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Review

Aromatase, aromatase inhibitors, and breast cancer

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ABSTRACT

Estrogens are known to be important in the growth of breast cancers in both pre and postmenopausal women. As the number of breast cancer patients increases with age, the majority of breast cancer patients are postmenopausal women. Although estrogens are no longer made in the ovaries after menopause, peripheral tissues produce sufficient concentrations to stimulate tumor growth. As aromatase catalyzes the final and rate-limiting step in the biosynthesis of estrogen, inhibitors of this enzyme are effective targeted therapy for breast cancer. Three aromatase inhibitors (AIs) are now FDA approved and have been shown to be more effective than the antiestrogen tamoxifen and are well tolerated. AIs are now a standard treatment for postmenopausal patients. AIs are effective in adjuvant and first-line metastatic setting. This review describes the development of AIs and their current use in breast cancer. Recent research focuses on elucidating mechanisms of acquired resistance that may develop in some patients with long term AI treatment and also in innate resistance. Preclinical data in resistance models demonstrated that the crosstalk between ER and other signaling pathways particularly MAPK and PI3K/Akt is an important resistant mechanism. Blockade of these other signaling pathways is an attractive strategy to circumvent the resistance to AI therapy in breast cancer. Several clinical trials are ongoing to evaluate the role of these novel targeted therapies to reverse resistance to AIs.

Article from the special issue on 'Targeted Inhibitors'.

Contents

Estrogens are a group of steroid hormones that are essential to normal female physiology and reproduction. Estrogen signaling pathway engages in several cellular processes particularly cell proliferation and cell survival. Beside the reproductive system, estrogens also have important functions in the musculoskeletal system, cardiovascular system, and brain [1]. The three main natural estrogens in women include estrone (E1), estradiol (E2), and estriol (E3). Estradiol or 17β-estradiol is a major form of estrogens in women of reproductive age. In contrast, estrone is a form of estrogens predominantly in postmenopausal women and estriol is formed primarily during pregnancy. In premenopausal women,

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estrogens are synthesized from androgens by the granulosa cells in the ovaries. The main source of steroids in the ovaries is cholesterol. When ovaries are no longer functional, the source of estrogens in postmenopausal women comes from the peripheral conversion of androgens by the aromatase enzyme. This enzyme is present in multiple organs including adipose tissue, brain, blood vessels, skin, bone, endometrium, and breast tissue. Estrogens exert their activity by binding to the specific high affinity estrogen receptors (ER) including ERα and ERβ [2]. ERα is the subtype of ER that is required for most of the known estrogenic responses [3]. With the presence of ligand, ERα is displaced from the heat shock proteins and interacts either directly through specific estrogen response elements (EREs) or indirectly through transcriptional factors like AP1, SP1, and NF-κB [1,4]. Beside its genomic action, recent data demonstrated that ER also has non-genomic activity by acting as a component of membrane and cytoplasmic signaling cascades [5].

The first and most successful targeted cancer therapies are those that target estrogen signaling pathway in breast cancer. Approximately three quarter of breast cancer tumors express hormone receptors like ER and/or progesterone receptors (PR). By modulating either its ligand or the receptor, this strategy has been shown to be effective in treating hormone receptor-positive breast cancer for over a century. In 1890s, Sir George Beatson demonstrated that the majority of breast cancers in premenopausal women respond to bilateral oophorectomy. At that time, it was hypothesized that most of the breast cancer tumors were dependent on "ovarian hormones" [6,7]. Subsequently, other surgical modalities like adrenalectomy and hypophysectomy were also performed for the treatment of breast cancer [7]. One of the breakthroughs in breast cancer treatment is the discovery of drugs targeting the estrogen signaling pathway early 1970s. Tamoxifen, previously known as ICI 46,474, was the first targeted cancer therapy against this pathway that was approved for the treatment of breast cancer in the early 1970s [8]. Tamoxifen is categorized as a selective estrogen receptor modulator (SERM) due to its distinct actions in different organ sites. While tamoxifen is quite an effective treatment for patients with hormone receptor-positive breast cancer, it has adverse partial estrogenic effects in the uterus and vascular system causing an increased risk of endometrial cancer and thromboembolism [9,10]. Due to these unfavorable side effects and incomplete blockade of estrogen action, the alternative approach to target the ligand production instead of the ER itself was hypothesized to be more effective with fewer side effects. This hypothesis came from Harry and Angela Brodie who were initially working on the biochemistry of aromatase and were developing the inhibitors of aromatase as potential contraceptive agents but also as improved treatment for breast cancer. They reported the first series of these compounds in 1973 [11] with the hope of blocking the production of estrogen with specific inhibitors of aromatase [11–13]. 4-Hydroxy-androstenedione (4-OH-A) was demonstrated to be the most potent aromatase inhibitor of more than 100 compounds either synthesized or acquired for testing [13,14]. Subsequently, this compound was found to act by rapid competitive inhibition as well as inactivation of the enzyme resulting in long lasting or irreversible effect [15]. It was further demonstrated that 4-OH-A could reduce estrogen concentrations which resulted in tumor regression in rat mammary tumors. Also, 4-OH-A appeared to be more effective than tamoxifen without the adverse estrogenic effect on other tissue particularly the uterus [13].

Given that the main source of estrogen production in postmenopausal women comes from the peripheral conversion by the aromatase enzyme, inhibition of this particular enzyme results in the significant further reduction of estrogens. AIs are now considered to be the standard of care for postmenopausal women with hormone receptor-positive breast cancer [16]. Nonetheless, the emergence of resistance to AIs continues to be problematic, particularly in metastatic breast cancer. This review article summarizes the structure and function of aromatase enzyme, discovery and evolution of AIs, clinical efficacy of AIs, and recent insights into the mechanisms of AI resistance.

1. Aromatase enzyme

The human aromatase enzyme is a member of the cytochrome P450 family and is the product of the CYP19A1 gene, located on chromosome 15 [17,18]. It functions to catalyze the rate-limiting and final step of estrogen biosynthesis; the aromatization of androgens to estrogens. It does this via three oxidation reactions of the androstenedione A ring, with each reaction consuming a molecule of both oxygen and NADPH per reaction. Of these three steps, the third is unique to aromatase, while the first two are common to P450 cytochrome proteins [19]. Breast cancer tissues have been shown to express aromatase and produce higher levels of estrogens than non-cancerous cells. This is one of the main reasons that aromatase has generated a high level of interest for treatment of breast cancer [20]. As described previously, aromatase has also been found in a wide variety of tissues, including ovary, placenta, bone, adipose, testis, skin, and the brain [17,21–25]. However, only in primates has aromatase been shown to function in tissues other than the gonads or brain. In primates, tissue specific expression of aromatase is controlled by the presence of tissue specific promoters [25,26]. Aromatase is the only known vertebrate enzyme that can aromatize a six-membered ring; aromatase is, therefore, the sole source of estrogen in the body [27].

Traditionally, research on human aromatase has been performed on purified native or recombinant protein, allowing for kinetic analysis of aromatase function [22,28]. It is well established that the microsomal enzymatic complex of human aromatase is a heterodimer made up of a cytochrome 450 aromatase, and a ubiquitous NADPH cytochrome P450 reductase [27]. The catalytic portion of cytochrome P450 aromatase contains a heme group as well as a steroid binding site [29]. There are still several areas of aromatase function that are not thoroughly understood, such as the third aromatization step, as well as the underlying reason for its high substrate specificity. To address this, some investigators have utilized homology models of aromatase, based on other P450 enzyme structures [30]. Additionally, a wide range of site directed mutagenesis studies have been conducted, assaying aromatase function on androgens and putative aromatase inhibitors, and combining this information with chemical studies of estrogen biosynthesis in order to elucidate the mechanism of aromatase function as well as functional elements [31,32].

