

Crosstalk between the Estrogen Receptor and the HER Tyrosine Kinase Receptor Family: Molecular Mechanism and Clinical Implications for Endocrine Therapy Resistance

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Breast cancer evolution and tumor progression are governed by the complex interactions between steroid receptor [estrogen receptor (ER) and progesterone receptor] and growth factor receptor signaling. In recent years, the field of cancer therapy has witnessed the emergence of multiple strategies targeting these specific cancer pathways and key molecules (ER and growth factor receptors) to arrest tumor growth and achieve tumor eradication; treatment success, however, has varied and both *de novo* (up front) and acquired resistance have proven a challenge. Recent studies of ER biology have revealed new insights into ER action in breast cancer and

have highlighted the role of an intimate crosstalk between the ER and HER family signaling pathways as a fundamental contributor to the development of resistance to endocrine therapies against the ER pathway. The aim of this review article is to summarize the current knowledge on mechanisms of resistance of breast cancer cells to endocrine therapies due to the crosstalk between the ER and the HER growth factor receptor signaling pathways and to explore new available therapeutic strategies that could prolong duration of response and circumvent endocrine resistant tumor growth. (*Endocrine Reviews* 29: 217–233, 2008)

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I. Introduction

BREAST CANCER EVOLUTION and progression are deeply influenced by both estrogen receptor (ER) and growth factor receptor signaling. In recent years, the field of cancer therapy has witnessed the emergence of multiple targeted strategies that inhibit specific key molecules and pathways important for tumor growth and progression. Among them, endocrine therapy to block ER activity and signaling, the first targeted therapy in oncology, is still the most successful systemic therapy in the management of ER-positive breast cancer.

Tamoxifen, which binds to and antagonizes ER, has been the mainstay of endocrine (hormonal) therapy in both early and advanced breast cancer patients for almost three decades (1, 2). Recently, its role has also expanded to preventive therapy in patients at high risk of developing the disease (3). Unfortunately, however, approximately 50% of patients with advanced disease do not respond to first line treatment with the selective estrogen receptor modulator (SERM) tamoxifen (*de novo* or up front, intrinsic resistance). Furthermore, almost all patients with metastatic disease and many that receive

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Abbreviations: AF, Activation function; AI, aromatase inhibitor; EGFR, epidermal growth factor receptor; ER, estrogen receptor; ERE, estrogen response element; HB-EGF, heparin-binding epidermal growth factor-like growth factor; IGFR, IGF-I receptor; MISS, membrane-initiated steroid signaling; MMP, matrix metalloproteinase; MNAR, modulator of nongenomic action of estrogen receptor; mTOR, mammalian target of Rapamycin; NCoA, nuclear receptor coactivator; PgR, progesterone receptor; PI3K, phosphatidylinositol 3-kinase; SERM, selective ER modulator; STI, signal transduction inhibitor; TKI, tyrosine kinase inhibitor.

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tamoxifen as adjuvant therapy eventually experience tumor relapse and die from their disease (acquired resistance). Thus, *de novo* and acquired resistance to tamoxifen occur frequently in breast cancer patients and seriously limit the efficacy of this treatment.

Aromatase inhibitors (AIs) are a class of drugs that inhibit the enzyme aromatase, which is responsible for converting androgens (produced by women in the adrenal glands) to estrogen, thereby lowering the circulating estrogen, and perhaps the tumor levels of estrogen. By depriving ER of its estrogen ligand (4–6), AIs inhibit tumor growth and are proving superior to tamoxifen at least in certain patient subsets (2, 7–9). However, the response rate to these compounds is only slightly higher than the response rate to tamoxifen in patients with advanced breast cancer, and both *de novo* (*i.e.*, immediate) and acquired resistance after an initial response commonly occur.

The membrane tyrosine kinase HER2 (c-ErbB2, HER2/neu) is gene-amplified in 20–25% of ER-positive breast cancer (10). There is clinical evidence that tamoxifen is less effective in HER2-positive tumors (11–13). Furthermore, pre-clinical models show that HER2 overexpression can cause tamoxifen-stimulated growth as a mechanism of *de novo* resistance (14–17) and that the HER family receptors are also implicated in acquired resistance to this drug (18). Advanced studies of ER biology have revealed new insights into ER action in breast cancer and have highlighted the role of an intimate crosstalk between the ER and the epidermal growth factor receptor (EGFR/HER1)/HER2 signaling pathways as a fundamental contributor to the development of resistance to endocrine therapies (19–21).

Accumulating knowledge of the mechanisms by which breast cancer cells become resistant to endocrine therapy, coupled with the availability of new compounds that can interfere with the growth factor-driven signaling pathways involved in resistance to endocrine therapy, might lead to the development of new strategies for treatment of ER-positive breast cancer patients (5). The aim of this review article is to summarize the current knowledge on mechanisms of breast cancer resistance to endocrine therapies, particularly those that are due to the crosstalk between the ER and growth factor receptor signaling pathways (focusing on the EGFR/HER2 pathway), and to explore emerging therapeutic strategies that prolong duration of response and circumvent endocrine resistant tumor growth.

II. Biology of the Estrogen Receptor (ER)

A. ER α and ER β subtypes

The ER signaling pathway and its estrogen ligands are believed to be key players in the etiology and progression of breast cancer. There are two different ER proteins, ER α and ER β , that are produced by distinct genes. Whereas clinical and experimental studies have confirmed the crucial role of ER α (22) in breast malignancies, the role of ER β in breast cancer is still controversial (23). Nonetheless, studies indicate that ER β can antagonize ER α activity (24) and suggest that reduced levels of ER β protein are associated with resistance

to tamoxifen therapy (25). If not otherwise specified, “ER” will refer to “ER α ” in the remainder of the manuscript.

