

## Estrogen Receptor Pathway: Resistance to Endocrine Therapy and New Therapeutic Approaches

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**Abstract** Endocrine therapy is widely accepted as the most important treatment for all patients with hormone receptor – positive breast cancer. However, despite the positive effect of endocrine therapy on clinical outcome, resistance to these drugs inevitably develops. This article reviews the problem of resistance to hormonal therapy and addresses potential approaches to overcome intrinsic or acquired mechanisms of resistance.

### Background

Endocrine therapy is the most important component of adjuvant therapy for patients with early stage hormone receptor – positive breast cancer. Tamoxifen, a selective estrogen receptor modulator (SERM), has been shown to improve relapse-free and overall survival in the adjuvant setting and to reduce the incidence of contralateral breast cancers (1). More recently, randomized data has shown that aromatase inhibitors (AI), which deplete extragonadal peripheral estrogen synthesis, substantially improve disease-free survival in postmenopausal women with operable breast cancer in the adjuvant setting (2–5). In addition to these benefits in early stage disease, endocrine therapy is also of significant importance in the treatment of advanced metastatic disease.

Despite these obvious benefits in a proportion of patients, initial or acquired resistance to endocrine therapies frequently occurs. Identification of the key mechanisms involved in resistance could help to predict patients who may or may not benefit from specific therapies, and identification of resistance mechanisms may also facilitate the development of new pharmaceutical compounds targeted at various molecular components of endocrine resistance pathways. Figure 1 illustrates some of the key signal transduction pathways that cross-talk with estrogen receptor signaling in endocrine-resistant breast cancer.

Resistance to endocrine therapy in hormone receptor – positive tumors can be classified as either

- Intrinsic—resistance occurs *de novo* at the initial exposure to endocrine therapy, or
- Acquired—resistance manifests over time after an initial response to endocrine therapy.

### Intrinsic Resistance

Estrogen receptor (ER) and progesterone receptor (PR) expression are currently the best predictors of response to endocrine therapy in the clinical setting (1, 6). However, 25% of ER+/PR+ tumors, 66% of ER+/PR– tumors, and 55% of ER–/PR+ tumors fail to respond to tamoxifen or develop early resistance to tamoxifen for reasons that are unclear (7–9).

Various mechanisms have been implicated in this observed intrinsic resistance to tamoxifen therapy. For example, a second ER has been cloned (ER $\beta$ ) that has different transcriptional activity compared with ER $\alpha$  and has been implicated in tamoxifen resistance (10). ER may also activate alternative DNA sequences such as activator protein 1 response elements that regulate genes involved in cell proliferation, motility, and apoptosis (11). The balance of coactivator and corepressor proteins in a cell may determine the response of ER to a particular ligand. For example, overexpression of the coactivator SRC-1 enhances the ER agonist response to tamoxifen (12). In contrast, reduced nuclear receptor corepressor N expression is associated with the development of tamoxifen resistance in breast cancer xenografts (13).

Nongenomic mechanisms have also been implicated in intrinsic resistance to endocrine therapy. ER interacting with several kinases, including insulin-like growth factor-1 receptor and the p85 regulatory subunit of phosphatidylinositol 3-kinase via adaptor proteins, Src and Shc, result in different cell survival and proliferative signals via the AKT (protein kinase B) and mitogen-activated protein kinase pathways (14–17). Tamoxifen may also exert agonist effects via the interaction of membrane ER with peptide growth factor signaling [epidermal growth factor receptor or human epidermal growth factor receptor 2 (HER-2; ref. 18)]. Similarly, cross-talk may occur in hormone receptor – positive tumors with HER-2 amplification by downstream activation from various HER-2-driven intracellular kinases. In this case, tamoxifen-treated tumors may result in the phosphorylation of nuclear tamoxifen-liganded ER and its associated coactivators. Therefore, bidirectional cross-talk pathways can result in tamoxifen stimulating the growth of hormone receptor – positive breast cancer.