Nevertheless, since aromatase was first characterized, research has been impeded by the lack of its three dimensional structure. In 2009, Ghosh et al. successfully solved the crystallized structure of human aromatase enzyme and provides a structural basis for the specificity to androgen [33,34]. The catalytic site of aromatase is located at the juncture of the I and F helices, β-sheet 3, and as the B-C loop. Androstenedione binds into the steroid binding pocket such that its β-face orientates toward the heme group of aromatase, placing C19 within 4.0 Å of the Fe atom. This binding site is only possible if the I-helix backbone is moved 3.5 Å, creating a binding pocket that is approximately 400 $Å^3$. This important distortion is created by residue P308, without which N309, steric hindrance would prevent catalytic activity [33]. P308 is not found in any other member of the cytochrome P450 family, and its location on the distal side of the I-helix from the androstenedione, has made it an item of interest in site-directed mutagenesis studies. However, its mutation resulted in an enzyme with catalytic activity similar to wild type [31]. The active site of human aromatase is situated within the enzyme, and contains several closely packed hydropho-

Table 1
Classification of AIs.

Generations	Type 1	Type 2
	Steroidal inhibitors	Non-steroidal inhibitors
Nonspecific inhibitor		Aminoglutethimide
Previous selective inhibitors not currently in clinical use	Formestane	Fadrozole Rogletimide Vorozole
Selective oral inhibitors currently in clinical use	Exemestane (Aromasin®)	Anastrozole (Arimidex®) Letrozole (Femara®)

bic residues, which serve to stack against the α-face backbone of androstenedione [34]. This, combined with the relatively long distance the steroid must travel to reach the active site on the deep interior of the generally spherical enzyme, yields a very high degree of substrate specificity. This crystal structure of aromatase will not only allow better structure-based drug design than previous models, but it has also allowed a direct analysis of why some currently available aromatase inhibitors function better than others.

2. Discovery and evolution of aromatase inhibitors

Around the same time that selective aromatase inhibitors were in development, aminoglutethimide, a drug that was initially used as an anti-epileptic drug, was found to suppress adrenal steroid production by inhibiting multiple cytochrome P450 enzymes. Since adrenalectomy has also been used to treat breast cancer, Richard Santen and colleagues started to use aminoglutethimide as a "medical" adrenalectomy for breast cancer. In late 1970s, they were able to show that aminoglutethimide was effective in treating breast cancer [14]. Subsequently, Santen demonstrated that the key beneficial effect of aminoglutethimide in fact was the inhibition of aromatase enzyme which resulted in the reduction of estrogens. Nevertheless, due to its inhibition of CYP11, cortisol replacement was also needed to be given in combination with aminoglutethimide. For this reason and a number of significant side effects, the use of aminoglutethimide to treat breast cancer has not been popularized [4,9].

In the fall of 1981, Angela Brodie went to give a presentation in Rome about her research. Hearing her presentation, Charles Coombes expressed an interest to conduct a clinical trial with 4-OH-A to treat breast cancer. The first batch of 4-OH-A was produced at Angela's laboratory at the University of Maryland. Subsequent toxicology testing was performed by the Cancer Research Campaign in the United Kingdom. In collaboration with Angela, Charles Coombes together with Paul Goss and Mitch Dowsett launched the first clinical trial of a selective AI using 4-OH-A for the treatment of breast cancer at the Royal Marsden Hospital in London. This and following clinical trials demonstrated that 4-OH-A was effect even in breast cancer patients who progressed on tamoxifen [4,14,15]. In the mid 1980s, 4-OH-A was renamed to formestane and became the first "selective" aromatase inhibitor used clinically for the treatment of breast cancer. This breakthrough has sparked and inspired the later development of a wide variety of AIs.

Current AIs can be classified into two subtypes, namely steroidal and non-steroidal AIs (Table 1). Given that some the AIs have steroid-like structure similar to the aromatase substrate, androstenedione, this subtype of AIs has been termed steroidal AIs or type I inhibitor. Due to its similarity, these AIs bind to the substrate-binding site of aromatase enzyme. After binding, they are converted to a reactive intermediate that covalently bind to the enzyme causing irreversible inaction. These inhibitors are also known as "suicide inhibitor" because the enzyme is inactivated by its own function [14,15]. This subtype of AIs includes formestane and exemestane. For type II inhibitor or non-steroidal AIs, these AIs bind non-covalently to the heme moiety of the aromatase enzyme and prevent binding of androgens by saturating the binding-site. Unlike steroidal inhibitors, inhibition by this type of AIs is reversible by competitive inhibition of androgens [4,18]. This particular subtype includes fadrozole, vorozole, rogletimide, letrozole and anastrozole. Although formestane, fadrozole, vorozole, and rogletimide showed some clinical activity in tamoxifen resistant breast cancer [14,15,16], they are no longer in clinical use as they were no more effective than tamoxifen or required intramuscular injection, had undesirable side effects, or suppression of aldosterone. These agents were superseded by the newer generation of AIs with better oral bioavailability and fewer side effects [35]. Currently, AIs that are now in clinical used and are approved by the US Food and Drug Administration (FDA) include anastrozole, letrozole, and exemestane. They are approved for postmenopausal women with hormone receptor-positive breast cancer in both the adjuvant and metastatic setting.

3. Clinical efficacy of aromatase inhibitors

Due to the specificity of the aromatase enzyme, selective inhibition of aromatase would not interfere with the other steroid biosynthesis. All three available AIs appear to have comparable efficacy. Even though there is evidence that letrozole is more potent in reducing plasma estrogen levels [36], the clinical significance of this finding remains unclear. The phase III clinical trial comparing steroidal (exemestane) vs. non-steroidal (anastrozole) AIs is currently ongoing.

In general, AIs are well tolerated drugs with minimal side effects. The common side effects include hot flashes, vaginal dryness, and headache which are typically mild. Due to the lack of partial estrogenic effects, AIs do not increase the risk of endometrial cancer and thromboembolism like tamoxifen [4,20–22]. However, AIs significantly increase musculoskeletal symptoms, osteopenia, osteoporosis and fracture rate when compared to tamoxifen (375 cases vs. 234 cases; incidence rate ratio [IRR] 1.55, $p < 0.0001$) [21]. Nevertheless, the long term follow of patients receiving AI in the ATAC (arimidex, tamoxifen, alone or in combination) trial demonstrated that the fracture risk appears to increase only while patients are on active treatment. After the treatment is completed, there was no significant difference in fracture risk (146 cases vs. 143 cases; IRR 1.03, $p = 0.79$) [20]. Furthermore, patients at risk of osteoporosis can be given bisphosphonates, such as zoledronic acid (Zometa®). This not only reduces bone loss but also appears to synergize with AI's anti-tumor effect.

Below, we reviewed the clinical trial experience of AIs according to disease stage including metastatic and early stage disease as well as the role of AIs for chemoprevention. Important clinical trials of AIs are summarized in Table 2 according to stage of the disease.

