Biologic Basis of Sequential and Combination Therapies for Hormone-Responsive Breast Cancer

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Key Words. Estrogen receptors • Growth factors • MAP kinase signaling pathway Genes, HER-2 • Aromatase inhibitors • Fulvestrant • AKT kinase signaling

Learning Objectives

After completing this course, the reader will be able to:

1. Explain the possible role of alternate growth factor signaling pathways in the development of resistance to endocrine therapies in estrogen receptor (ER)-positive breast cancer.

2. Describe how the proposed mechanisms of action of tamoxifen, fulvestrant, aromatase inhibitors, and estrogens differ from one another.

3. Summarize the rationale for use of sequential endocrine therapy in postmenopausal women with ER-positive breast cancer.

Abstract

Although pharmacologic therapies that reduce or block estrogen signaling are effective treatments of estrogen receptor (ER)-positive breast cancer, acquired resistance to individual drugs can develop. Furthermore, this approach is ineffective as initial therapy for a subgroup of receptor-positive patients. The mechanisms of drug resistance are not completely understood, but the presence of alternative signaling pathways for activating ER response appears to play a significant role. Cross-talk between signaling pathways can activate ERs when conventional ER pathways are blocked or inactivated. For example, signaling via epidermal growth factor or HER-2 receptors, mitogen-activated protein kinases, phosphatidylinositol 3' kinase/protein kinase B, and vascular endothelial growth factor receptor can lead to estrogen-independent stimulation of ERs and tumor growth. The discovery that alternative pathways are involved in estrogen signaling has prompted development of newer endocrine therapies, such as aromatase inhibitors and pure estrogen antagonists, with distinct mechanisms for interrupting signal transduction. The existence of multiple pathways may explain the effectiveness of follow-up therapy with a different class of endocrine agents after failure of prior endocrine treatment. Because they do not have the partial agonist activity of tamoxifen that is enhanced by the adaptive hypersensitivity process, these alternative endocrine agents may play an increasingly important role in the treatment of ER-positive breast cancer. Although optimal sequencing of these agents has not been determined and is continuing to evolve, current evidence allows rational recommendations to be made. The multiple pathways involved in activating ERs also provide a rationale for combining endocrine and non-endocrine therapies that block different signaling pathways, which may have synergistic and overlapping interactions. The Oncologist 2006;11:704–717

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**Introduction**

Estrogen receptors (ERs) and progesterone receptors (PRs) frequently are present in breast tumors and function as prognostic indicators for response to treatment. The presence of ERs and PRs is predictive for response to endocrine therapy and improved disease-free survival [1, 2]. Approximately 50%–60% of women with ER-positive breast cancer benefit from endocrine therapy [3]. In contrast, only a small minority of ER/PR-negative patients respond to endocrine therapy [2].

Based on its demonstrated efficacy, endocrine therapy has become an integral component of breast cancer therapy in both the metastatic and adjuvant settings [4]. In recent Early Breast Cancer Trialists’ Collaborative Group (EBCTCG) meta-analyses of randomized trials of adjuvant tamoxifen in early breast cancer, treatment with tamoxifen was associated with highly significant reductions in the risk for disease recurrence and in breast cancer-related mortality [5]. The most recent EBCTCG meta-analysis of 194 randomized trials reported that, in patients with ER-positive breast cancer who had been treated for approximately 5 years with adjuvant tamoxifen, the recurrence rate was nearly one half lower and the breast cancer-related mortality rate was one third lower than with placebo [5]. Since these trials were initiated, new endocrine therapies, such as aromatase inhibitors and the “pure” antiestrogen and ER downregulator fulvestrant (Faslodex®; AstraZeneca Pharmaceuticals, Wilmington, DE), have become available. Endocrine agents for breast cancer are targeted therapies; most of these agents have relatively low toxicity and allow an excellent quality of life [4].

Despite its demonstrated efficacy in patients with ER-positive breast cancer, tamoxifen is ineffective for a subgroup of ER-positive breast tumors [6]. The reason for tamoxifen’s lack of efficacy in some patients is not entirely clear; however, the effect may be related to the presence of alternative signaling pathways for activating estrogen receptors. Other ligands or signaling components, such as epidermal growth factor (EGF), HER-2/new-activating ligands, mitogen-activated protein kinase (MAPK), and phosphatidylinositol 3 kinase (PI3K)/protein kinase B (AKT), may activate ERs/cellular pathways in the absence of estrogen. These alternative pathways may present new targets for drug therapy. This article reviews some of the known and emerging complexities of ER signaling and discusses therapeutic implications of these findings.

**Mechanism of Action of Endocrine Therapies**

The rationale for the use of endocrine therapies in the treatment of breast cancer is that estrogen-dependent tumors use estrogen as a growth factor [7]. When estradiol binds to the ER, the estradiol–ER complex undergoes conformational changes followed by dimerization and subsequent binding to specific DNA sequences (i.e., estrogen response elements [EREs]), resulting in the promotion of transcription [8]. Estrogen-induced interaction between ERs and EREs historically has been considered the initiating event involved in estrogen-regulated gene expression [9]. The ER uses two independent functional domains to bind coactivator proteins required for transcription: activator function 1 (AF-1), which lies within the ER amino-terminal domain, and activator function 2 (AF-2), which lies in the ligand-binding domain (Fig. 1) [10, 11]. When a selective estrogen receptor modulator (SERM) binds to ER-α, conformational changes prevent AF-2 binding to the coactivator complex.