More recently, the metabolism of tamoxifen has been implicated in intrinsic resistance to therapy. Specifically, genetic polymorphisms in tamoxifen-metabolizing genes have been

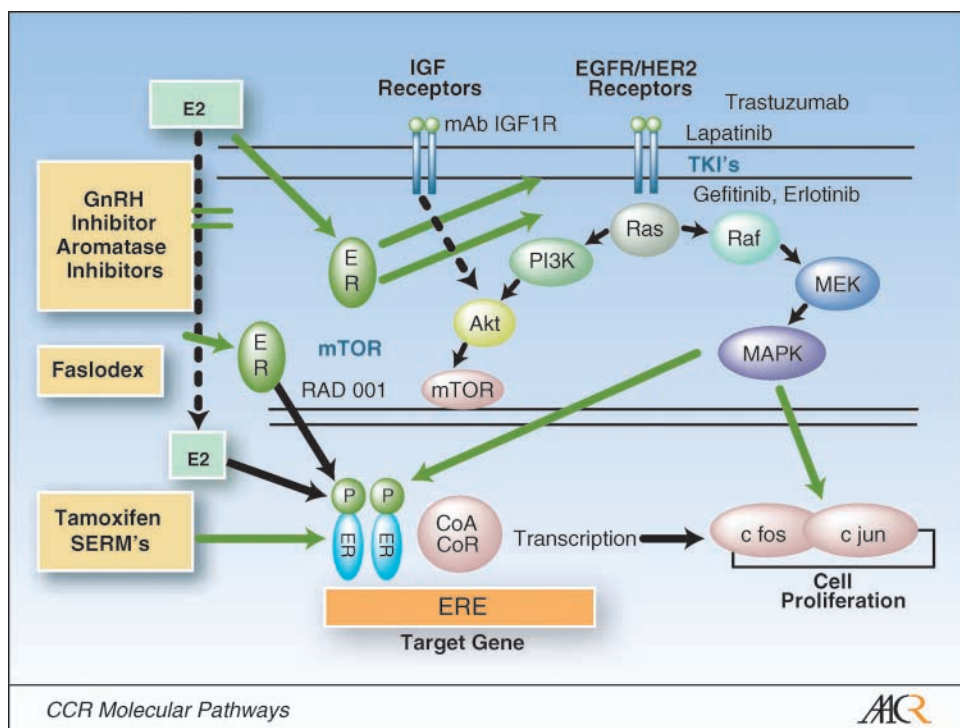
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**Fig. 1.** Cross-talk pathways that may be active in endocrine-resistant breast cancer and pharmaceutical compounds targeted at various molecular components of endocrine resistance pathways. Abbreviations: TKI, tyrosine kinase inhibitors; E2, estradiol; IGF1R, insulin-like growth factor receptor; EGFR, epidermal growth factor receptor; HER-2, human epidermal growth factor receptor 2; ERE, estrogen-responsive element; PI3K, phosphatidylinositol 3-kinase.



identified that affect the plasma concentration of tamoxifen metabolites. In tamoxifen-treated patients, women with certain cytochrome p450 2D6 (CYP2D6) genotypes tend to have a higher risk of disease relapse (19). In fact, preliminary data indicates that CYP2D6 status may be used to identify which patients should receive tamoxifen therapy and which tamoxifen-treated patients should not be coadministered potent CYP2D6 inhibitors (20).

### Acquired Resistance

Acquired resistance occurs when patients with hormone receptor-positive breast cancer initially respond to endocrine therapy, but over time, their tumors progress despite continued endocrine therapy. There is increasing recognition that growth factor pathways play a central role in this acquired resistance to endocrine therapy. Breast cancer cells may use alternative intracellular signaling pathways over time to enhance and activate ER signaling, allowing cells to escape from their initial endocrine therapy. Several experimental models and clinical studies have implicated various peptide growth factor receptor pathways in the development of acquired resistance to endocrine therapy. For example, the epidermal growth factor receptor and HER-2 pathways become selectively up-regulated in breast cancer cells that acquire resistance to tamoxifen during prolonged exposure. In preclinical models of tamoxifen resistance, overexpression of HER-2 results in phosphorylation and activation of ER and of coactivators such as AIB1. In this setting, tamoxifen, like estrogen, has a growth-stimulatory effect. Enhanced epidermal growth factor receptor expression and subsequent downstream mitogen-activated protein kinase activation have been found in MCF-7 breast cancer cells that become resistant over time to tamoxifen.

