels of the proto- oncogene with resistance to endocrine therapy. Two studies, however, failed to show a reduced clinical response to tamoxifen in HER2-overexpressing tumors (37, 38). The first of these studies, however, did show a statistically shorter time to tamoxifen treatment failure in patients bearing cancers with ≥10% HER2-positive cells by immunohistochemistry compared with patients whose tumors exhibited lower levels of HER2 (37). In the second study, patients with lymph node-positive, nonmetastatic breast cancer were assigned to tamoxifen therapy or not by physician discretion after completion of standard adjuvant chemotherapy. The lack of randomization and the immediately preceding therapy potentially confounded the rigorous interpretation of the results of this trial (38). The discrepancies between these last two reports and the former group of studies (28–36) could be explained by the variable and sometimes low number of patients in these studies, different antiestrogen therapies used, variable methods to assess HER2 overexpression, and the failure to rigorously exclude ER-negative tumors, among other potential inconsistencies. Nonetheless, a recent meta-analysis of ~1200 breast cancer patients, 71% ER positive and 6.2% ER unknown, showed a 2.8 odds ratio of treatment failure in tumors with high levels of HER2 compared with low-expressing tumors, as defined by clinical progression within 6 months of initiation of endocrine therapy (39).

**Inhibition of HER2 and MAPKs Restores Tamoxifen Action against Antiestrogen-resistant Breast Tumor Cells**

Two studies have reported the ability of antibodies against the ectodomain of HER2 to increase the inhibitory effect of tamoxifen or the pure antiestrogen ICI 182,780 against breast tumor cells with or without HER2 overexpression (40, 41). We have extended these reports by studying the mechanisms by which HER2 signaling confers antiestrogen resistance to ER-positive breast tumor cells. These results (15) will be summarized next. Forced expression of HER2 in MCF-7 breast cancer cells resulted in MAPK hyperactivity and tamoxifen resistance. Inhibition of HER2 and MAPKs with the HER1/2 kinase inhibitor AG1478 (42) and the MEK1/2 inhibitor U0126, respectively, as well as by transient transfection of dominant-negative MEK1/2 constructs, restored the inhibitory effect of tamoxifen on ER-reporter activity and colony survival. Similar results were observed in BT-474 human breast cancer cells, which naturally exhibit HER2 gene amplification. Both AG1478 and U0126 also restored the tamoxifen-mediated association of ER with the corepressors N-CoR (15) and SMRT4 in the antiestrogen-resistant MCF-7/HER2-18 cells. Finally, only treatment with the

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4 C. L. Arteaga, unpublished observations.
combination of tamoxifen and the HER2 kinase inhibitor reduced tumor MAPK activity and completely prevented growth of HER2-overexpressing MCF-7/HER2-18 xenografts in athymic mice in the absence of any host toxicity.

In this study, several results implicated MAPK signaling as the mediator of antiestrogen resistance in the HER2-overexpressing tumor cells: (a) overexpression of HER2 in MCF-7 cells resulted in both activation of MAPK and antiestrogen resistance; (b) inhibition of MAPK with U0126 enhanced the ability of tamoxifen to inhibit both ER-mediated transcription and colony formation of MCF-7/HER2-18 and BT-474 cells; (c) dominant-negative mutants of MEK1 and MEK2 also enhanced the inhibitory effect of tamoxifen on ER-mediated transcription; and (d) AG1478 markedly reduced active MAPK in MCF-7/HER2-18 xenografts, and this reduction was temporally associated with tamoxifen-induced growth restraint of tumors in vivo.