The underlying molecular mechanism regulating ER expression and the role of the ER in the pathogenesis of breast cancer are not completely understood; however, at least two promoter regions of estrogen-regulated genes have been identified [12–14]. The ER also plays a role in regulating its own transcription by mechanisms that are independent of ERE-mediated pathways (e.g., via the interaction with other transcription factors, such as activator protein 1 [AP-1]) [15, 16]. Transcription by ligand-bound ER from AP-1 sites also has been observed in some tissues, although ER does not bind directly to the DNA sequences of AP-1-enhancer elements [10]. For activation by ER from AP-1 sites, binding of AP-1 proteins (Jun and Fos) to the AP-1 site is required as are both AF-1 and AF-2 domains of the ER, which can interact with transcriptional coactivators [10]. This pathway also is important because increased AP-1 activity is associated with increased resistance to chemotherapeutic drugs through increased expression of the multidrug-resistant gene 1 (MDR-1) [9, 15, 17].

When tamoxifen binds to the ER, the tamoxifen–ER complex still can undergo dimerization, but the ability of this complex to promote transcription is reduced [8]. Thus the biologic events mediated by the tamoxifen–ER complex are different from those produced by the estradiol–ER complex [8]. Notably, the effects of tamoxifen appear to be tissue specific [18]. For example, although tamoxifen blocks estrogen activity in the breast, it also has estrogen agonist activity in some other tissues (e.g., endometrium) [19]. In addition to its antiproliferative and antitumor effects, long-term tamoxifen therapy irreversibly inhibits estrogen-dependent gene expression [20].

The effects of estrogen and antiestrogen are mediated through at least two different ER gene products, ER-α and ER-β. In normal breast tissue, only about 5%–10% of epithelial cells express ER-α, but up to 80% of these cells express ER-β [21]. However, invasive breast cancers...
tor gene product, ER-α, in metastatic tumors, and mutations in the classic receptor are generally reported to be relatively rare among primary tumors seen in breast cancers, mutations within the receptor sequence are intensifying. The PR is an ER-regulated gene, and congruence in the expression of ER and PR has been used as a marker for an intact estrogen signaling pathway in breast tumor cells [31]. Further, the PR has two isoforms that both appear to be significant to breast cancer progression but in different fashions, with PR-A linked to tamoxifen resistance and PR-B potentially increasing the risk for breast cancer itself [32]. Although ER-positive, PR-negative breast tumors (including those undergoing loss of PR expression during endocrine treatment) are characterized by a relatively poor response to tamoxifen and associated prognosis, there is some suggestive evidence from subgroup analyses that PR negativity may have less of an impact on the efficacy of some of the newer aromatase inhibitors [33]. However, it is best not to base clinical decisions solely on subgroup analyses when there are no clear reasons for a differential effect of a treatment. Interested readers should refer to the recently published reviews by Osborne et al. [32] and Cui et al. [34] that provide insightful discussions of the molecular biology of the PR and contemporary insights into its clinical significance, including potential mechanisms underlying differing responses of PR-negative tumors to tamoxifen versus aromatase inhibitors.

Aromatase inhibitors include both steroidal (e.g., exemestane and formestane) and nonsteroidal (e.g., anastrozole and letrozole) agents. These agents act by blocking the conversion of adrenal androgens to estrogen in peripheral (i.e., nonovarian) tissues through the inhibition of the aromatase enzyme, thereby decreasing circulating and tumor estrogen levels [35]. Steroidal agents irreversibly inactivate aromatase, while nonsteroidal agents reversibly bind to aromatase [35]. The formation of estrogens from C19 steroids generally contain significant levels of ER-α and ER-β, with approximately two thirds of tumors staining positive by immunohistochemical assays [21, 22]. Epithelial stem cells bearing ERs in normal breast may function as primary progenitor cells in the etiology of breast cancers and may eventually be targets for specific therapy [23, 24]. Despite enhanced expression of ER-α in primary breast cancers, mutations within the receptor sequence are reported to be relatively rare among primary tumors seen in the clinic [21]. The mutation frequency may be higher in metastatic tumors, and mutations in the classic receptor gene product, ER-α, are found in some tamoxifen-stimulated tumors. These alterations can result in reduced interaction of the ER with corepressors and an increase in the transcriptional activity of ER-α with tamoxifen treatment, resulting in tamoxifen-induced tumor stimulation [25]. Other ER-α alterations that may be associated with hormonal resistance also have been reviewed elsewhere [26–28], but the clinical significance of these mutations remains to be elucidated [21].

The function of the more recently identified ER-β remains to be established [22]. Recent data suggest that ER-β, expression is correlated with most prognostic factors in breast cancer and that ER-β is not a surrogate for ER-α in breast cancer prognosis [22]. However, additional confirmatory studies with improved assay reagents and methods are needed [21].

The partial agonism of tamoxifen appears to be related to the differential effects of the drug on the two distinct transcriptional activating functions (AF-1 and AF-2) of the ER. The binding of tamoxifen to the ER alters the conformation of the major ligand-dependent transcriptional activation domain (AF-2), while the other activation domain (AF-1) remains active [29]. The differential effect of tamoxifen on these two transcriptional activation domains may provide one explanation for the emergence of tamoxifen-resistant tumors [30]. In other words, activation of alternative molecular pathways may provide the cell with a mechanism to bypass the requirement for estrogen to regulate cell growth and apoptosis [30].