3.1. Metastatic setting

For first line treatment, the three oral AIs have been demonstrated to be well tolerated and superior or at least as good as tamoxifen in response rate, median time to progression and clinical benefit rate [37–40]. The largest study involving 916 patients demonstrated that letrozole is superior to tamoxifen with a longer time to disease progression of 42 weeks vs. 23 weeks (hazard ratio [HR] 0.70, $p = 0.0001$) [41]. The subsequent two trials for anastrozole showed somewhat conflicting results. While the smaller North American study with 353 patients showed a significant improvement in time to disease progression (11.1 months vs. 5.6 months, $p = 0.005$) [38], the similar study design with a larger population,

TARGET trial, failed to confirm these findings (8.2 months vs. 8.3 months, HR 0.99, $p = 0.941$) [40]. Thus, anastrozole appears to be at least as good as or superior to tamoxifen. An European phase III study comparing exemestane with tamoxifen (EORTC 10951) also demonstrated a significant improvement in progression free survival (PFS) of exemestane over tamoxifen (HR = 0.79, $p = 0.04$) [42]. For the second line treatment after progression on tamoxifen, all of the third-generation AIs seemed to have a marginal benefit over megestrol acetate with only a modest improvement in response, clinical benefit, and median time to progression but they appear to have better tolerability [43–47]. Due to the rapid adoption of AIs into the adjuvant and first-line metastatic setting, the second line treatment with AIs is currently less clinically relevant [35].

3.2. Adjuvant setting

Several strategies were employed to investigate the benefit of AIs as an adjuvant treatment for hormone receptor-positive breast cancer in postmenopausal women. These strategies include upfront treatment with AIs, switching therapy to AIs after 2–3 years of tamoxifen, and extended AI therapy after the completion of tamoxifen for 5 years. For the upfront strategy, there were three phase III clinical trials, namely ATAC [48], BIG 1-98 [49,50], and ABCSG-12 trial [51], comparing 5 years of anastrozole or letrozole to 5 years of tamoxifen. The HR for disease free survival (DFS) of these studies ranges from 0.88 to 1.10 with no significant improvement in overall survival (OS). The lack of OS benefit may be related to considerable subsequent crossover after these trials had been reported. Of note, the combination arm of anastrozole concurrently with tamoxifen in the ATAC trial not only showed no additional benefit of the combination but also showed that the benefit of anastrozole over tamoxifen was lost in the combination [52].

Since prolonged exposure to tamoxifen can potentially lead to acquired resistance, several clinical trials were launched to evaluate the switching approach after a few years of tamoxifen to non-cross resistant agents like AIs (Table 2). Five clinical trials (ABCSG-8 [53], BIG 1-98 [50], ARNO 95 [54], ITA [55], and IES [56]) comparing 5 years of tamoxifen to sequential treatment of AI after 2–3 years of tamoxifen demonstrated a significant benefit of the switching strategy over tamoxifen alone with the HR for DFS ranging from 0.57 to 0.76. There are only two trials comparing 5 years of AIs to the sequential therapy which include BIG 1-98 [50] and TEAM [57] trials. Both of the trials did not show statistically significant differences in DFS between AI alone group and tamoxifen sequenced with an AI group as well as an AI sequenced with tamoxifen group in BIG 1-98. However, there appears to be more early relapses in the tamoxifen followed by letrozole group comparing to upfront AI group particularly in women with lymph node involvement [50].

Given the prolong relapse prospect of hormone receptor-positive breast cancer, extended course of endocrine therapy has been evaluated in multiple clinical trials. Currently, extended therapy of tamoxifen beyond 5 years is still controversial. Although there appears to be some marginal benefit of extended tamoxifen, this is offset by its toxicities particularly increased risks of endometrial cancer and thromboembolism [58–60]. In contrast, three large randomized clinical trials (ABCSG 6a [61], MA.17 [62], NSABP B-33 [63]) have demonstrated that extending the duration of endocrine therapy with AIs after 5 years of tamoxifen can be beneficial.

3.3. Neoadjuvant setting

Neoadjuvant therapy refers to any treatment including chemotherapy, endocrine therapy, or radiation that is given prior to surgery. This can be a mean to down-stage the tumor to improve the chance for patients to undergo breast-conserving surgery (BCS) [64] and, more attractively, providing an *in vivo* measurement of tumor response [35,65]. As summarized in Table 2, two clinical trials (IMPACT [66] and PROACT [67] trial) evaluated the use of tamoxifen and anastrozole for 3 months prior to surgery. There was no significant difference in overall response and it appears that anastrozole is at least as effective as tamoxifen. In contrast, the PO24 [68] study showed a significant increase in overall response rate and BCS conversion rate with letrozole for 4 months. A smaller Russian trial also showed a significant improvement in overall response rate and BCS conversion rate with exemestane for 12 weeks. Nevertheless, a head-to-head comparison between all three AIs in the neoadjuvant setting (ACOSOG Z1031) demonstrated no significant difference in response rate between all three AI (anastrozole 66.7% vs. letrozole 70.9% vs. exemestane 60.5%).

3.4. Chemoprevention

Based on these promising results of superiority of AIs over tamoxifen and approximately 50% reduction in the risk of contralateral breast cancer demonstrated by ATAC trial [48], AIs are being investigated as chemopreventive agents for breast cancer. Two large phase III trials are currently ongoing to evaluate this aspect including the IBIS II trial in Europe and the MAP-3 trial in Canada.

4. Resistance to aromatase inhibitors

While AIs are a very effective treatment, their benefit is often limited by emergence of resistance which can occur in a significant number of patients in the adjuvant setting and is inevitable in metastatic breast cancer. There appeared to be no cross resistance between steroidal and non-steroidal AIs regardless of the sequence, switching between these two subtypes can produce 0–26% objective response rates [69–71]. While the response rates are small, the substantial percentage of patients (50–62%) achieved stable disease of more than 6 months [71–75]. The large randomized phase III trial comparing exemestane vs. fulvestrant for second line endocrine therapy after progressing on a non-steroidal AI showed a clinical benefit rate of 32.2% vs. 31.5% ($p = 0.853$) but a rather short median time to progression of 3.7 months in both groups [76].

Multiple resistant mechanisms to AIs have been described and are summarized below. These mechanisms can be categorized into two distinct pathways including ER signaling and growth factor receptor pathways.

4.1. ER signaling pathway

It has long been known that breast cancers that do not express either ER or PR would not respond to endocrine therapy like AIs. Several studies have suggested that patients with higher ER or PR level have better outcomes when treated with endocrine therapy [77–79]. The recent American Society of Clinical Oncology (ASCO)/College of American Pathologists (CAP) recommended that ER and PR tests should be considered being positive if there are at least 1% positive tumor nuclei in the breast cancer tumors [77]. Nevertheless, approximately a third of breast cancers do not express both ER and PR and this type of breast cancer is intrinsically resistant to endocrine therapy including AIs. Our recent study as well as others demonstrated that these ER/PR-negative cancer cells can be re-programmed by epigenetic modulators like hypomethylating agents (i.e., 5-azacytidine) and histone deacetylase inhibitors (HDACi) [80–84]. Several *in vitro* studies demonstrated that treatment with these epigenetic modulators can induce expression of ER and PR which rendered them to be sensitive to endocrine therapy like tamoxifen [82]. Our group further demonstrated that the combination of entinostat, a class 1 selective HDACi, and letrozole can induce durable regression of MDA-MB-231 xenograft tumors