MAPK has been shown to posttranslationally modify the ER by phosphorylating Ser118 (17, 18). We were unable to detect ER phosphorylation at Ser118. However, we have transfected wild-type ER and S118A ER-luciferase constructs into ER-negative, HER2-overexpressing SKBR-3 breast cancer cells. In these cells, tamoxifen failed to inhibit hormone-induced, wild-type ER reporter activity but completely inhibited hormone-induced S118A ER luciferase expression. These results clearly suggest that Ser118 phosphorylation is indeed involved in HER2-mediated repression of tamoxifen action. Neither HER2 overexpression nor inhibition of HER2 and MAPK altered ER levels, estradiol-induced ER reporter activity, or estradiol-induced proliferation, suggesting that the restoration of tamoxifen action was independent of ER levels or their intrinsic responsiveness to hormones. Of note, however, tamoxifen-induced association of ERα with N-CoR was markedly reduced in HER2-overexpressing cells compared with controls. Inhibition of HER2 or MAPK restored the antiestrogen-induced association of ER with N-CoR. This result implies that antiestrogen resistance in HER2-overexpressing tumor cells can, at least in part, be explained by unrestrained MAPK signaling that alters the association of ER with corepressors of transcription (Fig. 2). The molecular mechanisms by which MAPK regulates these protein-protein associations require further study. In a more recent report, however, another HER2-transfected MCF-7 cell line (27) exhibited significant down-regulation of ER levels, presumably sensitivity to antiestrogens (43). This result suggests that HER2 hyperactivity may be able to subvert the antiestrogen response by additional mechanisms involving ER down-regulation. Nonetheless, the data presented imply that: (a) MAPK may play a causal role in HER2-induced tamoxifen resistance in ER-positive breast tumor cells; and (b) that exogenous inhibitors of HER2 signal transduction can partially abrogate the antiestrogen-resistant phenotype, providing an evaluative therapeutic strategy in hormone-dependent breast cancer.

**Clinical Strategies to Inhibit HER (erbB) Receptor Signaling in ER-positive Mammary Tumors**

The causal association of HER2 with antiestrogen resistance, the preclinical data summarized above, and the availability of HER2 inhibitors provide a strong basis for the testing of combinations of HER2 inhibitors with antiestrogens in ER-positive breast cancers that overexpress HER2. We should note, however, that HER2 is a member of a highly interactive signaling network (23, 24) and that other members of this network can also mediate per se escape from antiestrogen therapy, e.g., transfection of EGFR into hormone-dependent MCF-7 and ZR75-1 breast cancer cells also induced tamoxifen resistance (44, 45). Furthermore, ER-positive, EGFR-positive tumors exhibit a lower response to antiestrogens compared with ER-positive, EGFR-negative tumors (46). For the other two members of this receptor family, HER3 and HER4, there are no reports associating them with antiestrogen resistance in breast cancer. In a recent report, Gee et al. (47) showed that MCF-7 cells selected in vitro for resistance to tamoxifen acquired markedly elevated levels of EGFR and HER2 compared with wild-type antiestrogen-responsive controls. Tamoxifen resistance did not occur if the selection was done in the presence of the small molecule EGFR tyrosine kinase inhibitor ZD1839 ("Iressa"). In addition, low concentrations of ZD1839 inhibited EGFR and HER2 phosphorylation and tumor cell growth (47). Taken together, these data provide a rationale for blocking not only HER2 but also other receptors and/or signal transducers in the HER network to maximally abrogate antiestrogen resistance. In addition, these results suggest that the abrogation of antiestrogen resistance by blockade of the HER network may not be limited to HER receptor-overexpressing tumor cells. The possibility that the clinical response of ER-positive tumors with low levels of EGFR and HER2, such as MCF-7 cells, may also benefit from blockade of the HER network has important implications for the selection of patients into trials testing this approach (see below). One anti-EGFR therapeutic strategy is based on the premise that EGFR receptors with mutations in the ATP-binding site lack tyrosine kinase function and do not display a full range of ligand-induced biochemical responses (23), implying that the...
receptor’s tyrosine kinase is essential for transformation. Therefore, in an attempt to generate antireceptor compounds, small molecules that compete for the Mg-ATP binding site of the catalytic domain of the EGFR tyrosine kinase have been identified by random screening of natural or synthetic compound libraries for specific EGFR kinase inhibitory activity (48). Table 1 shows a (partial) list of the small-molecular-weight EGFR tyrosine kinase inhibitors currently in clinical development. The concentrations of these compounds that inhibit the EGFR kinase in vitro are in the nM or sub-nM range, whereas, with some exceptions, the concentrations required to inhibit the >80% homologous HER2 kinase are logarithms higher (Table 1), supporting their overall EGFR specificity. This homology among HER receptors like EGFR and HER2, however, has been exploited in some cases for the generation of bifunctional inhibitors like CI1033, EKB-569, and GW2016. Chemical modification of some of these structures has led to the generation of irreversible inhibitors that bind covalently to specific cysteine residues in the ATP-binding pocket of either EGFR or HER2, such as CI1033 and EKB-569. In principle, a bifunctional inhibitor with low IC₅₀ against both EGFR and HER2 might be more attractive to block both kinases in tumor cells. However, recent data with the specific EGFR inhibitor ZD1839 have shown that it can effectively inhibit HER2 phosphorylation in vitro at concentrations that would not have been predicted from its reported IC₅₀ against the HER2 kinase in vivo shown in Table 1 (56). This result suggests that the EGFR may be a transactivating kinase for the ligandless HER2 receptor in tumor cells. It implies further that EGFR-specific inhibitors are a defensible strategy to block the HER2 kinase in tumor cells that also express EGFR.