Figure 1. Schematic representation of estrogen receptor (ER) activation upon ligand binding. When bound to ligand (estradiol), the ER dimerizes and binds to estrogen response elements (EREs) to transcribe target genes. This process requires both functional domains of the ER, activating function-1 (AF-1) and AF-2, to bind to DNA and recruit necessary coactivators of transcription (not shown). Reproduced with permission from Nature Reviews Cancer. Johnston SR, Dowsett M. Aromatase inhibitors for breast cancer: lessons from the laboratory. Nat Rev Cancer 2003;3:821–831. ©2003 Macmillan Magazines Ltd.
is catalyzed by a specific form of cytochrome P450, aromatase cytochrome P450 (estrogen synthetase; CYP19). Aromatase inhibitors are primarily effective in postmenopausal women because the primary source of estrogens in these women is the low-level conversion of androgen to estrogens (via aromatase) in peripheral tissues, as opposed to the much higher level of ovarian estrogen production in premenopausal women [35]. However, aromatase inhibitors can be used in premenopausal women who have undergone ovarian ablation, surgically or by either luteinizing hormone-releasing hormone (LHRH) agonists or radiation therapy (albeit rarely used at this time) [36, 37].

Fulvestrant is a novel antiestrogen that, unlike tamoxifen, has no known estrogen agonist activity [29, 38]. The drug produces multiple changes in ER function, including impaired dimerization, increased turnover, and disrupted nuclear localization [29, 39]. Thus, fulvestrant appears to block the ability of the ER to regulate transcription by any known mechanism [29]. Whereas tamoxifen blocks only the AF-2 transcriptional activation domain, fulvestrant blocks both AF-1 and AF-2 [40]. Therefore, unlike tamoxifen-like drugs that can produce increased cellular levels of ER, fulvestrant markedly reduces cellular levels of ER [29]. Furthermore, the novel mode of action of fulvestrant suggests that its use early in the therapy sequence would not lead to development of cross-resistance to subsequent endocrine therapies, although more studies must be done to determine its optimal placement in the therapy cascade [41].

**Signaling Pathways/Cross-Talk to Activate the ER**

Cross-talk between signaling pathways may underlie adaptive processes that can alter the hormone responsiveness of malignancies such as ER-positive breast cancer (Fig. 2A) [11, 42] and androgen-dependent prostate cancer [43]. By switching to an alternative growth factor signaling pathway, cells may circumvent the pharmacologic blockade of classical estrogen response pathways. Ligand-independent activation of steroid hormone receptors by growth factors has been examined in a variety of model systems. For example, in vitro model systems have demonstrated that long-term estrogen deprivation (which models low estrogen levels produced by aromatase inhibition) eventually produces a hypersensitivity of human breast cancer cells to estrogen and escalation in aromatase activity [44–47]. This hypersensitivity to estrogen is associated with the overexpression of growth factor pathways; it allows tumor cells to be stimulated by much smaller amounts of estrogen [45–47]. It has been postulated that the modulation of estrogen sensitivity may be one factor underlying the clinical effectiveness of sequential endocrine therapy [44].

Enhanced estradiol sensitivity among long-term estrogen-deprived breast cancer cells is associated with enhanced activation of MAPK and an increase in aromatase activity [48, 49]. This cross-talk between the ER and MAPK, presumably resulting in phosphorylation and subsequent activation of the ER [50], may be one explanation for estradiol hypersensitivity [49]. Increased MAPK activity may be the stimulus for additional adaptive changes, such as an increase in aromatase activity in the tumor cell [48, 51]. MAPK activation also may cause cells to be more responsive to growth effects of estrogen and, therefore, more responsive to the partial agonist activity of tamoxifen [46, 49].

Response to EGF, which is mediated in part through the MAP kinase pathway, is another alternative pathway by which the ER can be activated [52, 53]. There appears to be an inverse relationship between the level of ER-α and the level of EGF receptor in breast cancer [40]. The loss of ER-α associated with antiestrogen therapy may result in cells becoming more sensitive to growth stimuli by switching to EGF as a growth factor [40]. EGF receptor is upregulated in cells that have become resistant to antiestrogen treatment [40]. Thus, the enhanced expression [40] or activation [53] of EGF receptor may contribute to the development of resistant cells.

To understand the biologic basis of hormonal resistance, it is important to consider other complementary pathways in estrogen action. Numerous, rapid cell responses to estrogen treatment cannot be fully explained by the current nuclear model of estrogen action [54, 55]. In eliciting acute responses, estrogens appear to interact with an extranuclear ER to promote hormonal responses through a pathway that cross-communicates with a genomic mechanism (Fig. 2A) [11]. In addition, activation of extranuclear ER may occur by steroid-independent signaling, involving mediation by growth factor receptors [52, 53].

Liganded extranuclear ER or growth factor receptor-activated ER may affect one or more of several intracellular signaling pathways, including modulation of ion channels, leading to enhanced ion flux, interaction with membrane receptors, and activation of G-proteins, nucleotide cyclases, and/or MAPK or PI3K/AKT. Through these interrelated pathways, extranuclear and nuclear events may respond in a coordinated manner to estrogen [42, 56, 57]. Experimental evidence suggests that disruption of extranuclear ER signaling also is vital in the emergence of resistance to hormonal therapy (Fig. 2B) [42, 55, 56].