It is thought that resistance to estrogen suppression by aromatase inhibition may occur because tumor cells adapt to a

sustained low-estrogen environment, resulting in enhanced sensitivity to estrogen (21, 22). This may be due to an increase in ER protein or to relocation of ER to the plasma membrane in association with Shc and insulin-like growth factor-1 receptor. Increased activation of Src and RAS/RAF/MEK/MAPK signaling may also result in enhanced sensitivity to estrogen (17). Although xenograft tumor models exposed to AIs display some initial up-regulation of the ER, it seems that increased growth factor pathway signaling, particularly in the mitogen-activated protein kinase cascade, is critical to the process of adaptation and tumor resistance (21, 23). This suggests that tumor cells adapt to estrogen deprivation during treatment with AIs by the activation of alternate signaling pathways. Abrogation of alternative growth factor signaling may restore sensitivity to endocrine therapy.

### Clinical Translations Advances: Strategies to Treat or Prevent Endocrine Resistance

**Molecular targeted therapies.** Results of clinical studies of signal transduction inhibitors (STI) as monotherapy in the second-line treatment of metastatic breast cancer have been somewhat disappointing, with low clinical response rates and short times to further disease progression. However, the results of these monotherapy trials have provided a strong rationale that combined treatment of STIs with endocrine therapy may provide greater beneficial effects than either therapy alone. There are now several clinical trials under way to assess the efficacy of combinations of STI with various endocrine agents in the metastatic setting (Table 1).

Recently, clinical trial 0223, a phase 2 neoadjuvant trial of anastrozole ± gefitinib, determined whether the AI + STI combination would improve clinical outcome. 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 arm instead of the combination AI + STI arm [response rate, anastrozole (61%) versus anastrozole + gefitinib (48%);  $P = 0.067$ ; ref. 24]. A phase III study comparing the epidermal growth factor receptor and HER-2 inhibitor lapatinib + letrozole versus letrozole alone in metastatic breast cancer is ongoing.

Similar work is either ongoing or has been completed with farnesyltransferase inhibitors and mammalian target of rapamycin antagonists in which strong preclinical data suggests additive or synergistic effects for either of these agents in combination with endocrine therapy. The farnesyltransferase inhibitor tipifarnib, when combined with tamoxifen or estrogen deprivation, induced greater tumor regression than either endocrine therapy alone. However, a randomized phase II trial in 120 patients that compared letrozole and tipifarnib with letrozole alone showed a longer duration of objective response for the combination (23 versus 16 months) but no actual improvement in response rate (25).

There is a similar rationale for combining the mammalian target of rapamycin antagonists with an AI. Cotreatment with temsirolimus (CCI-779) inhibited mammalian target of rapamycin activity and restored sensitivity to tamoxifen primarily through the induction of apoptosis, suggesting that AKT-induced tamoxifen resistance may be partly mediated by signaling through the mammalian target of rapamycin pathway (26). Preliminary data from a randomized phase 2 study showed higher clinical benefit rates for temsirolimus combined with letrozole compared with letrozole alone, but a randomized phase 3 trial was recently terminated before patient accrual was completed, presumably for lack of efficacy (27). Currently, a randomized phase 2 neoadjuvant trial is addressing whether everolimus (RAD001) can enhance the efficacy of 4 months of preoperative letrozole. One could also speculate that more than one STI is required to prevent acquired resistance to endocrine therapy.