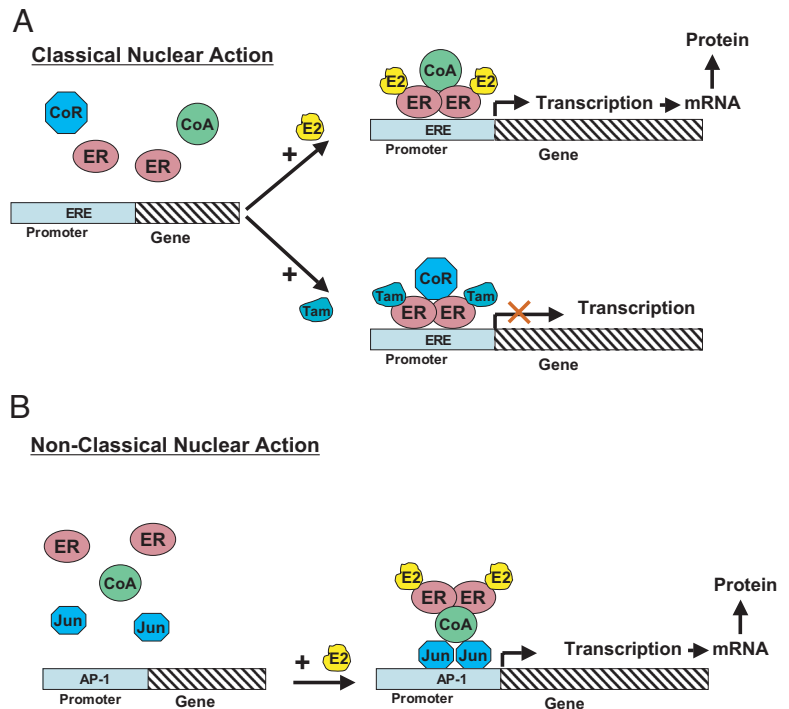
B. Genomic action of ER

ER is mainly a nuclear protein that shares a common structural and functional organization with many other nuclear receptors (22). Through its genomic nuclear activity, also known as nuclear-initiated steroid signaling (NISS) (26), ER functions as a ligand-dependent transcription factor and promotes expression of a variety of genes (19). Many of these gene products directly promote breast cancer cell proliferation and survival and tumor progression. Examples are the IGF-I receptor (IGFR), the cell cycle regulator cyclin D1, the antiapoptotic factor Bcl-2 (21, 27, 28), and the proangiogenic vascular endothelial growth factor (20, 29). Nuclear ER also induces the expression of different HER and other growth factor receptor ligands including TGF α and amphiregulin (30), which are able to bind and activate EGFR (31). Recently, microarray analysis of gene expression in the human breast cancer ER-positive MCF-7 cell lines suggests that in response to estrogen, ER is also able to *inhibit* expression of a subclass of genes (32), many of these being transcriptional repressors, or genes with antiproliferative or proapoptotic function. The ER protein is a composite of multiple domains including a DNA-binding domain and two major transcriptional activation function (AF) domains, AF-1 and AF-2, which usually act synergistically, although some gene promoters have been shown to be activated independently by AF-1 or AF-2 (22, 33). The antineoplastic effects of endocrine therapeutic agents are largely mediated by countering or eliminating the transcriptional effects on gene expression of estrogen when bound to ER.

Ligand binding to ER induces a specific conformational change in the receptor, releases it from an inhibitory complex consisting of several chaperone proteins (34), and triggers receptor dimerization (34). This change further facilitates the binding of coregulatory proteins (35) that alter ER transcriptional activity on specific consensus DNA elements [also known as estrogen response elements (EREs)], which are present in the promoter regions of target genes (classic action, Fig. 1A). In particular, the transcriptional activity of ER is enhanced by the binding of coactivators such as members of the p160 family of nuclear receptor coactivators [*e.g.*, nuclear receptor coactivator 1 (NCoA1 or SRC-1), NCoA2 (SRC-2), and NCoA3 (AIB1, SRC-3, TRAM1, RAC3, p/CIP or ACTR) (36, 37) (Fig. 1A)]. These proteins lead to the formation of large complexes that enhance ER-driven transcription by different mechanisms, including recruitment of histone-acetyltransferases that modulate the chromatin structure at the promoter site (36).

In contrast to estrogens, estrogen antagonists induce a distinct receptor conformation leading to ER association with corepressor complexes, such as nuclear-receptor corepressor 1 (NCoR1) and NCoR2 (SMRT), rather than with coactivators, thereby shutting off gene transcription (38, 39) (Fig. 1A). Interestingly, SERMs, including tamoxifen and raloxifene, have mixed agonist/antagonist activity and may either stimulate or antagonize ER function depending on the tissue, cell, and gene context (40).

FIG. 1. Nuclear genomic ER activity. ER, in its classical action (A), directly binds to DNA sequences called estrogen response elements (EREs) residing in the promoter region of target genes, and by recruiting coregulatory proteins regulates gene transcription. Estrogen (E2)-bound ER generally recruits coactivator (CoA) complexes to induce gene transcription, whereas estrogen antagonists such as tamoxifen (Tam) mostly lead to ER association with corepressor (CoR) complexes, thereby turning off gene transcription. In its nonclassical action (B), ER regulates gene transcription via protein-protein interaction (*e.g.*, with Fos/Jun family members) that tether ER to DNA sites responsive to other transcription factors such as AP-1. Together, all of these nuclear ER genomic activities also are called nuclear-initiated steroid signaling (NISS).



Many coregulatory proteins may be present at rate-limiting levels in the nucleus, so that changes in their level of expression and/or activity can lead to alterations of ER signaling. In particular, overexpression of coactivators and down-regulation of corepressors can negate the inhibitory effects of endocrine therapy, especially in the case of SERMs (41–45). In this regard, recent studies have observed that high AIB1 expression in patients who received tamoxifen adjuvant therapy was associated with an inferior clinical outcome, which is indicative of tamoxifen resistance (12, 46).

In addition to the “classical” mode of action of ER regulating the expression of genes that harbor EREs in their promoter region, ER can also regulate gene transcription at DNA sites responsive to other transcription factors (40). Via this so-called “nonclassical” mode, nuclear ER protein interacts with other transcription factors such as specificity protein 1 (SP-1) and members of the Fos/Jun activating protein 1 (AP-1) transcription complex, leading to regulation of gene expression at non-ERE regulatory DNA sequences (Fig. 1B) (40, 47, 48).

Importantly, signaling from different growth factor receptor-dependently kinases phosphorylates various factors in the ER pathway, including ER itself; this potentiates ER genomic signaling activity on gene transcription. As an example, kinase-induced phosphorylation of nuclear ER on serine 305 (49–51) enhances cyclin D1 transcription in breast cancer. Similarly, activation of the growth factor-dependent signaling of p42/44 MAPK (ERK 1/2) and phosphatidylinositol 3-kinase (PI3K)/AKT leads to an increase in ER serine 118 and serine 167 phosphorylation and ER AF-1 activity (52–54). This phosphorylation of ER and its coregulatory proteins by growth factor receptor-dependent kinases is an essential component of the ordinary regulation and function of genomic ER activity. However, in the presence of hyperac-

tive growth factor receptor signaling, as often occurs in breast cancer (*e.g.*, HER2 overexpression), an excessive phosphorylation of ER and its coregulators may severely weaken the inhibitory effects of various endocrine therapies and lead to endocrine resistance, as will be detailed in *Sections II and III* of this review.

C. Nongenomic rapid ER activity

Estrogen, as well as some SERMs like tamoxifen, has also been shown to exert rapid stimulatory effects on a variety of signal transduction pathways and molecules. This rapid nongenomic activity, also called MISS (for membrane-initiated steroid signaling) (26), begins outside the nucleus and is initially independent of gene transcription. The identity and mode of function of the receptors responsible for this steroid-induced rapid signaling are not completely clear at this point. In the case of estrogen, however, it has been shown that this activity is mediated, at least in part, by a small fraction of the traditional ER protein or perhaps by its closely related short-form splicing/translational variants (55–57) that are localized near or at the plasma membrane. Cytoplasmic signaling molecules related to growth factor receptor signaling such as the short form variant of metastasis associated gene 1 (17) and the modulator of nongenomic action of estrogen receptor (MNAR) (58) can increase this non-nuclear fraction of ER. Molecular evidence suggests several mechanisms by which non-nuclear/membrane or cytoplasmic ER couples with components of signaling complexes and triggers their responses (59) (Fig. 2). Membrane ER may exist as a cytoplasmic pool tethered to the inner face of the plasma membrane bilayer through binding to membrane proteins of lipid rafts such as caveolin-1 (60, 61), flotillin-2 (62), or the caveolin-binding protein striatin (63), or possibly through association