Vascular endothelial growth factor (VEGF), a potent stimulator of angiogenesis, is another growth factor that appears to be involved in cross-talk with ERs [58, 59]. Expression of VEGF is upregulated in a number of cancer types (including breast cancer), and there is a correlation between VEGF expression and microvessel density in...
Figure 2. Cross-talk between growth factor receptors and estrogen receptor (ER). (A): Interactions between cell surface growth factor receptors and nuclear estrogen receptor. Ligand binding to cell surface growth factor receptor tyrosine kinases can lead to activation of major downstream signaling pathways involved in survival (phosphatidylinositol 3’ kinase [PI3K]/protein kinase B [AKT]), proliferation (extracellular-regulated kinase [ERK]), and cytokine/stress response (p-38-mitogen-activated protein kinase [p38-MAPK]). Activation of these pathways can result in phosphorylation of the ER or steroid receptor coactivators, such as p160, enhancing transcription of estrogen-responsive genes. Some ligands that may be involved in phosphorylation of ERs are illustrated: epidermal growth factor (EGF) and vascular endothelial growth factor (VEGF). These ligands can activate downstream signaling pathways by binding to their receptors, such as the EGF receptor (EGFR), VEGF receptor (VEGFR), and human EGFR-related 2 (HER-2/neu). Reproduced with permission from Nature Reviews Cancer Johnston SR, Dowsett M. Aromatase inhibitors for breast cancer: lessons from the laboratory. Nat Rev Cancer 2003;3:821–831 ©2003 Macmillan Magazines Ltd.

(B): Working model of estrogen action in target cells. Nuclear as well as extranuclear/nuclear models of hormone action are depicted. In many prevailing models (a, b), estrogen freely enters all cells and is retained only in those cells in which it homes in on its receptor, localized within the nucleus. There, estrogen binding to ER is believed to promote alterations in receptor conformation favoring enhanced association with coactivator proteins and with specific estrogen-responsive elements (EREs), leading, in turn, to selective gene transcription (a). However, this model fails to account for ERE-independent genomic activation. As shown in panel B, the steroid may also interact with activating protein-1 (AP-1) to facilitate gene transcription via a pathway that does not require ERE. Neither nuclear model (a, b) explains the numerous, rapid cell responses to estrogen treatment, such as the downstream signaling cascade. In eliciting these acute responses, estrogens bind to an extranuclear, plasma membrane-associated ER to promote hormonal responses through a complementary pathway that cross-communicates, or interacts directly, with a genomic mechanism (c). In addition, activation of ER may occur by steroid-independent signaling, involving mediation by growth factor receptors (d). Liganded membrane ER or growth factor receptor-activated ER may affect one or more of several pathways, including modulation of ion channels, leading to enhanced flux of ions, notably Ca^{2+}; interaction with peptide membrane receptors; and activation of G-proteins, nucleotide cyclases, and/or mitogen-activated protein kinases (MAPKs), with resultant increases in their catalytic products. These membrane interactions may elicit phosphorylation of ER itself through steroid-promoted (c) or steroid-independent pathways (d). Accordingly, through these interrelated pathways, extranuclear and nuclear events respond in a coordinated manner to estrogen levels in the external environment. The intricate array of physiologic responses of cells to estrogens may occur as a consequence of a synergistic feed-forward circuit, where estrogens activate cell membrane signaling pathways that act, in turn, to enhance transcriptional activity of ER in the nucleus. Abbreviation: SRC, steroid receptor coactivator. From Pietras RJ, Szego CM. Plasma membrane for steroid hormones in cell signaling and nuclear function. In: Melmed S, Conn PM, eds. Endocrinology: Basic and Clinical Principles, Second Edition. Totowa, NJ: Humana Press, 2005:67–84, with permission.
primary breast cancer sections [58, 59]. Moreover, an elevated level of VEGF is a negative prognostic indicator for endocrine-responsive tumors, including breast cancer [58, 59]. VEGF mRNA and protein expression are increased by estradiol in breast cancer cells, and the effect appears to be mediated by the activation of ERs [55, 59, 60]. Furthermore, fulvestrant can block estrogen-stimulated transcription of VEGF [59, 60]. In contrast, the partial ER agonists tamoxifen and toremifene induce VEGF expression [60].

Tumor-associated angiogenesis also may be affected by the interaction of estradiol directly with vascular endothelial cells that contain ERs and exhibit a proliferative response to treatment with estradiol [54, 55]. The reported expression of VEGF receptor-2 (VEGFR-2) in breast tumor cells and correlation of tumor-specific VEGFR-2 expression with an impaired response to tamoxifen is another area that requires further investigation [61].

HER-2/neu is a proto-oncogene that is overexpressed in approximately 20%–30% of primary breast cancers [62, 63]. Overexpression of HER-2/neu in the presence of ER-positive metastatic breast cancer is a negative prognostic factor and may predict weaker response to tamoxifen therapy [62, 64]. There is increasing evidence of cross-talk between the HER-2/neu and ER signal transduction pathways [55, 62]. For example, a direct interaction between the HER-2/neu signal pathway and ERs in human breast cancer cells has been demonstrated [64, 65]. HER-2/neu overexpression triggers the Ras/MAPK signaling pathway and increases phosphorylation of serine and tyrosine residues in the ER [50, 65]. The result is a loss of the inhibitory effect of tamoxifen on ER-mediated transcription [56, 64], which is evidenced by studies demonstrating that patients who are ER-positive with elevated serum levels of HER-2/neu are less likely to respond to endocrine therapy than are those who are ER-positive and HER-2/neu-negative [62]. There is some evidence of differential activity among endocrine therapies in HER-2/neu-positive, ER-positive tumors, with tamoxifen demonstrating a lower level of activity than with aromatase inhibitors or fulvestrant [6, 64]. Furthermore, the addition of anti-HER-2/neu antibody (trastuzumab) has been shown to promote the antitumor activity of antiestrogens in breast cancer cells overexpressing HER-2/neu [64].

The activation and modulation of these signal transduction pathways, and probably others, likely are responsible for the adaptive mechanisms by which tumors begin to regrow after initial therapy with antiestrogens. The observed clinical response to second-line (and subsequent) hormonal therapy may be a result of the different mechanism of action of each new agent. Because they use different mechanisms to interrupt signaling pathways, alternative agents (i.e., aromatase inhibitors, pure estrogen antagonists) can be effective for tumors that have relapsed following tamoxifen. Also, unlike tamoxifen, these other agents do not have partial agonist activity that might become enhanced by the adaptive hypersensitivity process [46].