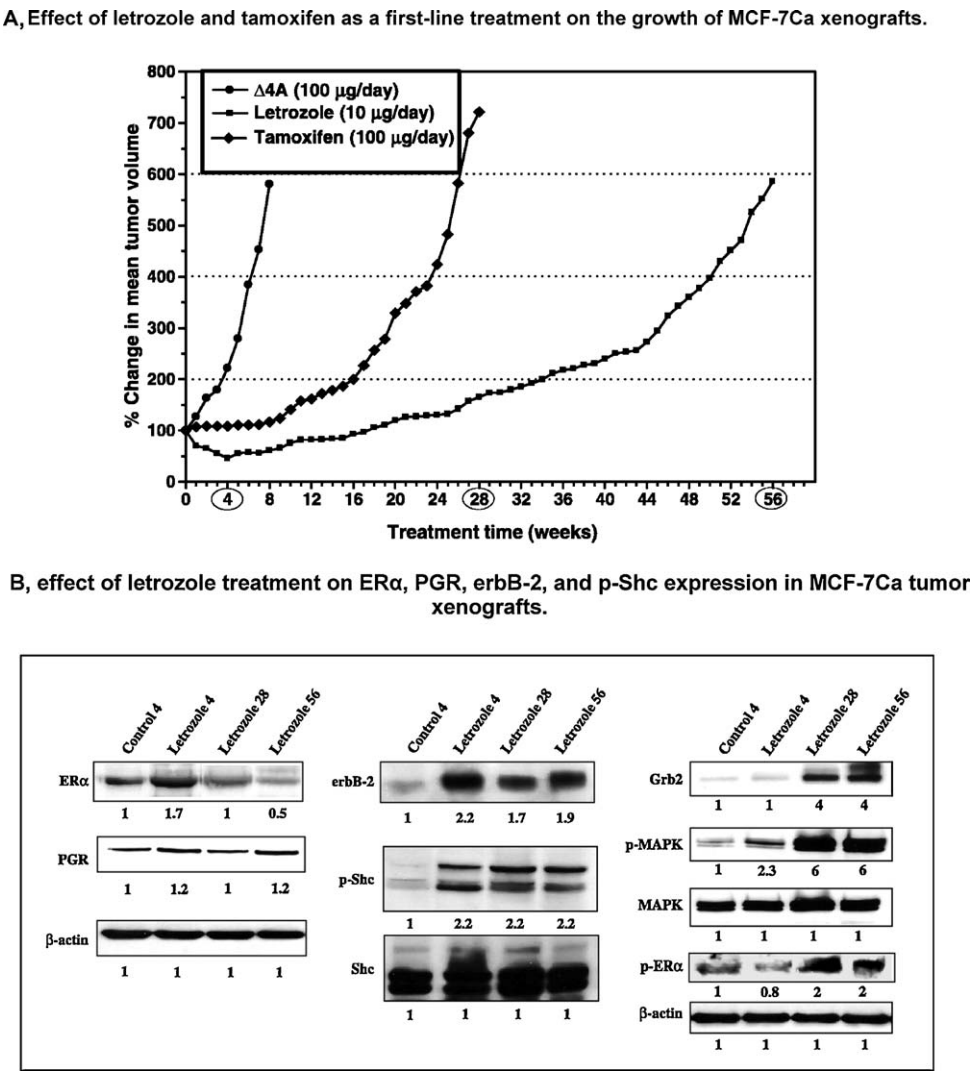

Fig. 1. (A) Effect of letrozole and tamoxifen as a first-line treatment on the growth of MCF-7Ca xenografts. Animals were inoculated with MCF-7Ca cells at two sites on each flank and were supplemented with androstenedione (100 Ag/d) for the duration of experiment. When the tumors reached a measurable size (~300 mm^3), animals were assigned to three groups ($n = 20$ per group) and injected s.c. daily with vehicle (control), or tamoxifen (100 μg/d), or letrozole (10 μg/d). Tumor volumes were measured weekly and were expressed as the percent change relative to the initial tumor volume. Two mice per group were sacrificed and tumors were collected for analysis at 4, 28, and 56 weeks. (B) The effect of letrozole treatment on Her2, p-Shc, Shc, Grb2, p-MAPK, MAPK, ER-α, p-ER-α, and PR expression in MCF-7Ca tumor xenografts. Letrozole-treated tumors collected at 4, 28, and 56 weeks (A), were analyzed by Western immunoblotting, and compared to vehicle-treated tumors collected at week 4 [12,89].

in vivo [85–87]. These results open a new avenue for the treatment of these de novo endocrine resistant breast cancers.

For acquired resistance to AIs, we have developed an intratumoral aromatase breast cancer model to evaluate mechanisms of resistance to different AIs [12,88]. This model mimics hormone receptor-positive breast cancer in postmenopausal women. In our experiments, xenografts with tumors of MCF-7 cells overexpressing aromatase (MCF-7Ca) were treated with letrozole and tumors were collected at different time points [89]. We found that by 28 weeks after treatment, there was a significant decrease in total ERα expression. However, the levels of phosphorylated ERα (p-ERα) in letrozole-resistant tumors were significantly higher than p-ERα level at baseline. Moreover, PR which is the downstream effecter of ER also remained unchanged from the baseline (Fig. 1). This signifies that the ER signaling cascades continue to be an active driving force in AI resistant tumors despite the loss in its expression as discussed below.

Other mechanisms of resistance centering on ER signaling pathways include ERα mutation [90] and truncated ERα variant (ERα36) [91]. Moreover, upregulation of the ER-related transcription factors like activator protein 1 (AP1) [92] and NF-κB [93] as well as co-activators of ER such as AIB1 [94] have also been described to confer resistant to endocrine therapy [5]. Nonetheless, these studies were described as resistant mechanisms to tamoxifen and the role of these mechanisms in AI resistance remain unclear.

4.2. Growth factor receptor pathways

In our previously described experiment, beside loss of ERα expression, we also found that in tumors treated with letrozole for 28 weeks, there was significant upregulation of the human epidermal growth factor receptor 2 (Her2)/mitogen-activated protein kinase (MAPK) signaling pathway in letrozole resistant tumors. The expressions of Her2, p-Shc, Grb-2, p-Raf, p-Mek1/2, and p-MAPK were all significantly increased in the treatment group compared to baseline (Fig. 1). This effect can be observed as early as 4 weeks while tumors still responded to letrozole. Downstream targets of MAPK like p90 ribosomal S6 kinase (p90RSK) and the ETS-domain containing protein (Elk) were also found to be activated by phosphorylation [89]. Our subsequent studies demonstrated that overexpression of Her2 and subsequent activation of MAPK pathway in the letrozole resistant tumors appeared to be secondary