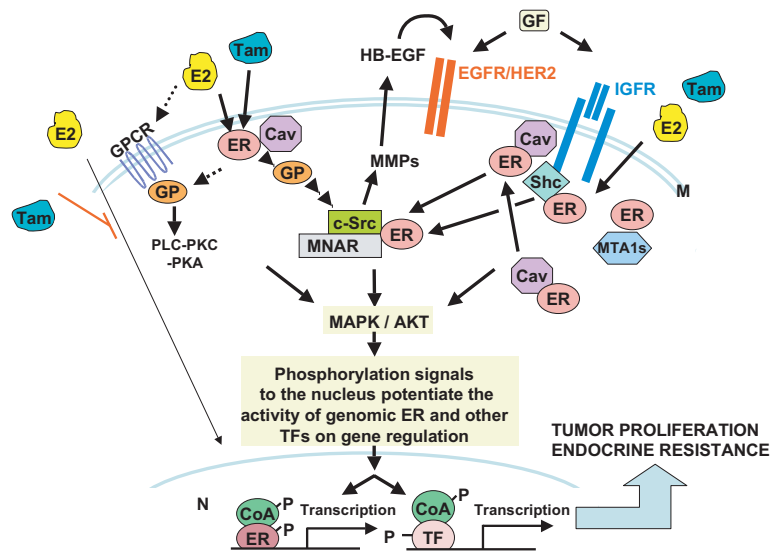


FIG. 2. Integration of genomic and nongenomic/rapid ER signaling and its crosstalk with growth factor receptor and cell kinase pathways in endocrine resistance: a working model. The ligand estrogen (E2) induces genomic ER activity in the nucleus (N), which results in increased gene transcription, including important genes in the growth factor receptor pathways. The SERM tamoxifen (Tam) antagonizes this activity. However, both estrogen and tamoxifen can turn on nongenomic signaling acting on ER that resides at the membrane (M) and/or cytoplasm (a signaling mode also known as membrane-initiated steroid signaling, or MISS). This induction, in turn, through multiple interactions with signaling intermediate molecules such as Shc and MNAR, can activate growth factor (GF) tyrosine kinase receptors (TKRs), such as EGFR and HER2, and the IGFR and cellular kinases such as c-Src. Cytoplasmic signaling molecules such as metastasis-associated gene 1 (MTA1) can increase this non-nuclear fraction of ER. The interaction between membrane/cytoplasmic ER and TKRs and their downstream kinases, *e.g.*, p42/44 MAPK and AKT. Other potential MISS activity involves the activation, either directly or indirectly, of G protein (GP)-coupled receptors (GPCRs), which can then trigger various signaling processes including the activation of c-Src and MMPs and subsequent cleavage and release of HB-EGF. The HB-EGF can then stimulate and activate the EGFR/2 signaling pathway. TKR-induced kinases phosphorylate (P) nuclear ER and its coactivators (CoA) as well as other transcription factors (TF), thus potentiating genomic ER activity, which results in enhanced gene expression including genes in the TKR pathways. These gene products in turn further augment GF-TKR signaling, thus completing the cooperative cycle between the two activities of ER and their crosstalk with the growth factor receptor and cellular kinase pathways. In the presence of excessive TKR signaling, such as in HER2-overexpressing tumors, the nongenomic rapid ER action may become more prominent. The resulting activation of downstream kinases can lead to endocrine resistance by modifying the activity of various transcription factors and/or negating the inhibitory effects of tamoxifen on nuclear ER. PKA, Protein kinase A.

with other membrane receptors [*e.g.*, IGFR (64), EGFR (65, 66), or HER2 (62, 67)], or with signaling adaptor molecules such as Shc (64). Recent laboratory data further suggest that the ER protein within isolated caveolar vesicles typically spreads throughout the cell membrane in a similar fashion to growth factor receptors (60, 68–70) and assembles as part of a large signalsome complex that includes receptor tyrosine kinases (EGFR, IGFR, HER2 receptor), nonreceptor tyrosine kinases such as Src (71), and G proteins (68, 72, 73). Additional evidence from endothelial (56, 74) and breast cancer (75) cell culture models suggests that estrogen-bound ER coimmunoprecipitates with and/or activates distinct G protein subunits. These interactions trigger secondary downstream signaling pathways including effectors of p21Ras, leading to activation of Raf/Mek/MAPK and of the AKT/PI3K modules.

More detailed recent work has identified and defined motifs in a specific domain of ER that are critical to membrane localization and function of the receptor (61, 76, 77). This domain, also known as the E domain, resides in the carboxyl (C) part of ER and harbors the ligand-binding domain and AF-2 function. Mutations in these motifs prevent ER dimerization, association with caveolin-1, and nongenomic ER signaling to activate p42/44 MAPK, PI3K, and cAMP (76, 78). Similarly, specific deletions at the caveolin-1 scaffolding do

main largely prevent the localization of ER at the plasma membrane (60, 76). Interestingly, however, ER interaction with the signaling molecule MNAR involves both the amino (N) terminus and portions of the E domain of ER (79). In contrast, interaction of ER with Shc and striatin has been reported to involve the N terminus of the receptor (64). ER with deleted portions of its N terminus still localizes to the membrane and signals to p42/44 MAPK equivalently to wild-type ER (76), consistent with the idea that it is the E domain at the C terminus of the receptor protein that contains most of the information for both localization and function of ER at the membrane.

Studies in breast cancer culture models have shown that the endogenous membrane ER can directly or indirectly activate EGFR, HER2, and IGFR1 (80). This process involves the sequential activation of the cellular tyrosine kinase Src (81), matrix metalloproteinases (MMPs) 2 and 9, and the release of the EGFR ligand heparin-binding epidermal growth factor-like growth factor (HB-EGF), which, in turn, activates the EGFR downstream kinase cascades (*i.e.*, Ras/Mek/MAPK and PI3K/AKT) (75, 76). These downstream activated kinases, consequently, phosphorylate and thereby activate ER and its coregulators, thus augmenting genomic activities of ER on gene transcription (20, 52, 82) (Fig. 2). The genomic and nongenomic mechanisms of action of ER, therefore, do

not appear to be mutually exclusive, but rather are complementary to one another, and many interactions between these two signaling forms exist. These two ER activities also intimately interact at multiple levels with many cellular (growth factor-dependent and other) kinase networks to sustain bidirectional crosstalk that augments signaling of both ER and kinase-related pathways.

In breast cancer, it is becoming evident that this crosstalk of ER with the EGFR/HER2 pathway, and presumably with additional growth factor receptor pathways, plays a very important role not only in the physiological action of ER as well as growth factor receptor signaling, but also in endocrine therapy resistance (42), as will be discussed in the next section.

III. The HER Tyrosine Kinase Receptor Family and Its Role in the Development of Endocrine Resistance: Studies in Preclinical Models

As briefly discussed above, whereas the ER, via its membrane and nuclear activities, up-regulates growth factor signaling, the molecular crosstalk between these two pathways is continuous and bidirectional. Signaling from multiple signal transduction pathways can modulate both the genomic and nongenomic activities of the ER pathway and their ligand dependency. In the section that follows, we will discuss in detail how this crosstalk, especially between the ER and HER family, contributes to endocrine resistance.

A. HER/ER crosstalk as a mechanism for endocrine therapy resistance: molecular determinants

Growth factor signaling plays an important role in the development of both *de novo* and acquired resistance of breast cancer cells to endocrine manipulation.

It has been demonstrated that ER can be phosphorylated and activated by several intracellular kinases (42, 83). In particular, ER is phosphorylated at key residues (including serine 106/107, 118, 167, 305, and threonine 311) residing mainly in the AF-1 domain, after p42/44 MAPK, PI3K/AKT, p90rsk, p21-activated kinase 1 Pak1, protein kinase A, or p38 MAPK pathway activation by various cytokines and growth factors including ligands of EGFR or IGFR (42, 49–52, 54, 84). ER phosphorylation has been shown to change ER pharmacology and can result in ligand-independent or tamoxifen-mediated activation of the receptor (53, 54, 85). Phosphorylation of ER coregulators is probably no less important than phosphorylation of ER itself in communicating these signal transduction effects on the ER pathway. Phosphorylation of coactivators, similarly to that of the receptor, enhances the activity of the coactivators themselves on the genomic ER even in the absence of its ligand or in the presence of antiestrogens (42, 86). This phosphorylation potentiates the ability of estrogen and SERMs to interact with ER and to recruit other transcriptional coregulators to its transcriptional complex (87); furthermore, it can directly activate their intrinsic enzymatic activities (88). Phosphorylation of corepressors, on the other hand, can result in their export from the nucleus, thereby preventing their access to and inhibition of ER transcriptional complexes in the nucleus (89).