### Sequencing Endocrine Therapies and Combination Approaches

#### Sequencing Therapy

**Adjuvant Setting**

Anastrozole and letrozole have become first-line alternatives to tamoxifen for treating postmenopausal women in the adjuvant setting, based on data from the phase III (ATAC) and Breast International Group (BIG) 1-98 trials, respectively (Table 1). In the Arimidex®, Tamoxifen, Alone or in Combination (ATAC) trial, adjuvant therapy with anastrozole, tamoxifen, and the combination of these two treatments was evaluated in more than 9,000 postmenopausal women with early breast cancer [66]. Anastrozole was found to be more effective than tamoxifen or combination therapy and was better tolerated with respect to a number of adverse events [66]. Follow-up at 68 months in that trial revealed that, compared with tamoxifen, anastrozole resulted in a significantly longer disease-free survival time and time to recurrence and fewer distant metastases and contralateral breast cancers in postmenopausal women with hormone receptor-positive localized breast cancer [67]. In the first analysis, with a 33.3-month median follow-up, there was no significant difference between the tamoxifen monotherapy and combination arms [66]. The investigators speculated that this equivalence may have been related to a minor level of tamoxifen-induced estrogenic signaling (stemming from its partial agonist activity and unlikely to be affected by the anastrozole in the combination) or potential differences in resistance mechanisms between anastrozole and tamoxifen [66]. More recently, a report of BIG 1-98 (based on data for approximately 8,000 evaluable patients) revealed significantly higher disease-free and distant recurrence rates for initial adjuvant treatment with letrozole versus tamoxifen after a 25.8-month median follow-up [68]. Whereas an ATAC subanalysis demonstrated a significantly greater disease-free survival benefit for anastrozole in ER-positive, PR-negative versus ER-positive, PR-positive disease [33], preliminary results of a similar analysis of BIG 1-98 found no significant difference between these patient subsets with respect to letrozole [69].

A series of commentaries in response to the BIG 1-98 results has raised concerns regarding interpretation of subset analyses of the large adjuvant trials and the higher incidence of cardiac events seen with letrozole versus tamoxifen [70–73]. These issues require further investigation in future work.
Preplanned sequencing of endocrine agents has become an intense area of investigation in the adjuvant setting, as there are data to support that switching to an aromatase inhibitor after a prespecified course of tamoxifen confers disease-free survival benefits over the traditional 5-year course of tamoxifen alone (Table 1). In BIG 1-98, patients may have received letrozole and tamoxifen in sequence (i.e., letrozole for 2 years followed by tamoxifen for 3 years or tamoxifen for 2 years followed by letrozole for 3 years) rather than a 5-year course of monotherapy [68]; however, comparative data for sequencing versus monotherapy are not yet available. The Intergroup Exemestane Study (IES) was a double-blind, randomized trial investigating the sequential use of tamoxifen for 2–3 years followed by exemestane for 2–3 years compared with 5 years of tamoxifen in 4,742 patients [74]. Switching to exemestane versus continuing on tamoxifen was associated with a 32% lower risk; in terms of disease-free survival, this corresponds to an absolute benefit of 4.7% at 3 years after randomization. In three European trials—the Arimidex®-Nolvadex® (ARNO) 95, Austrian Breast and Colorectal Cancer Study Group (ABCSG) 8, and Italian Tamoxifen Anastrozole (ITA) trials—investigators are evaluating the benefits of switching to anastrozole after 2 years (ARNO 95 and ABCSG 8) or 2–3 years (ITA trial) of adjuvant tamoxifen in postmenopausal women with hormone-sensitive early breast cancer [75, 76]. At a median follow-up of 26 months in a combined analysis of the ARNO 95 and ABCSG 8 trials [76] and at a follow-up of 36 months for the ITA trial [75], recurrence-free survival was significantly longer with anastrozole than with continuing on tamoxifen alone; 68-month follow-up data for the combination are not available.

### Table 1. Recent reports of phase III trials of aromatase inhibitors as adjuvant breast cancer therapy

<table>
<thead>
<tr>
<th>Study</th>
<th>Arms</th>
<th>Disease-free survival</th>
<th>Hazard ratio for disease-free survival; 95% confidence interval; p-value</th>
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<tr>
<td><strong>Sequential therapy for 5 yrs</strong></td>
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<tr>
<td>IES: Intergroup Exemestane Study</td>
<td>Tamoxifen 20 mg/d × 2–3 yrs ⇒ exemestane 25 mg/d</td>
<td>91.5%</td>
<td>0.68;</td>
</tr>
<tr>
<td>ITA: Italian Tamoxifen Arimidex® trial</td>
<td>Tamoxifen 20 mg/d × 2–3 yrs ⇒ anastrozole 1 mg/d × 2–3 yrs</td>
<td>86.8%</td>
<td>0.56–0.82; p = .00005</td>
</tr>
<tr>
<td>Combined analysis of ARNO 95 and ABCSG 8: Arimidex®-Nolvadex®-95 and Austrian Breast and Colorectal Cancer Study Group 8</td>
<td>Tamoxifen (dose NR) × 2 yrs ⇒ anastrozole (dose NR)</td>
<td>95.8%</td>
<td>0.60;</td>
</tr>
<tr>
<td>Meta-analysis of ITA, ARNO 95, and ABCSG 8 (as per the ITA and ARNO 95 and ABCSG 8 descriptions above)</td>
<td>Tamoxifen (dose NR) × 2 yrs ⇒ tamoxifen (dose NR)</td>
<td>92.7%</td>
<td>0.44–0.81; p = .0009</td>
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<tr>
<td><strong>Extension use of aromatase inhibitors</strong></td>
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<tr>
<td>National Cancer Institute of Canada Clinical Trials Group MA.17</td>
<td>Tamoxifen × ~5 yrs ⇒ letrozole 2.5 mg/d × 5 yrs</td>
<td>94.4%</td>
<td>0.58;</td>
</tr>
<tr>
<td></td>
<td>Tamoxifen × ~5 yrs ⇒ placebo × 5 yrs</td>
<td>89.8%</td>
<td>0.45–0.76; p &lt; .001</td>
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Kaplan-Meier estimates, if available, are included in the table; otherwise, this outcome is reported as the number of events after a specified median follow-up.