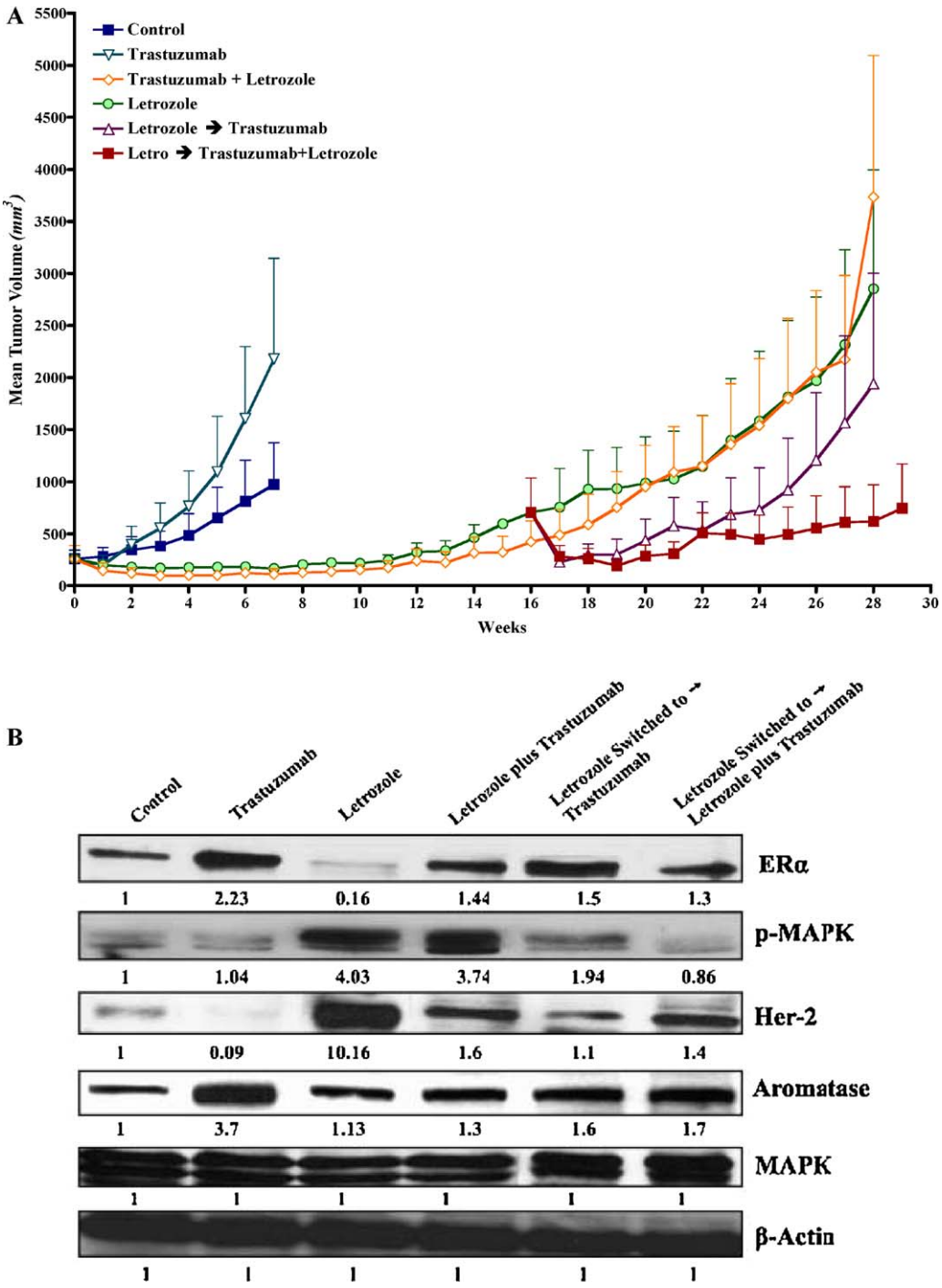

Fig. 2. (A) effect of trastuzumab alone or in combination with letrozole on the growth of MCF-7Ca xenografts. Trastuzumab (5 mg/kg/wk) did not inhibit the growth of MCF-7Ca tumors. The difference in the exponential variable governing growth rate of control vs. trastuzumab treatment was 0.02 F 0.14, which was not statistically significant ($p = 0.86$). The difference in the exponential variable governing growth rate of trastuzumab vs. trastuzumab plus letrozole was 0.49 ($p = 0.0001$). The difference in the exponential variable governing growth rate of trastuzumab vs. letrozole was 0.32 ($p = 0.0009$). The difference in the exponential variable governing rate of letrozole vs. letrozole switched to letrozole plus trastuzumab was 0.21 F 0.08 ($p = 0.008$). The difference in the exponential variable governing tumor growth rate of letrozole plus trastuzumab vs. letrozole switched to letrozole plus trastuzumab was 0.39 F 0.09 ($p < 0.0001$). The difference in the exponential variable governing rate of letrozole switched to trastuzumab vs. letrozole switched to letrozole plus trastuzumab was 0.2 F 0.08 ($p = 0.011$) over weeks 15–28. When compared with week 29, the difference in the exponential variable governing growth rate of letrozole vs. letrozole switched to trastuzumab was 0.005 F 0.08 ($p = 0.97$). (B) Effect of trastuzumab and letrozole alone or in combination on protein expression of ERa, Her2, MAPK, and CYP-19 in MCF-7Ca xenografts. Expression of proteins was examined using Western immunoblotting. Blot shows ERa at 66 kDa, Her2 at 185 kDa, p-MAPK and MAPK at 42–44 kDa, CYP-19 at 55 kDa, and h-actin at 45 kDa. The blots show a single representative of three independent experiments. The blots were stripped and reprobed for h-actin to verify equal loading.

to the change in the stability of Her2 protein and not due to Her2 gene amplification [95]. In contrast to letrozole, we found that resistance to anastrozole is associated with upregulations of insulin-like growth factor 1 (IGF-1) receptor and phosphatidylinositol 3-kinase (PI3K)/Akt pathway [96]. In a similar experiment, tumors treated with anastrozole were collected at 14 weeks when they became resistant to anastrozole. Although there was a reduction in total MAPK and p-MAPK, the expressions of IGF-1R and mammalian

target of rapamycin (mTOR) as well as phosphorylated mTOR, a critical downstream effecter of PI3K/Akt, were increased compared to the vehicle control group. Multiple previous studies demonstrated that other signaling pathways like MAPK and PI3K/Akt pathways can cross-talk and activate ERα signaling pathways in a ligand-independent manner [97–99]. MAPK has been shown to phosphorylate ERα directly or indirectly via Elk-1 and p90RSK and result in the transcription of genes involved in growth regulation and tumor progression [97–99]. Both MAPK and Akt can directly phosphorylate ERα within the AF-1 domain at serine 118 and serine 167, respectively [100]. Besides phosphorylation of the ERα itself, these two pathways can also stimulate ER signaling pathway by phosphorylation of the ER co-activator AIB1 [101,102].

Nonetheless, this cross-talk between ER and these 2 major signaling pathways appears to be a dynamic interface. Using the similar model, we found that interrupting letrozole treatment can reverse tumors into their baseline state. There are significant increases in ERα and aromatase expression levels as well as a reduction in phosphorylated MAPK to the similar levels at baseline. These changes after interrupting the treatment also restore the sensitivity to AIs. At 22 weeks, after LTLT-Ca (letrozole resistant) xenograft tumors have become resistant to letrozole, a short interruption of letrozole for 6 weeks can induce regress of tumors again after resuming letrozole treatment [95,103]. Notably, intermittent treatment with letrozole (6 weeks on and 6 weeks off) in letrozole responsive tumors (MCF-7Ca) is inferior to continuous treatment as tumors can rapidly acquire resistance [95].

Given that overactive growth factor receptor signaling pathways confer resistance to AIs; our laboratory further demonstrated that disrupting these pathways with specific inhibitors can also restore AI sensitivity. Adding trastuzumab, a monoclonal antibody against Her2, to letrozole when MCF-7ca tumors have become resistant to letrozole can restore the sensitivity to letrozole and induce tumor regression (Fig. 2). Trastuzumab alone did not have anti-tumor activity in the parental endocrine responsive MCF-7Ca and the upfront combination of trastuzumab and letrozole did not prolong or avert the resistance [104]. Analysis of protein expression levels (Fig. 2B) in the tumors at the end of treatment showed reduced levels of Her2 with trastuzumab alone or in combination and increased expression of ER. However, in presence of the combined treatment, the effect of trastuzumab to increase ER expression and aromatase was blocked by letrozole resulting in reduced tumor growth (Fig. 2A). We also demonstrate that inhibition of PI3K/Akt pathway by wortmannin together with fulvestrant, an estrogen receptor antagonist that down-regulates the ER, results in more effective tumor regression compared to either of the agent alone in the long-term estrogen-deprived aromatase-transfected ER-positive breast cancer model [105].