The ER coactivator AIB1 has been shown to be phosphorylated and activated by multiple kinases including MAPKs and other cellular kinases (86, 87, 90). Two independent recent retrospective studies demonstrate that tumors with high levels of both AIB1 and HER receptors (HER2 or HER3) are less responsive to tamoxifen therapy, probably because of increased estrogen agonistic activity of tamoxifen-bound ER (12, 46). Such findings support the hypothesis that increased signaling from the HER family activates downstream kinases, which in turn activates ER and AIB1, increasing transcriptional activity, including in the presence of tamoxifen.

Finally, membrane functions of ER appear to depend not only on ER, but also on the levels of growth factor receptors and their ligands (14, 17). This mode of ER signaling might, therefore, be predominant in breast cancer cells that express high levels of tyrosine kinase receptors such as EGFR and HER2 (Fig. 2). Importantly, it has also been suggested that SERMs such as tamoxifen may behave as estrogen agonists for these membrane effects of ER (14, 20).

B. HER2/ER crosstalk in *de novo* endocrine resistance models

The involvement of HER2 in *de novo* resistance of breast cancer cells to tamoxifen has long been hypothesized (67, 91). In the BT474 HER2-overexpressing breast cancer model, HER2 signaling has been shown to induce resistance to tamoxifen by inhibiting the apoptotic effects of the drug (15). Most recently, using the MCF7/HER2–18 model, which is a derivative line of MCF7 cells that stably overexpresses endogenous AIB1 and exogenous HER2, Shou *et al.* (14) demonstrated that in a low estrogen environment, tamoxifen acts as a potent agonist on tumor growth. In both the above HER2-positive breast cancer cell lines, both estrogen and tamoxifen induce rapid (nongenomic) activation of EGFR/HER2 signaling, which leads to activation of both p42/44 MAPK and AKT signal transduction pathways. These intracellular kinases, as shown in the MCF7/HER2–18 cells, then phosphorylate and functionally activate both ER and the coactivator AIB1. Furthermore, culture of these MCF-7/HER2–18 cells under short-term tamoxifen treatment increases the expression of estrogen-regulated genes nearly as well as estradiol itself. This phenomenon is due to the ability of tamoxifen-ER complexes to recruit coactivators such as AIB1 rather than corepressors to ER-targeted promoters in these HER2-overexpressing cells. Interestingly, all of these phenomena could be blocked by treatment with the selective EGFR-tyrosine kinase inhibitor (TKI) gefitinib (Iressa; AstraZeneca, Macclesfield, Cheshire, UK), which can block signaling from EGFR/HER2 heterodimers, suggesting that EGFR/HER2 signaling is directly involved in the growth-promoting activity of tamoxifen in HER2-overexpressing cells. In this respect, gefitinib was highly effective in inhibiting the tamoxifen-induced growth of MCF-7/HER2–18 cells, whereas it had only a modest effect on estrogen-induced growth (14). The above-mentioned findings are in agreement with the clinical observations noted earlier indicating that tumors that coexpress HER2 and AIB1 have poor outcome when treated with tamoxifen (12, 46).

C. *HER2/ER* crosstalk in acquired endocrine resistance models

Recent laboratory and clinical studies have shown that acquired resistance to tamoxifen in tumors that originally express low levels of EGFR and HER2 is also associated with increased EGFR/HER2 signaling, including HER2 gene amplification (92, 93). Experimental evidence by Nicholson and Gee's group (94) has demonstrated that EGFR/HER2 signaling is involved in acquired tamoxifen resistance of breast cancer cells in long-term culture. These cells show increased levels of expression of EGFR and HER2, increased activation of EGFR/HER2 heterodimers, and increased phosphorylation of p42/44 MAPK, AKT, and nuclear ER (on serine residues 118 and 167) (95, 96). As in the *de novo* experimental models of tamoxifen resistance, the growth of these cells after acquiring resistance is significantly inhibited by treatment with the EGFR/HER2 inhibitor gefitinib or the monoclonal anti-HER2 antibody trastuzumab (Herceptin; Genentech, Inc., South San Francisco, CA) (96, 97). Another tyrosine kinase receptor, the IGF1R, has also been associated with tamoxifen resistance. In fact, it has recently been reported that IGF-II treatment activates both IGF1R and EGFR/HER2 in tamoxifen-resistant cells (98). Taken together, these findings suggest that enhanced growth factor signaling, which up-regulates both the genomic and nongenomic activities of ER, is a key contributor to the mechanism of acquired resistance to tamoxifen.

Enhanced expression of EGFR and HER2, together with subsequent downstream activation of signaling pathways regulated by p42/44 MAPK, has also been identified in breast cancer cell models that have become resistant over time to estrogen depletion or to AI therapy (99–101). Interestingly, MCF-7 cells adapted to grow in the presence of the potent antiestrogen fulvestrant also show increased EGFR signaling, suggesting that growth factor receptors play central roles in resistance to various endocrine therapies such as AIs and pure ER antagonists in addition to tamoxifen (94, 102, 103).

IV. The HER Tyrosine Kinase Receptor Family and Its Role in the Development of Endocrine Resistance: Clinical Evidence

A. *De novo* endocrine resistance

Cumulative clinical data suggest that patients with HER2- and EGFR-overexpressing tumors have a poorer outcome when treated with tamoxifen (13, 104, 105). In a subset of patients with metastatic breast cancer, results of published studies have been somewhat inconsistent due to different study designs, different techniques for measuring HER2, and the relatively small numbers of patients included in each study. Despite this heterogeneity, a recently published meta-analysis examining the interaction between HER2 expression and response to endocrine treatment in metastatic disease clearly shows that HER2-positive breast cancer is less responsive to endocrine treatment (13). EGFR generates similar, although not identical, downstream signals as HER2. In metastatic breast cancer patients, EGFR overexpression is

also predictive of a decreased benefit from tamoxifen (18, 106). In a recent study (104), tumors with higher EGFR were less likely to respond to tamoxifen, and these patients had a significantly shorter time to treatment failure. Even when ER and progesterone receptor (PgR) levels were taken into consideration, EGFR remained predictive of a less sustained response, supporting the hypothesis that signaling from other HER family members besides HER2 can also contribute to the development of tamoxifen resistance.

In patients with early breast cancer, several studies suggest that patients with tumors overexpressing HER2 may derive less benefit from adjuvant tamoxifen than those with HER2-negative tumors (107) and that HER2 expression is a risk factor for tamoxifen failure (108). However, this is not a universal finding, probably because in many adjuvant studies chemotherapy use may have obscured the interaction (109, 110). The difficulty in making a conclusive judgment is illustrated by data from the collection of samples from the NATO and Cancer Research Campaign adjuvant breast cancer trials with 2 yr of tamoxifen *vs.* no treatment (111). In this study, the relative risk of recurrence for tamoxifen was 0.54 for patients negative for both HER2 and EGFR and 1.17 for HER2-positive and/or EGFR-positive patients. However, despite the big difference in the relative risk of recurrence between the different patient groups and the more than 800 patients in the study overall, the small size of the EGFR-/HER2-positive tumor group resulted in SE estimates that overlapped with those of the HER-negative population. A positive association between overexpression of HER family receptors (EGFR and/or HER2 and/or HER3) and tamoxifen outcome has recently been shown in two additional independent datasets of patients treated with adjuvant tamoxifen (112, 113).