To support this speculation, we have tested the EGFR kinase inhibitor ZD1839 in combination with 4-hydroxytamoxifen against MDA-361 and MCF-7/HER2 breast cancer cells in a colony-forming assay. These cells express EGFR and high levels of HER2 but are only modestly growth inhibited by high concentrations (1 µM) of 4-hydroxytamoxifen. In both tumor cell lines, the inhibitory effect of tamoxifen on colony formation was increased by the addition of 1 µM ZD1839 (Fig. 3). At this concentration, ZD1839 inhibited HER2 phosphorylation without altering HER2 levels as measured by phosphotyrosine and HER2 immunoblot analyses of HER2 precipitates (data not shown). Based on these cumulative data, there is a strong rationale for randomized Phase III trials of an antiestrogen or an aromatase inhibitor with or without HER kinase inhibitors as first-line therapy in patients with metastatic ER- or Progesterone receptor-positive breast tumors and any levels of EGFR or HER2. To address whether inhibition of EGFR/HER2 reverses acquired antiestrogen resistance, patients progressing on the SERM or aromatase inhibitor arm can be switched to the combination arm of such study and evaluated for clinical benefit (Fig. 4). The choice of the small molecule kinase inhibitor may depend on the toxicity profile of the small molecule, its ability to inhibit both EGFR (HER1) and HER2 in vivo, and its pharmacokinetic properties, as well as other issues beyond the scope of this report. Nonetheless, it is not unreasonable to expect that blockade of peptide growth factor receptor and other oncogenic signaling pathways will have a profound impact in the natural history of hormone-dependent breast cancer.

**Table 1** Small molecule inhibitors of the EGFR tyrosine kinase in clinical development

<table>
<thead>
<tr>
<th>Compound</th>
<th>EGFR IC₅₀ (µM)ᵃ</th>
<th>HER2 IC₅₀ (µM)ᵇ</th>
<th>Ref.</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>CI1033 (PD183805)</td>
<td>0.0014</td>
<td>0.009</td>
<td>49, 50</td>
<td>Pfizer</td>
</tr>
<tr>
<td>ZD1839 (&quot;Iressa&quot;)</td>
<td>0.033</td>
<td>&gt;3.7</td>
<td>51, 56</td>
<td>AstraZeneca</td>
</tr>
<tr>
<td>OSI-774</td>
<td>0.02</td>
<td>0.35</td>
<td>52</td>
<td>OSI/Genentech</td>
</tr>
<tr>
<td>EKB-569</td>
<td>0.039 ± 0.007</td>
<td>1.3 ± 0.16</td>
<td>53</td>
<td>Wyeth-Ayerst</td>
</tr>
<tr>
<td>PKI-166 (CP59326)</td>
<td>0.025</td>
<td>NRᵇ</td>
<td>54</td>
<td>Novartis</td>
</tr>
<tr>
<td>GW2016</td>
<td>0.016</td>
<td>0.009</td>
<td>55</td>
<td>Glaxo SmithKline</td>
</tr>
</tbody>
</table>