Multiple emerging clinical data have supported our preclinical findings. Lipton et al. demonstrated that approximately 26% of patients treated with letrozole converted from serum Her2 negative to positive at the time of disease progression [106]. The extracellular domain of Her2 protein can be detected in the peripheral blood has been demonstrated to correlate with the overexpression of Her2 protein in tumor cells [107]. Several clinical trials have confirmed the benefit of targeting both ER and Her2 pathways. A phase II trial of letrozole and trastuzumab in ER-positive/Her2-negative metastatic breast cancer patients demonstrated that the combination was well tolerated with a clinical benefit rate of 50% [108]. A subsequent phase III trial (TANDEM trial) of anastrozole in combination with trastuzumab demonstrated a significant improvement in PFS with an addition of trastuzumab (3.8 months vs. 5.6 months; $p = 0.0059$) [109]. Moreover, recent randomized phase III trial of letrozole in combination with lapatinib, an oral dual tyrosine kinase inhibitor of Her2 and EGFR, also demonstrated a significant benefit of adding lapatinib to letrozole with the PFS of 8.2 months vs. 3.0 months (HR of 0.71; $p = 0.019$) [110]. However, the benefit only appears to be in the group of patients with Her2 overexpression. Nonetheless, the preplanned Cox regression analysis of patients with Her2-negative tumors who relapse less than 6 months after tamoxifen discontinuation demonstrated a non-significant trend toward improvement in PFS for the combination (HR 0.78; $p = 0.117$) [110]. This result supports our preclinical finding that the upfront combination of AI with anti-Her2 therapy does not prolong or avert the resistance but the combination is more beneficial at the time of the resistance.

5. Conclusions

Given that the aromatase enzyme has a very specific function in the steroid biosynthesis, selective aromatase inhibition is truly a targeted approach for breast cancer treatment that confers only minimal side effects. In the past decade, multiple clinical trials have not only demonstrated a superior efficacy of AIs over tamoxifen, but also better side effect profile of these agents. AI is now considered to be a standard treatment for postmenopausal women with hormone receptor-positive breast cancer [16]. Nevertheless, the benefit of AIs is often limited by the emergence of resistance. Our preclinical data as well as others demonstrated that the crosstalk between ER and other signaling pathways particularly MAPK and PI3K/Akt is the key resistant mechanism. Interfering with these other signaling pathways is an attractive strategy to circumvent the resistance to AI therapy in breast cancer. Several clinical trials are ongoing to evaluate the role of these novel targeted therapies to reverse resistance to AIs. These agents include MEK inhibitors, Raf inhibitors, PI3K inhibitors, mTOR inhibitors, and Akt inhibitors. However, future studies are still needed to determine the strategy to prolong or avert the AI resistance. Furthermore, obtaining the tumor specimens when it is feasible in the patients with AI resistance is also critical. Global gene expression analysis of these biopsied specimens would allow us to have a better insight to the mechanisms underlying AI resistance.

References

[1] D.P. McDonnell, J.D. Norris, Connections and regulation of the human estrogen receptor, Science 296 (5573) (2002) 1642–1644.
[2] G.G. Kuiper, E. Enmark, M. Pelto-Huikko, S. Nilsson, J.A. Gustafsson, Cloning of a novel receptor expressed in rat prostate and ovary, Proc. Natl. Acad. Sci. U.S.A. 93 (12) (1996) 5925–5930.
[3] J.F. Couse, K.S. Korach, Estrogen receptor null mice: what have we learned and where will they lead us? Endocr. Rev. 20 (3) (1999) 358–417.
[4] D.P. McDonnell, The molecular pharmacology of SERMs, Trends Endocrinol. Metab. 10 (8) (1999) 301–311.
[5] E.A. Musgrove, R.L. Sutherland, Biological determinants of endocrine resistance in breast cancer, Nat. Rev. Cancer 9 (9) (2009) 631–643.
[6] G. Beatson, On the treatment of inoperable cases of carcinoma of themamma. Suggestions for a new method of treatment with illustrative cases, Lancet II (1896) 104–107.
[7] R.R. Love, J. Philips, Oophorectomy for breast cancer: history revisited, J. Natl. Cancer Inst. 94 (19) (2002) 1433–1434.
[8] V.C. Jordan, A.M. Brodie, Development and evolution of therapies targeted to the estrogen receptor for the treatment and prevention of breast cancer, Steroids 72 (1) (2007) 7–25.
[9] B. Fisher, J.P. Costantino, C.K. Redmond, E.R. Fisher, D.L. Wickerham, W.M. Cronin, Endometrial cancer in tamoxifen-treated breast cancer patients: findings from the National Surgical Adjuvant Breast and Bowel Project (NSABP) B-14, J. Natl. Cancer Inst. 86 (7) (1994) 527–537.
[10] V.C. Jordan, Tamoxifen: toxicities and drug resistance during the treatment and prevention of breast cancer, Annu. Rev. Pharmacol. Toxicol. 35 (1995) 195–211.
[11] W.C. Schwarzel, W.G. Kruggel, H.J. Brodie, Studies on the mechanism of estrogen biosynthesis. 8. The development of inhibitors of the enzyme system in human placenta, Endocrinology 92 (3) (1973) 866–880.
[12] L.F. Macedo, G. Sabnis, A. Brodie, Aromatase inhibitors and breast cancer, Ann. N. Y. Acad. Sci. 1155 (2009) 162–173.
[13] A.M. Brodie, W.C. Schwarzel, A.A. Shaikh, H.J. Brodie, The effect of an aromatase inhibitor 4-hydroxy-4-androstene-3,17-dione, on estrogen-dependent processes in reproduction and breast cancer, Endocrinology 100 (6) (1977) 1684–1695.