At present there are no clear outcome data related to HER2 status from adjuvant trials of AIs. Data on the influence of HER2 status on the relative benefits of tamoxifen and the AI letrozole as adjuvant therapy in the Breast International Group (BIG) 1-98 trial (114, 115) are, therefore, of considerable interest. Both preliminary and updated reports indicated that HER2-positive status was associated with significantly higher relapse rate in the BIG 1-98 trial, regardless of whether letrozole or tamoxifen was used. Additional studies are currently underway to determine the significance of HER2 status in the large randomized adjuvant ATAC (Arimidex, Tamoxifen, alone or in combination) trial comparing the AI anastrozole, the SERM tamoxifen, and the combination (116).

Neoadjuvant trials conducted in women with locally advanced breast cancer before surgery provide a unique opportunity to integrate the molecular determinants of response and resistance with the clinical response of primary breast cancer to medical therapy. Results in the neoadjuvant setting are less controversial and provide solid evidence for the role of HER2 and to a lesser extent of EGFR in tamoxifen resistance (Table 1). Ellis *et al.* (117), in a neoadjuvant study that randomized for treatment with the AI letrozole *vs.* tamoxifen, provided a context to study in further detail the relationship between EGFR and HER2 expression and response to AIs *vs.* tamoxifen. In this report, biopsy-confirmed ER-positive and/or PgR-positive cases that received letro-

TABLE 1. Response to estrogen deprivation/SERMs in the neoadjuvant setting

	Study 1 Ellis (117)		Study 2 Zhu (118)		Study 3 Smith (11)	
	Letrozole	Tamoxifen	Letrozole	Tamoxifen	Letrozole	Tamoxifen
EGFR/HER2 negative	54%	42%	35%	–	41%	43%
EGFR/HER2 positive	88%	21%	75%	–	58%	22%

zole had a statistically significant better response rate compared with those receiving tamoxifen (60 *vs.* 41%, respectively). In retrospective analysis, differences in response rates between the two drugs were most marked for the ER-positive tumors that were also positive for EGFR and/or HER2 (88 *vs.* 21%), suggesting that EGFR and HER2 signaling through ER is ligand-dependent (estrogen or tamoxifen) and that the growth-promoting effects of these receptor tyrosine kinases on ER-positive breast cancer can be inhibited by potent estrogen deprivation therapy.

In a different neoadjuvant study in locally advanced breast cancer patients, Zhu *et al.* (118) analyzed levels of HER2 expression before and after AI treatment and correlated them to the clinical outcome of patients. Using both immunohistochemistry and fluorescence *in situ* hybridization (FISH), data from this study showed that response to the treatment was significantly influenced by HER2 status, with a response rate of 75% for HER2-positive and 35% for HER2-negative tumors ($P = 0.017$). In addition, the response rate was also significantly affected by the decrease in HER2 status after the treatment, with a response rate of 73% for tumors showing decreased HER2 overexpression and 38% for tumors showing no change in HER2 expression ($P = 0.037$).

Most recently, Smith and associates (11, 107) carried out a series of biomarker studies in the IMPACT neoadjuvant trial, a double-blind study that randomized 330 patients to 3-month treatment with the AI anastrozole or tamoxifen or a combination of the two agents. The primary marker of biological efficacy in this study was the nuclear proliferation antigen Ki67 (119). Of particular importance, it was shown that suppression of Ki67 was greater at both 2- and 12-wk timepoints when using anastrozole compared with either tamoxifen or the combination, confirming the results obtained in the earlier ATAC trial (7) in which the superiority of anastrozole over both tamoxifen and the combination was also demonstrated. This study indicates that analysis of Ki67 may be considered a useful marker of early response or resistance to endocrine agents. Further subanalysis of the IMPACT study investigated the effect of ER, PgR, and HER2 on the antiproliferative effects of, and the clinical response to, the agents. Only four patients were EGFR-positive and ER-positive, so the analysis was confined to the 34 patients who were both HER2- and ER-positive. Seven of 12 (58%) HER2-positive patients responded to anastrozole compared with two of nine (22%, $P = 0.09$) to tamoxifen and four of 13 (31%) to the combination (11). In the HER2-negative population, responses were 28 of 68 (41%) for anastrozole, 31 of 73 (43%) for tamoxifen, and 33 of 64 (52%) for the combination. Thus, although the numbers of patients are small in this subgroup analysis, in the HER2-positive group, the data tend to support the findings of the letrozole study (117). Regarding Ki67, in the overall population, there was marginally greater Ki67 suppression in the HER2-negative group than in the HER2-

positive group after 2 wk, and this difference seemed to be confined to the group treated with tamoxifen (120). At 12 wk, there was a statistically significant greater suppression by anastrozole of Ki67 in the HER2-negative group compared with the HER2-positive group: mean Ki67 suppression in the HER2-positive group was 45% and in the HER2-negative group was 85%. This contrasts with the good clinical response witnessed in this small HER2-positive group of patients and may suggest that resistance dependent on HER2 signaling might rapidly emerge as was reported in a recent preclinical model (102). Furthermore, a recent follow-up report by Ellis *et al.* (121) also supports the idea that in ER-positive, HER2-positive tumors, estrogen-independent signaling that leads to increased proliferation is present even during therapy with AIs. Therefore, in such tumors, although estrogen deprivation may be highly effective initially, the addition of HER signaling inhibitors may be needed for a sustained response.

B. Progesterone receptor (PgR) negativity associated with endocrine resistance and HER signaling

Progesterone regulates cell growth in normal breast tissue and in the uterus. The PgR, which is transcriptionally up-regulated as a downstream effect of activated ER, plays an important role in mammary growth and development, especially during pregnancy. Its role in breast cancer is somewhat less established than that of ER, but epidemiological, experimental, clinical, and molecular data suggest that PgR signaling plays a critical role in breast cancer development and progression (122–126). Numerous studies have identified different mechanisms of PgR activation resembling ER signaling, including ligand-dependent and ligand-independent activation (124–127). Ligand-dependent action is based on PgR functioning as a classical ligand-activated transcription factor in the nucleus (genomic action), and PgR can up-regulate the expression of various genes involved in cell proliferation, survival, and tumor progression, including cyclin D1, Bcl2, and vascular endothelial growth factor. Progesterone action is also based on PgR activating p42/44 MAPK pathways by direct interaction with c-Src-family tyrosine kinases, an event that requires progesterone binding to PgR and occurs in the cytoplasm and/or in association with cell membranes (nongenomic or MISS action) (124, 127).

Intriguing preclinical and clinical studies have recently suggested that PgR negativity in ER-positive breast cancer may be a marker of hyperactive growth factor signaling (128) rather than a result of a nonfunctional ER signaling pathway, as previously suggested (129). Recent laboratory studies suggest that growth factors in the IGF and EGF families that activate the PI3K-AKT-mTOR (mammalian target of Rapamycin) pathway can reduce expression of PgR at its transcriptional (mRNA) level (129). Alternatively, studies from

Lange *et al.* (130, 131) have demonstrated a coupling between transcriptional hyperactivity of PgR and increased PgR protein turnover. Accordingly, HER downstream kinases, such as p42/44 MAPK and cyclin-dependent kinase 2, which phosphorylate PgR and its accessory proteins and dramatically increase PgR transcriptional activity including in the presence of low levels of progesterone, also greatly increase PgR turnover, resulting in PgR-low or PgR-negative tumors (128, 129, 132–135). Finally, increased ligand-independent activation and transcriptional hypersensitivity of PgR associated with breast cancer cell growth have also been recognized as deriving from the phosphorylation of PgR under EGFR/EGF signaling (134).