A combination regimen was also evaluated as a third arm (anastrozole 1 mg/d + tamoxifen 20 mg/d), which in the original analysis (33.3-month median follow-up) was statistically inferior to anastrozole alone and statistically equivalent to tamoxifen alone; 68-month follow-up data for the combination are not available.

Two sequential regimens were also evaluated as third and fourth arms (letrozole 2.5 mg/d × 2 years ⇒ tamoxifen 20 mg/d × 3 years and tamoxifen 20 mg/d × 2 years ⇒ letrozole 2.5 mg/d × 3 years), for which efficacy data are not yet available.

Total treatment period of 5 years. Abbreviation: NR, not reported.
tamoxifen. A recently presented meta-analysis of all three trials (median follow-up of 30 months) revealed a highly significant disease-free survival advantage for switching to anastrozole versus continuing on tamoxifen, translating into the first reported overall survival advantage for sequential hormonal therapy over tamoxifen alone (hazard ratio, 0.71; \( p = .038 \)) [77]. Lastly, in the MA-17 trial, 5,187 postmenopausal women who were recurrence free after 5 years of tamoxifen were randomized to 5 years of letrozole or placebo [78, 79]. At a median follow-up of 2.4 years, patients receiving letrozole showed a significantly higher rate of estimated 4-year disease-free survival compared with those receiving placebo (93% vs. 87%), prompting early closure of the trial [78]. An updated report concluded that disease-free and distant disease-free survival were significantly longer with letrozole, with equivalent rates of contralateral breast cancer and overall survival between the two arms (however, letrozole significantly improved overall survival in node-positive patients) [79]. Additionally, among patients initially randomized to placebo who were then offered letrozole on an open-label basis, significant improvements were seen in all evaluated end points—namely, disease-free, distant disease-free, and overall survival as well as the rate of contralateral breast cancer [80].

The adjuvant studies cited here suggest that these agents are at least partially non-cross-resistant, and sequential or combination treatment with multiple endocrine therapies may have an added clinical benefit. The identification of an optimal sequencing strategy for endocrine therapy has the potential to improve clinical outcomes and prolong the treatment period during which hormonal therapy can be used [36].

**Advanced Breast Cancer**

The clinical role of aromatase inhibitors in the treatment of breast cancer has increased dramatically in recent years. Among patients in whom first-line therapy with tamoxifen failed, aromatase inhibitors have improved clinical outcomes, including longer survival, and are associated with better tolerability than with megestrol acetate [81–83]. Although the mechanism is unclear, one study showed that switching to a different aromatase inhibitor was also effective in some patients in whom prior therapy with another aromatase inhibitor was unsuccessful [84]. A combined analysis found that the aromatase inhibitor anastrozole was comparable with tamoxifen with respect to response rates, but had the added benefit of prolonging time to disease progression and was better tolerated [85]. Similarly, letrozole compared with tamoxifen had a longer mean time to disease progression, longer mean time to treatment failure, and higher overall response rate [86]. Fulvestrant has been shown to be as effective as anastrozole in women who have progressed after initial therapy with tamoxifen [87–90].

In postmenopausal breast cancer, options for first-line endocrine therapy of advanced breast cancer include an aromatase inhibitor (if the patient has already received adjuvant tamoxifen) or tamoxifen (if the patient has already received an adjuvant aromatase inhibitor) (Fig. 3) [91, 92]. Following progression on first-line endocrine therapy, viable second-line options include fulvestrant, tamoxifen, or an aromatase inhibitor, depending on prior treatment [91]. In patients who received aromatase inhibitors as first-line therapy, fulvestrant currently is being investigated as an appropriate next step in treatment [93, 94]. Third-line therapy may consist of fulvestrant, tamoxifen, or an aromatase inhibitor, varying from the patient’s previous treatment. Recommended fourth-line therapy is an endocrine therapy not previously used, such as megestrol acetate [91], and fourth- or fifth-line therapeutics also may include androgens or other novel approaches such as low- or high-dose estrogen therapy to reverse endocrine resistance [92, 95–99]. From a historical standpoint, it is interesting that high-dose estrogens were commonly used in the treatment of advanced breast cancer before the introduction of tamoxifen (which became the preferred endocrine therapy because of its more favorable toxicity profile). In fact, long-term follow-up of a trial conducted during the late 1970s reported a survival benefit for first-line hormonal therapy with diethylstilbestrol (DES) versus tamoxifen in postmenopausal women with metastatic breast cancer [100]. In recent years, estrogen therapy has emerged as an intriguing approach to restoring the hormonal sensitivity of tumors developing resistance to hormonal therapy. There is both clinical and preclinical evidence supporting such an approach. Lonnning et al. [95] reported a 32% objective response rate and two cases of prolonged stable disease among 32 postmenopausal women receiving DES for heavily endocrine-pretreated advanced breast cancer, and there is accumulating preclinical evidence that estrogens can induce apoptosis and tumor regression of breast tumors resistant to other hormonal approaches [96–98]. Evaluation of high-dose estrogen is being further evaluated in the clinical setting [99].