**Complete estrogen blockade.** Because the goal of endocrine therapy is to eliminate the estrogenic signal and prevent all

downstream signaling of ER, it has been hypothesized that administration of only one drug may not be sufficient to achieve complete estrogen blockade. A preclinical nude mouse model with MCF-7 p450Arom up-regulated tumor cell implants has shown that whereas both the AI letrozole and the ER down-regulator fulvestrant slow tumor growth considerably, a combination of the two causes considerably more growth inhibition (45%) and remained free of growth for the duration of the experiment (29 weeks; ref. 28). Thus, achieving a more complete estrogen blockade may delay the development of hormone-independent signaling pathways that regulate proliferation.

This concept of complete estrogen blockade was the rationale behind the combination arm of AI (arimidex) + SERM (tamoxifen) in the adjuvant Arimidex, Tamoxifen, Alone or in Combination trial (3). The subsequent finding that the AI + SERM combination did no better than SERM alone and was, in fact, inferior to AI alone, considerably dampened enthusiasm for attempting complete estrogen blockade. Reasons why this result may apply to an AI specifically in combination with tamoxifen have been offered. Some have hypothesized that tamoxifen's estrogen agonist effect may be amplified in the presence of ultra-low estradiol levels. More recently, the efficacy of a different combination AI (atamestane) + SERM (toremifene) was attempted in the hope of exploiting the weaker estrogen agonist effect of toremifene in a low estrogen environment. Of note, the combination was found to be equivalent to an AI (letrozole) alone among women with metastatic breast cancer (29). This finding suggests that the concept of complete estrogen blockade should be pursued with novel, less ER-agonistic SERMs in combination with AIs. Thus, a North American Breast Intergroup/Southwest Oncology Group/Cancer Trials Support Unit trial of anastrozole + fulvestrant versus fulvestrant alone in women with metastatic breast cancer is currently enrolling patients.

**Strategies to restore estrogen sensitivity.** An alternative strategy to address acquired resistance to endocrine therapy might be to manipulate the hormonal milieu with a view to restore the baseline "estrogen sensitivity" of cancer cells.

**Table 1.** Clinical trials of STIs in combination with endocrine therapy

Trial design	Trial phase	Disease setting	Status
<b>Phase II</b>			
Sorafenib and Anastrozole	I/II	Metastatic	Open
Trastuzumab + Exemestane	II	Metastatic	Completed
Bevacizumab + Letrozole	II	Metastatic	Open
Gefitinib ± Tamoxifen	II RCT	Metastatic	Open
Gefitinib + Anastrozole	II RCT	Metastatic	Open
Lapatinib + Tamoxifen	II	Metastatic second line	Open
Letrozole ± Tipifarnib	II RCT	Metastatic	Completed
Tipifarnib + Fulvestrant	II	Metastatic second line	Open
Tipifarnib + Tamoxifen	II	Post-tamoxifen	Terminated
Everolimus ± Letrozole	II RCT	Neoadjuvant	Open
Insulin-like growth factor receptor monoclonal antibody CP-751,871 + Exemestane	II	Metastatic	Planned
Anastrozole ± Gefitinib	II RCT	Neoadjuvant	Completed
<b>Phase III</b>			
Temsirolimus ± Letrozole	III	Metastatic	Terminated
Lapatinib ± Letrozole	III	Metastatic	Open
Trastuzumab ± Tamoxifen	III	Metastatic	Planned
Letrozole ± Everolimus	III	Metastatic	Terminated
Letrozole ± Everolimus	III	Neoadjuvant	Open

Administration of estrogen has been found to induce apoptosis and tumor regression in a breast cancer cell line resistant to estrogen deprivation (30, 31). In fact, a clinical trial of high-dose estrogen in 32 heavily pretreated women who had developed resistance to prior endocrine therapy showed a remarkable overall response rate of 31% (32).

Currently, a clinical trial is analyzing whether therapeutic doses of estradiol, followed by the antiestrogen fulvestrant, to patients with metastatic breast cancer who have progressive

disease on an AI.<sup>1</sup> The rationale behind this study is that the cells may have lost sensitivity to estrogen and that exposure to high doses of estrogen may restore the tumor's normal estrogen sensitivity, rendering fulvestrant more effective.

Results of clinical trials exploring these concepts are eagerly awaited.

<sup>1</sup>SE Come, personal communication, 2006.

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