These molecular data, in concert with clinical findings that follow, suggest that loss of PgR expression in some tumors, at the mRNA and/or the protein level, may be due to increased growth factor receptor activity potentially leading to endocrine therapeutic resistance. Although more studies are needed on this topic, these results suggest that suppressed/reduced PgR levels may derive from and indicate hyperactivity in the signaling cascade generated by EGFR, HER2, and other kinase activation.

Studies in the clinical setting in patients with metastatic breast cancer indicate that PgR is a predictive factor for the outcome of endocrine therapy (136). However, there is still no definitive evidence on the predictive role of PgR in women with early breast cancer receiving adjuvant endocrine therapy. The overview data from 2005 (137) shows a 41% reduction in recurrence in ER-positive, PgR-poor patients receiving about 5 yr of tamoxifen compared with a 40% reduction in the ER-positive, PgR-positive patients. This suggests that there is no underperformance of tamoxifen in ER-positive, PgR-negative patients, although this data set originally suggested benefit from tamoxifen in patients with ER-negative tumors, raising questions about receptor assay quality. The overview data conflict with those from a large, albeit nonrandomized series, where biochemical ER and PgR assays were identically performed in two different central laboratories and in which benefit from tamoxifen was substantially less in PgR-negative tumors (138).

Many clinical studies have confirmed in both metastatic and early breast cancer that the benefit of tamoxifen is less in ER-positive/PgR-negative *vs.* ER-positive/PgR-positive tumors (138–142). A retrospective analysis from the ATAC trial showed that patients with ER-positive/PgR-positive tumors had a lower recurrence rate than those with ER-positive/PgR-negative tumors (7.6 *vs.* 14.8%) (143, 144). This difference, however, was largely due to the reduced efficacy of tamoxifen in the subgroup of patients with ER-positive/PgR-negative tumors. In contrast, there was little difference in the recurrence rate with anastrozole in the PgR-positive *vs.* PgR-negative subsets. The observation that patients with ER-positive/PgR-negative tumors respond nearly as well to the AI as those with PgR-positive tumors suggests that the ER signaling pathway is functional in many ER-positive/PgR-negative tumors and that these tumors are still dependent on estrogen for growth despite somewhat lower ER levels. Importantly however, a recently updated conference report of this trial, analyzing the differential response of PgR-negative tu-

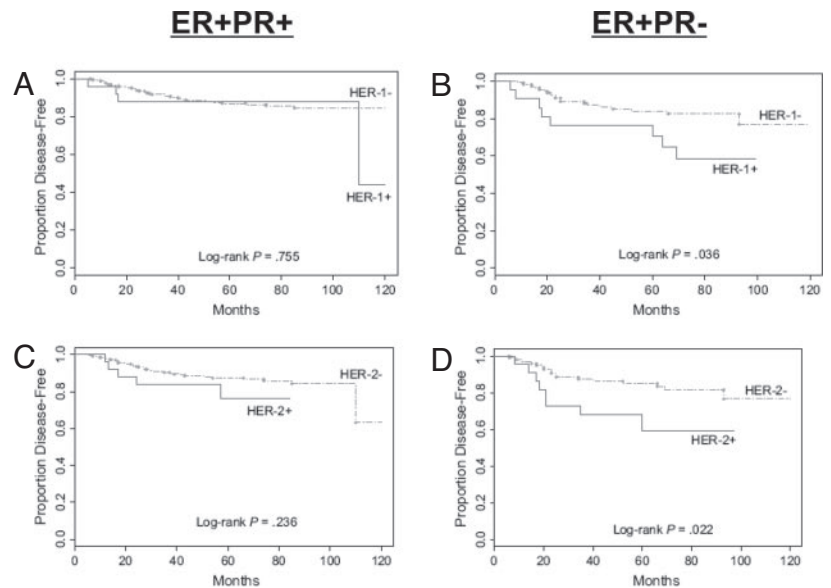
mors to AIs *vs.* tamoxifen only in specimens with centrally reviewed steroid receptor expression, failed to confirm the selective benefit of AIs *vs.* tamoxifen in this group of tumors. A worse outcome was, nonetheless, demonstrated in patients with PgR-negative tumors, regardless of the endocrine therapy used (145). A similar interaction between PgR-negative status and endocrine resistance in the adjuvant tamoxifen setting was also found in a recent study in an independent group of patients (112). Finally, a report from the BIG 1-98 trial that compared letrozole and tamoxifen with sequences of each agent, while confirming the finding that patients with PgR-negative tumors have a worse clinical outcome, failed to demonstrate an effect of PgR expression on the relative efficacy of letrozole over tamoxifen (114). Whether PgR negativity is a predictive factor for poor clinical outcome on endocrine therapy or a prognostic factor, as recently suggested by RT-PCR-based assays (146), remains controversial and needs to be further investigated.

A recent retrospective analysis from Arpino *et al.* (147) considered a large number of patients with tumor receptor assays all performed centrally by standardized techniques; this work provides clues to the origin of the distinct ER-positive/PgR-negative phenotype. PgR-negative tumors have more aggressive features: they are larger, have more nodal metastases, are more likely to be aneuploid, and are more rapidly proliferating. Interestingly, PgR-negative tumors are also associated with a significantly higher frequency of HER2 overexpression and high expression of EGFR. About 30% of ER-positive, PgR-negative tumors are HER2- and/or EGFR-positive compared with only 10% of ER-positive, PgR-positive tumors (147). As indicated in the preclinical setting, several recent clinical reports also suggest that high growth factor receptor activity may be associated with reduced PgR levels in breast cancer (148–151). For example, tamoxifen-treated women with ER-positive/PgR-negative and either EGFR or HER2-positive tumors are found to have a significantly worse outcome than patients in the same subgroup whose tumors display low EGFR or HER2. In contrast, deleterious effects of high EGFR or HER2 are less apparent in patients with ER-positive/PgR-positive tumors (147) (Fig. 3). One possible explanation is that less active EGFR and HER2 signaling despite receptor overexpression occurs in ER-positive/PgR-positive tumors and results in a smaller impact on response to tamoxifen. In this context, PgR loss may be a marker of active EGFR/HER2 signaling networks in these HER2-positive tumors.

C. HER2/ER crosstalk in acquired endocrine resistance

Recent provocative clinical studies document that acquired resistance to tamoxifen may also be associated with an increase in HER2 expression and/or gene amplification as shown in preclinical models. One study analyzed the changes in growth factor receptors and their associated downstream kinases in tumors after the development of tamoxifen resistance (92). A tissue microarray was constructed of pairs of samples from the same patient, first at presentation, and second at the development of tamoxifen resistance during adjuvant therapy. A total

FIG. 3. Kaplan-Meier curves for disease-free survival (DFS) in tamoxifen-treated patients according to the HER family receptor and PgR status. Among tamoxifen-treated patients with ER+/PR+ tumors, neither EGFR (HER1) nor HER2 had a significant effect on DFS, although there was a suggestion of a trend in HER2-positive tumors. In contrast, among patients whose tumors were ER+/PR-, both EGFR and HER2 expression were associated with significantly poorer DFS (for HER-1, HR = 2.4, 95% CI = 1.0 to 5.4, $P = 0.04$; and for HER2, HR = 2.6, 95% CI = 1.1 to 6.0, $P = 0.03$). [Adapted with permission of Oxford University Press from Arpino *et al.* (147).]



of 39 patients had sufficient tissue in both samples on the arrays to provide near-complete sets of data. Pretreatment, strong positive correlations between ER, PgR, and Bcl-2, and an inverse correlation between ER and HER2, were found as expected. These correlations were lost in the tamoxifen-resistant tumors and replaced by strong correlations between ER and phosphorylated (p) p38 MAPK (p-p38) and phosphorylated p42/44 MAPK (p-p42/44 MAPK). ER expression was lost in 17% of resistant tumors. Three (11%) of the 26 tumors originally negative for HER2 became amplified and/or overexpressed at resistance. All ER-positive tumors that overexpressed HER2 originally or at resistance expressed high levels of p-p38. In the pretreatment and tamoxifen-resistant specimens, there were strong correlations between p-p38 and p-p42/44 MAPK. Therefore, the molecular pathways driving tumor growth can change as the tumor progresses, and the HER family may play an important role in the development of acquired tamoxifen resistance.