Overall, the process of selecting hormonal therapy for advanced breast cancer is likely to become increasingly complex as adjuvant hormonal regimens become increasingly sophisticated. Also of note is the potentially changing landscape of breast cancer prevention, where raloxifene appears to be as effective as tamoxifen in preventing invasive breast cancer among postmenopausal women considered at elevated risk but with a lower incidence of life-threatening adverse events (based on first results of the Study of Tamoxifen and Raloxifene [STAR] trial, released by teleconference on April 17, 2006).
A number of clinical trials are evaluating the tolerability and effectiveness of sequential hormonal therapies for advanced breast cancer. The North Central Cancer Treatment Group (NCCTG) conducted a phase II trial involving postmenopausal women who progressed on nonsteroidal aromatase inhibitors with or without prior treatment with tamoxifen [93]. Fulvestrant (250 mg/month) was administered i.m., and the primary endpoint was the objective response rate. Recently published results include a clinical benefit rate of 35% among the 77 eligible patients (including 11 partial responses and 16 cases of stable disease for ≥6 months), with a median time to progression of 3 months and a median survival time of 20.2 months [93]. Another phase II trial, the SAKK study by the Swiss Group for Clinical Cancer Research, is intended to evaluate the effect of fulvestrant (250 mg/month i.m.) in postmenopausal women who have progressed on treatment with tamoxifen and an aromatase inhibitor [94]. Based on preliminary results of this study, which were presented at the 2004 San Antonio Breast Cancer Symposium, the clinical benefit rate (i.e., partial response or stable disease for ≥24 weeks) was 29% among patients previously responding to aromatase inhibitor therapy for advanced breast cancer; accrual into a second stratum of aromatase inhibitor-resistant patients (i.e., failed to respond or had stable disease for <24 weeks) was ongoing [94]. Both the NCCTG and SAKK trials found that fulvestrant was well tolerated, with primarily grade 1–2 toxicities and low adverse event-related discontinuation rates [93, 94]. The phase III Evaluation of Fulvestrant and Exemestane Clinical Trial (EFFECT), which involved postmenopausal women with hormone receptor-positive breast cancer who had recurrence or progression following treatment with a nonsteroidal aromatase inhibitor, evaluated the efficacy of fulvestrant (500 mg i.m. on day 0, followed by 250 mg/month i.m.) versus the steroidal aromatase inhibitor exemestane (25 mg/day orally [p.o.]) [101]. The EFFECT trial is closed, and it is anticipated that initial results will become available during 2006.

Based on the biologic rationale that multiple signaling pathways are involved in activating ERs, combination therapies (involving both endocrine and nonendocrine agents) that block different pathways are plausible and testable. Recent preclinical studies have provided evidence that such combination therapies may be clinically feasible and may be an effective method for overcoming antiestrogen resistance in breast cancer [56, 64]. The Faslodex® and Arimidex® in Combination Trial (FACT), an ongoing European phase III trial, is evaluating anastrozole (1 mg/day p.o.) alone or with fulvestrant (same regimen as the aforementioned EFFECT
in patients with postmenopausal women with ER-positive and/or PR-positive breast cancer. Patients must have a history of relapse following or during primary adjuvant therapy but no prior hormonal therapy for advanced disease. Outcome data on this trial are not expected until 2007–2008. Other ongoing trials of hormonal agents used concurrently or in combination with trastuzumab or targeted therapies (as indexed in the National Cancer Institutes’ PDQ® Clinical Trials database) are summarized in Table 2. Additional clinical trials assessing combinations

Table 2. Ongoing clinical trials of endocrine-based combination therapy for advanced breast cancer