ᵃ Concentration required to inhibit 50% of the EGFR kinase in vitro.
ᵇ Concentration required to inhibit 50% of the HER2 kinase in vitro.
ᶜ NR, not reported.

Fig. 3 ZD1839 enhances tamoxifen-mediated inhibition of tumor colony survival. MDA-361 or MCF-7/HER2-18 cells were plated as a single-cell suspension in 0.8% agarose/10% fetal calf serum/25 mM Hepes in 35-mm dishes (3 × 10⁴ cells/dish), as described previously (42). Where indicated, 4-OH tamoxifen and/or ZD1839 were added at the time of plating. Controls contained 0.1% ethanol and 0.1% DMSO to correct for the respective diluents of 4-OH tamoxifen and ZD1839. Dishes were incubated in a humidified CO₂ incubator at 37°C, and colonies measuring ≥50 µm in diameter were counted after 7 days using an OMNICON 3800 Tumor Colony Analyzer (Biologics, Gainesville, FL). Bar, the mean colony number of four dishes ± SD. These results were reproduced in each cell line in two independent experiments.
Open Discussion

**Dr. Kent Osborne:** You haven’t mentioned tamoxifen. When you use tamoxifen with one of these kinase inhibitors, you have the same antiestrogen effects as with an aromatase inhibitor, but you still have the advantages on bone and other parameters.

**Dr. Arteaga:** One reason is that fewer and fewer patients with metastatic disease have tamoxifen as first-line therapy. Certainly that has hurt recruitment for a trial we have at Vanderbilt. We recently closed a Vanderbilt Cancer Center Network trial of tamoxifen plus Herceptin in ER+ HER2/neu-positive metastatic disease. The trial accrued poorly until we changed tamoxifen for an aromatase inhibitor.

**Dr. Stephen Johnston:** If the aromatase inhibitors have the response rate that Dr. Ellis cites, 88% in ER+, HER1/2+ disease, maybe the control arm is going to have as high a response rate as the experimental arm.

**Dr. Arteaga:** That is possible, but I doubt it. In the preclinical models, the response to estrogen deprivation in HER2-overexpressing tumors is dramatic but very short-lived.

**Dr. Johnston:** That’s why progression-free response is the important end point.

**Dr. Osborne:** If resistance to tamoxifen is frequently related to its intrinsic “agonist” activity, it might be augmented by the growth factor pathways after a period of treatment. If you could overcome that by concomitant blockade, then tamoxifen plus kinase inhibitor would be a better combination to use in adjuvant patients than aromatase inhibitor or antitumor growth factor inhibitor, because of the bone-sparing effects and the other advantages that tamoxifen offers. So tamoxifen may be a worse drug right now because of its intrinsic agonist activity. If you can get rid of that agonist activity with fressa or another newer agent, then you’ve got an ideal treatment.

**Dr. Matthew Ellis:** The experiment is a good one, and in fact this might be something you could do in the perioperative treatment-naïve setting, comparing a week or 2 of tamoxifen versus tamoxifen plus tyrosine kinase inhibitor to get some data on cell growth and apoptosis. But it is tough to accrue patients to tamoxifen trials in the metastatic setting, because many patients are becoming resistant to tamoxifen during their adjuvant treatment or soon after.

**Dr. Johnston:** You may not need that many patients, though. There really is a major effect on time to progression. You may not need the power if you have good clinical response numbers. It may be difficult to do, but it seems to me a very important question.

**References**

4442s Aromatase Inhibition/Inactivation of the interaction between HER2 and the response to endocrine therapy


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