Acquired HER2 gene amplification during cancer progression after tamoxifen adjuvant therapy has also been recently reported in patients' circulating tumor cells (93). Similarly, serum HER2 conversion from negative to positive has also been shown in patients with advanced disease at the time of disease progression on endocrine therapy (152). These data suggest that acquired HER2 overexpression can occur during endocrine treatment, perhaps as an adaptive mechanism for tumor cell survival on these therapies or as a consequence of reversing ER-induced down-regulation of HER expression by endocrine therapy (152).

At present, there are no biological data on samples derived at relapse from patients on AIs. This is an area of substantial clinical importance over the next few years, because these agents are more frequently used in the adjuvant setting. Sampling tumors for molecular studies before and after treatment will enhance our understanding of the mechanisms of response and resistance to these therapies (11, 116).

V. Novel Therapeutic Strategies to Overcome HER/ER Pathway Crosstalk and Endocrine Resistance

A. Preclinical studies

Treatment with various growth factor receptor inhibitors and other signal transduction inhibitors (STIs) has been used in preclinical tumor models as an attempt to block growth factor signaling pathways (96, 153–156). However, several reports have shown that in hormone-sensitive, ER-positive breast cancer, these inhibitors as monotherapy (used one at a time) may have only a minimal effect on tumor growth (14, 102, 157, 158). As discussed above, during prolonged endocrine therapy, adaptive changes occur such as up-regulation of growth factor signaling (96, 153–156), which may lead to endocrine resistance. Thus, strategies to combine endocrine therapies with STIs against growth factor receptor downstream signaling molecules such as farnesyltransferase inhibitors (directed against the Ras signaling pathway) and mTOR inhibitors (159, 160), have been used in hopes of preventing resistance and improving therapeutic efficacy of endocrine agents (83).

In vitro, the combination of tamoxifen and the selective EGFR TKI gefitinib provided nearly complete inhibition of p42/44 MAPK and AKT phosphorylation, greater suppression of the cell-survival protein Bcl-2, and more complete G₀/G₁ cell-cycle arrest than that observed with tamoxifen alone (97). In particular, this combined therapy prevented acquired EGFR/p42/44 MAPK signaling and significantly delayed the subsequent resistance that started to develop after 5 wk in tamoxifen-alone-treated cells. For *de novo* tamoxifen-resistant breast cancer models made by stable transfection of HER2, the strategy of combining endocrine therapy with different HER inhibitors or other STIs is also more effective than using either therapy alone (14, 154, 157). A synergistic effect has also been reported for the anti-HER2 antibody trastuzumab combined with tamoxifen in ER-positive, endogenously HER2-positive BT474 breast cancer cells,

with enhanced accumulation of cells in G_0/G_1 and a reduction in S-phase compared with either therapy alone (161). Recently, the dual EGFR/HER2 TKI lapatinib (Tykerb, GW57016; GlaxoSmithKline, Brentford, UK) has been shown to cooperate with tamoxifen and other endocrine agents to provide more rapid and profound cell-cycle arrest than either therapy alone in endocrine-resistant cells (162, 163).

In vivo, it has been shown that gefitinib added to tamoxifen can completely overcome the agonist activity of tamoxifen and significantly delay the growth of stably transfected HER2-positive MCF-7 xenografts (14). Similar beneficial effects were seen with gefitinib combined with estrogen deprivation. The combination more effectively inhibited growth and delayed the acquired resistance that develops rapidly with estrogen deprivation alone (102).

Finally, data from a more recent study (157) demonstrated in both MCF7/HER2-18 and BT474 ER-positive, HER2-positive xenograft tumors that a combination of several HER inhibitors designed to completely inhibit signaling from all HER dimer pairs, together with, where applicable (MCF7-HER2-18), either tamoxifen or estrogen deprivation, is much more effective in inducing apoptosis and slowing proliferation than each individual drug. Multidrug anti-HER combinations such as those used in the above study can completely eradicate tumors in a substantial number of mice. These provocative data suggest that one type of resistance to the HER inhibitors used currently in the clinic is incomplete blockade of downstream signals generated by the various homo- and heterodimers of the HER family, and also suggest that tumors, to survive, can switch or use an alternative HER dimer pathway when the pathway being used by the cells is blocked. Importantly, however, in one of the above two ER-positive, HER2-positive xenograft models, this potent combination anti-HER therapy was only modestly effective when implemented without endocrine therapy, supporting the concept that initial complete blockade of all HER dimer pairs, together with ER blockade, may be necessary for optimal treatment in some breast tumors.

B. Clinical studies

Taken together, the experimental data indicate that various growth factor receptor and other intracellular signaling pathways may be activated or overexpressed in breast cancer, especially in endocrine-resistant cells, and suggest that targeting such pathways simultaneously with the ER pathway may be an effective therapy.

Proof of principle for therapies that specifically target growth factor pathways in breast cancer has already been provided with the HER2 monoclonal antibody trastuzumab (Herceptin). Successful clinical use of trastuzumab has been demonstrated in HER2-positive breast cancer, and significant activity has been seen as first-line monotherapy, with up to a 48% clinical benefit rate in HER2-positive tumors and a 34% objective response rate in tumors reported positive for HER2 amplification by fluorescence *in situ* hybridization (164). Thus, it is hoped that various small molecule TKIs and STIs might also prove effective anticancer strategies in the appropriate setting. Early phase II clinical studies have been initiated with EGFR/HER2 TKIs, farnesyltransferase inhib-

itors, and more recently mTOR antagonists, as monotherapy in patients with advanced (often heavily pretreated) disease; such studies, however, have been somewhat disappointing, with low clinical response rates and rapid disease progression (83).

The EGFR inhibitor gefitinib, an orally active, low-molecular-weight, selective EGFR-TKI, has been studied in patients with breast cancer. There have been several phase II monotherapy studies of gefitinib in patients with advanced breast cancer (165–167). Overall, the data are relatively disappointing. The only trial in the metastatic setting to report a significant number of responses includes patients with ER-positive tumors progressing on tamoxifen (167), a setting in which preclinical models have shown the best evidence of activity for gefitinib. Pharmacodynamic studies performed in one of these trials confirmed that EGFR tyrosine kinase signaling is inhibited in both skin and tumor biopsies by the doses of gefitinib delivered orally (166). However, discordance in the effect of gefitinib on downstream intracellular signaling in treated tumor biopsies has been noted, with lack of inhibition of Ki67 in tumors but not in matched skin biopsies. This suggests that activation of other intracellular pathways downstream of EGFR (especially in breast cancer as opposed to normal skin cells) may determine the clinical response to gefitinib. More research is required to establish tumor phenotypes and specific predictive biomarkers in responding *vs.* nonresponding patients (168). Indeed, a recent neoadjuvant trial that randomized ER-positive, EGFR-positive patients to gefitinib plus the AI anastrozole or to gefitinib alone (169) demonstrated greater reduction in Ki67 with the combination. Fewer clinical data exist regarding other EGFR-TKIs in breast cancer, although a phase II monotherapy trial of the selective EGFR TKI erlotinib (OSI-774) in breast cancer has proven relatively disappointing (170).