[14] R.J. Santen, H. Brodie, E.R. Simpson, P.K. Siiteri, A. Brodie, History of aromatase: saga of an important biological mediator and therapeutic target, Endocr. Rev. 30(4) 343–375.
[15] A.M. Brodie, W.M. Garrett, J.R. Hendrickson, C.H. Tsai-Morris, P.A. Marcotte, C.H. Robinson, Inactivation of aromatase in vitro by 4-hydroxy-4-androstene-3 17-dione and 4-acetoxy-4-androstene-3,17-dione and sustained effects in vivo, Steroids 38 (6) (1981) 693–702.
[16] H.J. Burstein, A.A. Prestrud, J. Seidenfeld, H. Anderson, T.A. Buchholz, N.E. Davidson, K.E. Gelmon, S.H. Giordano, C.A. Hudis, J. Malin, E.P. Mamounas, D. Rowden, A.J. Solky, M.R. Sowers, V. Stearns, E.P. Winer, M.R. Somerfield, J.J. Griggs, American society of clinical oncology clinical practice guideline: update on adjuvant endocrine therapy for women with hormone receptor-positive breast cancer, J. Clin. Oncol.
[17] E.A. Thompson Jr., P.K. Siiteri, Utilization of oxygen and reduced nicotinamide adenine dinucleotide phosphate by human placental microsomes during aromatization of androstenedione, J. Biol. Chem. 249 (17) (1974) 5364–5372.
[18] S.A. Chen, M.J. Besman, R.S. Sparkes, S. Zollman, I. Klisak, T. Mohandas, P.F. Hall, J.E. Shively, Human aromatase: cDNA cloning, Southern blot analysis, and assignment of the gene to chromosome 15, DNA 7 (1) (1988) 27–38.
[19] M. Akhtar, J.N. Wright, P. Lee-Robichaud, A review of mechanistic studies on aromatase (CYP19) and 17alpha-hydroxylase-17,20-lyase (CYP17), J. Steroid Biochem. Mol. Biol.
[20] N. Harada, Aberrant expression of aromatase in breast cancer tissues, J. Steroid Biochem. Mol. Biol. 61 (3–6) (1997) 175–184.
[21] L.E. Valladares, A.H. Payne, Induction of testicular aromatization by luteinizing hormone in mature rats, Endocrinology 105 (2) (1979) 431–436.
[22] K.C. Reed, S. Ohno, Kinetic properties of human placental aromatase. Application of an assay measuring $3H_2O$ release from 1beta,2beta-3H-androgens, J. Biol. Chem. 251 (6) (1976) 1625–1631.
[23] C.R. Durham, H. Zhu, B.S. Masters, E.R. Simpson, C.R. Mendelson, Regulation of aromatase activity of rat granulosa cells: induction of synthesis of NADPH-cytochrome P-450 reductase by FSH and dibutyryl cyclic AMP, Mol. Cell Endocrinol. 40 (2–3) (1985) 211–219.
[24] E.F. Hahn, S. Miyairi, J. Fishman, 19-Hydroxylation of androgens in the rat brain, Proc. Natl. Acad. Sci. U.S.A. 82 (9) (1985) 2728–2730.
[25] E.R. Simpson, M.S. Mahendroo, G.D. Means, M.W. Kilgore, M.M. Hinshelwood, S. Graham-Lorence, B. Amarneh, Y. Ito, C.R. Fisher, M.D. Michael, et al., Aromatase cytochrome P450, the enzyme responsible for estrogen biosynthesis, Endocr. Rev. 15 (3) (1994) 342–355.
[26] S. Sebastian, S.E. Bulun, A highly complex organization of the regulatory region of the human CYP19 (aromatase) gene revealed by the Human Genome Project, J. Clin. Endocrinol. Metab. 86 (10) (2001) 4600–4602.
[27] B. Amarneh, C.J. Corbin, J.A. Peterson, E.R. Simpson, S. Graham-Lorence, Functional domains of human aromatase cytochrome P450 characterized by linear alignment and site-directed mutagenesis, Mol. Endocrinol. 7 (12) (1993) 1617–1624.
[28] J.T. Kellis Jr., L.E. Vickery, Purification and characterization of human placental aromatase cytochrome P-450, J. Biol. Chem. 262 (9) (1987) 4413–4420.
[29] E.D. Lephart, K.G. Peterson, J.F. Noble, F.W. George, M.J. McPhaul, The structure of cDNA clones encoding the aromatase P-450 isolated from a rat Leydig cell tumor line demonstrates differential processing of aromatase mRNA in rat ovary and a neoplastic cell line, Mol. Cell Endocrinol. 70 (1) (1990) 31–40.
[30] S. Karkola, H.D. Holtje, K. Wahala, A three-dimensional model of CYP19 aromatase for structure-based drug design, J. Steroid Biochem. Mol. Biol. 105 (1–5) (2007) 63–70.
[31] Y.C. Kao, L.L. Cam, C.A. Laughton, D. Zhou, S. Chen, Binding characteristics of seven inhibitors of human aromatase: a site-directed mutagenesis study, Cancer Res. 56 (15) (1996) 3451–3460.
[32] J. Fishman, H. Guzik, D. Dixon, Sterochemistry of estrogen biosynthesis, Biochemistry 8 (11) (1969) 4304–4309.
[33] D. Ghosh, J. Griswold, M. Erman, W. Pangborn, Structural basis for androgen specificity and oestrogen synthesis in human aromatase, Nature 457 (7226) (2009) 219–223.
[34] D. Ghosh, J. Griswold, M. Erman, W. Pangborn, X-ray structure of human aromatase reveals an androgen-specific active site, J. Steroid Biochem. Mol. Biol. 118(4–5) 197–202.
[35] I.E. Smith, M. Dowsett, Aromatase inhibitors in breast cancer, N. Engl. J. Med. 348 (24) (2003) 2431–2442.
[36] J. Geisler, B. Haynes, G. Anker, M. Dowsett, P.E. Lonning, Influence of letrozole and anastrozole on total body aromatization and plasma estrogen levels in postmenopausal breast cancer patients evaluated in a randomized, cross-over study, J. Clin. Oncol. 20 (3) (2002) 751–757.
[37] J. Bonneterre, A. Buzdar, J.M. Nabholtz, J.F. Robertson, B. Thurlimann, M. von Euler, T. Sahmoud, A. Webster, M. Steinberg, Anastrozole is superior to tamoxifen as first-line therapy in hormone receptor positive advanced breast carcinoma, Cancer 92 (9) (2001) 2247–2258.
[38] J.M. Nabholtz, A. Buzdar, M. Pollak, W. Harwin, G. Burton, A. Mangalik, M. Steinberg, A. Webster, M. von Euler, Anastrozole is superior to tamoxifen as first-line therapy for advanced breast cancer in postmenopausal women: results of a North American multicenter randomized trial. Arimidex Study Group, J. Clin. Oncol. 18 (22) (2000) 3758–3767.
[39] H.T. Mouridsen, Aromatase inhibitors in advanced breast cancer, Semin. Oncol. 31 (6 (Suppl. 12)) (2004) 3–8.
[40] J. Bonneterre, B. Thurlimann, J.F. Robertson, M. Krzakowski, L. Mauriac, P. Koralewski, I. Vergote, A. Webster, M. Steinberg, M. von Euler, Anastrozole versus tamoxifen as first-line therapy for advanced breast cancer in 668 postmenopausal women: results of the Tamoxifen or Arimidex randomized group efficacy and tolerability study, J. Clin. Oncol. 18 (22) (2000) 3748–3757.
[41] H. Mouridsen, M. Gershanovich, Y. Sun, R. Perez-Carrion, C. Boni, A. Monnier, J. Apffelstaedt, R. Smith, H.P. Sleeboom, F. Janicke, A. Pluzanska, M. Dank, D. Becquart, P.P. Bapsy, E. Salminen, R. Snyder, M. Lassus, J.A. Verbeek, B. Staffler, H.A. Chaudri-Ross, M. Dugan, Superior efficacy of letrozole versus tamoxifen as first-line therapy for postmenopausal women with advanced breast cancer: results of a phase III study of the International Letrozole Breast Cancer Group, J. Clin. Oncol. 19 (10) (2001) 2596–2606.
[42] T.P. Paridaens, L. Dirix, First line hormonal treatment for metastatic breast cancer with exemestane or tamoxifen in postmenopausal patients: a randomized phase III trial of the EORTC Breast Group, Proc. Am. Soc. Clin. Oncol. (2004) 6.
[43] A. Buzdar, J. Douma, N. Davidson, R. Elledge, M. Morgan, R. Smith, L. Porter, J. Nabholtz, X. Xiang, C. Brady, Phase III, multicenter, double-blind, randomized study of letrozole, an aromatase inhibitor, for advanced breast cancer versus megestrol acetate, J. Clin. Oncol. 19 (14) (2001) 3357–3366.
[44] A. Buzdar, W. Jonat, A. Howell, S.E. Jones, C. Blomqvist, C.L. Vogel, W. Eiermann, J.M. Wolter, M. Azab, A. Webster, P.V. Plourde, Anastrozole: a potent and selective aromatase inhibitor, versus megestrol acetate in postmenopausal women with advanced breast cancer: results of overview analysis of two phase III trials. Arimidex Study Group, J. Clin. Oncol. 14 (7) (1996) 2000–2011.
[45] P. Dombernowsky, I. Smith, G. Falkson, R. Leonard, L. Panasci, J. Bellmunt, W. Bezwoda, G. Gardin, A. Gudgeon, M. Morgan, A. Fornasiero, W. Hoffmann, J. Michel, T. Hatschek, T. Tjabbes, H.A. Chaudri, U. Hornberger, P.F. Trunet, Letrozole, a new oral aromatase inhibitor for advanced breast cancer: double-blind randomized trial showing a dose effect and improved efficacy and tolerability compared with megestrol acetate, J. Clin. Oncol. 16 (2) (1998) 453–461.
[46] M. Kaufmann, E. Bajetta, L.Y. Dirix, L.E. Fein, S.E. Jones, N. Zilembo, J.L. Dugardyn, C. Nasurdi, R.G. Mennel, J. Cervek, C. Fowst, A. Polli, E. di Salle, A. Arkhipov, G. Piscitelli, L.L. Miller, G. Massimini, Exemestane is superior to megestrol acetate after tamoxifen failure in postmenopausal women with advanced breast cancer: results of a phase III randomized double-blind trial. The Exemestane Study Group, J. Clin. Oncol. 18 (7) (2000) 1399–1411.
[47] P.E. Goss, E.P. Winer, I.F. Tannock, L.H. Schwartz, Randomized phase III trial comparing the new potent and selective third-generation aromatase inhibitor vorozole with megestrol acetate in postmenopausal advanced breast cancer patients. North American Vorozole Study Group, J. Clin. Oncol. 17 (1) (1999) 52–63.
[48] J.F. Forbes, J. Cuzick, A. Buzdar, A. Howell, J.S. Tobias, M. Baum, Effect of anastrozole and tamoxifen as adjuvant treatment for early-stage breast cancer: 100-month analysis of the ATAC trial, Lancet Oncol. 9 (1) (2008) 45–53.
[49] B. Thurlimann, A. Keshaviah, A.S. Coates, H. Mouridsen, L. Mauriac, J.F. Forbes, R. Paridaens, M. Castiglione-Gertsch, R.D. Gelber, M. Rabaglio, I. Smith, A. Wardley, K.N. Price, A. Goldhirsch, A comparison of letrozole and tamoxifen in postmenopausal women with early breast cancer, N. Engl. J. Med. 353 (26) (2005) 2747–2757.
[50] H. Mouridsen, A. Giobbie-Hurder, A. Goldhirsch, B. Thurlimann, R. Paridaens, I. Smith, L. Mauriac, J.F. Forbes, K.N. Price, M.M. Regan, R.D. Gelber, A.S. Coates, Letrozole therapy alone or in sequence with tamoxifen in women with breast cancer, N. Engl. J. Med. 361 (8) (2009) 766–776.
[51] M. Gnant, B. Mlineritsch, W. Schippinger, G. Luschin-Ebengreuth, S. Postlberger, C. Menzel, R. Jakesz, M. Seifert, M. Hubalek, V. Bjelic-Radisic, H. Samonigg, C. Tausch, H. Eidtmann, G. Steger, W. Kwasny, P. Dubsky, M. Fridrik, F. Fitzal, M. Stierer, E. Rucklinger, R. Greil, C. Marth, Endocrine therapy plus zoledronic acid in premenopausal breast cancer, N. Engl. J. Med. 360 (7) (2009) 679–691.
[52] N.U. Lin, E.P. Winer, Advances in adjuvant endocrine therapy for postmenopausal women, J. Clin. Oncol. 26 (5) (2008) 798–805.
[53] R. Jakesz, M. Gnant, R. Greil, The benefits of sequencing adjuvant tamoxifen and anastrozole in postmenopausal women with hormone responsive early breast cancer: 5 year-analysis of ABCSG trial 8, in: The 28th Annual San Antonio Breast Cancer Symposium, San Antonio, TX, 2005.
[54] M. Kaufmann, W. Jonat, J. Hilfrich, H. Eidtmann, G. Gademann, I. Zuna, G. von Minckwitz, Improved overall survival in postmenopausal women with early breast cancer after anastrozole initiated after treatment with tamoxifen compared with continued tamoxifen: the ARNO 95 study, J. Clin. Oncol. 25 (19) (2007) 2664–2670.
[55] F. Boccardo, A. Rubagotti, P. Guglielmini, A. Fini, G. Paladini, M. Mesiti, M. Rinaldini, S. Scali, M. Porpiglia, C. Benedetto, N. Restuccia, F. Buzzi, R. Franchi, B. Massidda, V. Distante, D. Amadori, P. Sismondi, Switching to anastrozole versus continued tamoxifen treatment of early breast cancer. Updated results of the Italian tamoxifen anastrozole (ITA) trial, Ann. Oncol. 17 (Suppl. 7) (2006) 10–14.
[56] R.C. Coombes, L.S. Kilburn, C.F. Snowdon, R. Paridaens, R.E. Coleman, S.E. Jones, J. Jassem, C.J. Van de Velde, T. Delozier, I. Alvarez, L. Del Mastro, O. Ortmann, K. Diedrich, A.S. Coates, E. Bajetta, S.B. Holmberg, D. Dodwell, E. Mickiewicz, J. Andersen, P.E. Lonning, G. Cocconi, J. Forbes, M. Castiglione, N. Stuart, A. Stewart, L.J. Fallowfield, G. Bertelli, E. Hall, R.G. Bogle, M. Carpentieri, E. Colajori, M. Subar, E. Ireland, J.M. Bliss, Survival and safety of exemestane versus tamoxifen after 2–3 years' tamoxifen treatment (Intergroup Exemestane study): a randomised controlled trial, Lancet 369 (9561) (2007) 559–570.
[57] D. Rea, A. Hasenburg, C. Seynaeve, Five years of exemestane as initial therapy compared to 5 years of tamoxifen followed by exemestane: The TEAM trial, a prospective, randomized, phase III trial in postmenopausal women