<table>
<thead>
<tr>
<th>Regimen</th>
<th>Protocol numbers</th>
<th>Phase</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Combination endocrine therapy</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anastrozole ± fulvestrant in postmenopausal, ER+ and/or PR+ MBC</td>
<td>SWOG-S0226, NCT00075764, CAN-NCIC-SWOG-S0226</td>
<td>III</td>
</tr>
<tr>
<td>Fulvestrant ± anastrozole vs. exemestane alone in postmenopausal, ER+ and/or PR+ LABC or MBC relapsing or progressing during prior nonsteroidal AI therapy</td>
<td>ICR-CTSU-SOFEA EU-20531, EUDRACT-2004-00093-30, ISRCTN44195747, MREC-03677, SSA-04Q200635, NCT00253422</td>
<td>III</td>
</tr>
<tr>
<td>Exemestane + fulvestrant in postmenopausal, ER+ and/or PR+ ABC</td>
<td>OSU-0494 NCT00201864</td>
<td>II</td>
</tr>
<tr>
<td><strong>Endocrine therapy + trastuzumab</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anastrozole ± trastuzumab in postmenopausal, ER+ and/or PR+, HER-2-overexpressing MBC</td>
<td>ROCHE-BO16216 CWRU-030118, GENENTECH-H2223g, ROCHE-1100, ROCHE-B016216E, NCT00022672</td>
<td>II/III</td>
</tr>
<tr>
<td>Letrozole + trastuzumab in postmenopausal, ER+ and/or PR+, HER-2-overexpressing MBC</td>
<td>CFEM345C2403 NCT00171847</td>
<td>IV</td>
</tr>
<tr>
<td>Trastuzumab alone followed by letrozole + trastuzumab in postmenopausal, ER+ and/or PR+, progressive ABC</td>
<td>SWS-SACK-23/03 EU-20527, NCT00238290</td>
<td>II</td>
</tr>
<tr>
<td>Trastuzumab ± fulvestrant in postmenopausal, ER+ and/or PR+, HER-2-overexpressing stage IV breast cancer</td>
<td>UCLA-0502057-01 TORI-B-04, NCT00138125</td>
<td>II</td>
</tr>
<tr>
<td><strong>Endocrine therapy + small molecules</strong></td>
<td></td>
<td></td>
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<tr>
<td>Tamoxifen + lapatinib (dual kinase inhibitor) in ER+ and/or PR+, tamoxifen-resistant LABC or MBC</td>
<td>WSU-C-2876 NCT00118157, NCI-6724</td>
<td>II</td>
</tr>
<tr>
<td>Letrozole + lapatinib (dual kinase inhibitor) in postmenopausal, ER+ and/or PR+, stage IIIB–IV breast cancer</td>
<td>GSK-EGF30008 UCLA-0311034-01, NCT00073528</td>
<td>III</td>
</tr>
<tr>
<td>Letrozole + CCI-779 (mTOR inhibitor) in postmenopausal LABC or MBC</td>
<td>3066A1-303 NCT00083993</td>
<td>III</td>
</tr>
<tr>
<td>Letrozole + bevacizumab (VEGF inhibitor) in postmenopausal, ER+ and/or PR+, unresectable LABC or MBC</td>
<td>UCSF-037518 UCSF-H6961-24611-02, NCT00187694</td>
<td>II</td>
</tr>
<tr>
<td>Anastrozole + sorafenib (VEGF inhibitor) in postmenopausal, ER+ and/or PR+ MBC</td>
<td>GUMC-2004-251 NCT00217399, NCI-6584</td>
<td>I/II</td>
</tr>
<tr>
<td>Tamoxifen + gefitinib (EGFR inhibitor) in ER+, HER-2-overexpressing breast cancer</td>
<td>H 13546 NCT00206492</td>
<td>II</td>
</tr>
<tr>
<td>Tamoxifen + gefitinib or placebo in MBC</td>
<td>ZDI839IL/0225 NCT00069290</td>
<td>II</td>
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<tr>
<td>Anastrozole ± gefitinib in postmenopausal, ER+ and/or PR+, locally recurrent breast cancer or MBC</td>
<td>EORTC-10021 NCT00066378, IDBBC-10021</td>
<td>II</td>
</tr>
<tr>
<td>Anastrozole + gefitinib or placebo in postmenopausal, ER+ and/or PR+ MBC</td>
<td>ZDI839US/0713 NCT00077025</td>
<td>II</td>
</tr>
<tr>
<td>Fulvestrant + gefitinib in postmenopausal, ER+ and/or PR+ ABC or MBC</td>
<td>1839IL/0141 NCT00234403</td>
<td>II</td>
</tr>
<tr>
<td>Anastrozole + gefitinib versus fulvestrant + gefitinib in postmenopausal, ER+ and/or PR+ recurrent breast cancer or MBC</td>
<td>ECOG-4101 NCT00057941</td>
<td>II</td>
</tr>
<tr>
<td>Anastrozole ± lonafarnib (FTI) in postmenopausal, ER+ and/or PR+, stage IIIB–IV breast cancer</td>
<td>UCLA-0403073-01 SPRI-P03480, NCT00089404</td>
<td>II</td>
</tr>
<tr>
<td>Fulvestrant + tipifarnib (FTI) in postmenopausal, ER+ and/or PR+, inoperable ABC or MBC progressing after prior first-line endocrine therapy</td>
<td>NYWCCC-NCI-6205 NCT00082810, NCI-6205</td>
<td>II</td>
</tr>
</tbody>
</table>

Abbreviations: ABC, advanced breast cancer; AI, aromatase inhibitor; EGFR, epidermal growth factor receptor; FTI, farnesyl transferase inhibitor; ER, estrogen receptor; HER-2, human epidermal growth factor receptor-2; LABC, locally advanced breast cancer; MBC, metastatic breast cancer; mTOR, mammalian target of rapamycin; PR, progesterone receptor; VEGF, vascular endothelial growth factor.
of hormonal agents with signal transduction inhibitors as a strategy to overcome endocrine resistance are reviewed elsewhere [102].

**Summary and Future Directions**

The ER signaling pathway is complex, and there are multiple mechanisms by which ER-mediated cellular pathways may be activated. Such complexity likely enables tumors to use adaptive mechanisms for growth after the initiation of first-line endocrine therapy. This adaptation has important therapeutic implications because resistance to one agent is not necessarily conferred to others, primarily because different cellular mechanisms are affected by alternative endocrine therapies. For example, ER ligand therapy does not confer cross-resistance to estrogen deprivation therapy.

The concept of cycling antihormone treatment with estrogen therapy to resensitize tamoxifen-resistant breast tumors is being explored in the laboratory. Some growth factor signaling pathways also can activate the ER, even in the absence of estradiol. Blockade of such pathways may enhance the antitumor activity of endocrine agents. The existence of multiple mechanisms to block ER activation partially explains the effectiveness of sequential endocrine therapies and suggests that an optimal sequencing of therapies may prolong the duration of response. In addition, combination therapy with growth factor and estrogen-signaling agents appears promising and warrants further investigation. Similarly, novel antiestrogens without any agonistic activity (e.g., fulvestrant) may overcome resistance to conventional endocrine therapies, such as tamoxifen, in the treatment of hormone-responsive breast cancer.

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**Disclosure of Potential Conflicts of Interest**

The author indicates no potential conflicts of interest.

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**ADDITIONAL READING**