As mentioned above, cooperative activation of different growth factor receptors, and especially between HER receptors (EGFR/HER1-HER4), may limit the efficacy of targeting just one single receptor (157). Lapatinib is a potent dual inhibitor of both EGFR and HER2 and blocks autophosphorylation of both receptors (171–173). As noted above, it was effective with tamoxifen in cell culture studies of hormone-resistant cells (162, 163, 171). In phase I/II clinical studies, clinical activity was reported in heavily pretreated patients with EGFR-positive and/or HER2-positive tumors and in trastuzumab-resistant breast cancer patients (172–175), and diarrhea and skin rash were the main toxicities. Several biological and pharmacological reasons may account for the efficacy of a small molecule inhibitor of HER2 in patients resistant to therapy with trastuzumab. A phase II trial of lapatinib has been completed in heavily pretreated patients with advanced breast cancer that progressed on prior trastuzumab-containing regimens. A recent analysis of the first 41 patients confirmed clinical activity for lapatinib in breast cancer, with partial response in 7% of patients and/or stable disease in 24% of patients after 16 wk of therapy (174).

Based on the preclinical and clinical evidence suggesting benefit, several phase II/III trials have been initiated with TKIs or monoclonal antibodies in combination with tamoxifen, fulvestrant, or AIs (Table 2). Some of these trials are in the second-line setting, including patients whose tumors

TABLE 2. Selected recent ongoing and completed phase II/III clinical trials of inhibitors of HER family receptors in combination with endocrine therapy

Trial design	Disease setting	Trial phase	Primary endpoints	Outcomes
Gefitinib				
Tamoxifen ± gefitinib	Metastatic	II RCT	TTP	Completed
Anastrozole ± gefitinib (178)	Neoadjuvant	II RCT	ORR	NS ORR favoring the AI alone instead of the combination
Gefitinib ± anastrozole (169) ^a	Neoadjuvant	II RCT	↓ Ki67 labeling index ↓ in tumor size	↓ Ki67 labeling index 98% <i>vs.</i> 92.4% ↓ in tumor size no difference
Gefitinib ± anastrozole	Metastatic	II RCT	TTP	Ongoing
Gefitinib + anastrozole <i>vs.</i> gefitinib + fulvestrant	Metastatic	II RCT	ORR	Ongoing
Lapatinib				
Lapatinib + tamoxifen	Metastatic 2nd line	II	ORR/CBR	Ongoing
Lapatinib ± letrozole	Metastatic	III RCT	TTP	Ongoing
Lapatinib ± fulvestrant	Metastatic	II RCT	ORR/PK	Ongoing
Trastuzumab				
Trastuzumab + letrozole (176)	Metastatic	II	ORR/TTP	ORR 26% TTP 5.5 months
Anastrozole <i>vs.</i> anastrozole + trastuzumab (177)	Metastatic	III	ORR/TTP	TTP 2.4 months <i>vs.</i> 4.8 months ORR 6.8% <i>vs.</i> 20.3%
Trastuzumab + exemestane	Metastatic	II	TTP	Completed

RCT, Randomized controlled trial; ORR, objective response rate; CBR, clinical benefit rate; NS, not significant; Tam, tamoxifen; PK, pharmacokinetics. Also see information in Johnston *et al.* (83).

^a Only in EGFR-positive patients.

were progressing on tamoxifen, and then lapatinib was added to tamoxifen to see whether clinical responses could be observed and resistance reversed. The majority, however, are randomized phase II studies with only 100–200 patients, and in some studies the primary efficacy endpoint is objective response rate rather than time to progression. Such studies are asking whether the combination may provide greater initial antineoplastic activity than endocrine therapy alone, expecting to enhance or restore the response in tumors with *de novo* endocrine resistance. However, given the preclinical data, prolongation of time to progression (*i.e.*, delaying resistance onset) rather than response rate might be a better endpoint for these trials.

The first safety and efficacy data for the combination of an AI (letrozole) with trastuzumab for the treatment of ER and/or PgR-positive and HER2-positive advanced breast cancer have recently been published (176). In this study, although the response rate to the combination of trastuzumab plus letrozole was not enhanced over trastuzumab monotherapy from previous data, the responses were more durable. Further phase III studies that compare letrozole alone *vs.* trastuzumab alone *vs.* the combination will be necessary to fully outline the efficacy of the combination regimen. In addition, results from an open label phase III trial evaluating the safety and efficacy of trastuzumab plus anastrozole *vs.* anastrozole alone, demonstrated that trastuzumab prolonged progression-free survival in hormone-dependent/HER2-positive metastatic breast cancer (177).

Recently, a randomized phase II neoadjuvant trial of anastrozole ± gefitinib explored whether the AI + gefitinib combination might improve clinical outcome (178). The results of this trial were disappointing in that there was no significant difference in biological or clinical outcomes between the two treatment arms. In fact, there was a nonsignificant trend in response rate favoring the AI alone instead of the combination (response rate, anastrozole 61%, *vs.* anastrozole + ge-

fitinib 48%; $P = 0.067$) (169, 178). Whether this result might be different in patients selected for EGFR or HER2 overexpression remains to be seen in other studies. The results of the aforementioned trial (169), which demonstrated greater suppression of Ki67 by the addition of AI therapy to the EGFR inhibitor gefitinib in patients with ER-positive/EGFR-positive breast cancer, strongly supports this concept. A large randomized phase III trial comparing the dual EGFR and HER2 inhibitor lapatinib with or without letrozole in ER-positive advanced metastatic breast cancer is currently ongoing. Again, the recently published preclinical data showing the ability of lapatinib to reverse hormone resistance and to cooperate with tamoxifen in providing maximal growth arrest provide the rationale for this study (162).

It will be important in all of the trials mentioned in Table 2 and similar other studies to stratify for prior endocrine therapy (usually tamoxifen) given in the adjuvant setting and, in particular, the interval since completion of such therapy. This is important because it may have implications for the presence or absence of activated growth factor signaling in the relapsed tumor, which could determine the efficacy of added gefitinib or lapatinib. In addition, biological studies are required to help predict those patients who are more likely to benefit from combined endocrine/anti-HER therapy. For example, the tamoxifen/ gefitinib trial (Table 2) will undertake studies to look at downstream intracellular signaling components of the HER family, in addition to assessment of ER and ER co-activators such as AIB1.

VI. Future Challenges

As the complexity of ER biology is being revealed, so is the complexity of the mechanisms responsible for endocrine sen-

sitivity and resistance in breast cancer. The genomic and nongenomic activities of ER, as well as their complex interplay with many other growth factors and cellular kinase pathways, influence tumor sensitivity to various types of endocrine therapies. Although ER was first identified more than 30 yr ago, we are still trying to clarify and understand its multiple roles in normal physiology and in disease. In breast cancer, there is convincing evidence that ER does not act alone to stimulate tumor growth; rather, a complex interacting network operates to ensure the viability of cancer cells. Understanding this network will offer therapeutic advantages. The crosstalk between ER and growth factor receptor pathways is the cause of endocrine therapy resistance in many patients. The combination of ER-targeted therapies with growth factor receptor inhibitors or inhibitors of more downstream kinases is a novel strategy currently undergoing more intensive clinical investigation. Future and ongoing clinical trials will determine the true potential and applicability of this combined therapeutic approach. Further clinical trials are needed to evaluate various signaling elements from the multiple networks that crosstalk with and modulate ER activity as predictive markers for initial endocrine therapy. In the future, a molecular profile of these different components in a given patient's tumor immediately before treatment or at the time of disease progression after therapy might permit the individualization of both the initial type of endocrine therapy and the appropriate signaling inhibitor needed to block *de novo* or acquired resistance, a strategy that should improve the treatment of breast cancer in the future.

Acknowledgments

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