with hormone-sensitive early breast cancer, in: The 32nd Annual San Antonio Breast Cancer Symposium, San Antonio, TX, 2009.
[58] H.J. Stewart, R.J. Prescott, A.P. Forrest, Scottish adjuvant tamoxifen trial: a randomized study updated to 15 years, J. Natl. Cancer Inst. 93 (6) (2001) 456–462.
[59] B. Fisher, J. Dignam, J. Bryant, N. Wolmark, Five versus more than five years of tamoxifen for lymph node-negative breast cancer: updated findings from the National Surgical Adjuvant Breast and Bowel Project B-14 randomized trial, J. Natl. Cancer Inst. 93 (9) (2001) 684–690.
[60] R. Peto, C. Davies, ATLAS (Adjuvant Tamoxifen, Longer Against Shorter): international randomized trial of 10 versus 5 years of adjuvant tamoxifen among 11,500 women – preliminary results, in: The 30th Annual San Antonio Breast Cancer Symposium, San Antonio, TX, 2007.
[61] R. Jakesz, R. Greil, M. Gnant, M. Schmid, W. Kwasny, E. Kubista, B. Mlineritsch, C. Tausch, M. Stierer, F. Hofbauer, K. Renner, C. Dadak, E. Rucklinger, H. Samonigg, Extended adjuvant therapy with anastrozole among postmenopausal breast cancer patients: results from the randomized Austrian Breast and Colorectal Cancer Study Group Trial 6a, J. Natl. Cancer Inst. 99 (24) (2007) 1845–1853.
[62] P.E. Goss, J.N. Ingle, S. Martino, N.J. Robert, H.B. Muss, M.J. Piccart, M. Castiglione, D. Tu, L.E. Shepherd, K.I. Pritchard, R.B. Livingston, N.E. Davidson, L. Norton, E.A. Perez, J.S. Abrams, D.A. Cameron, M.J. Palmer, J.L. Pater, Efficacy of letrozole extended adjuvant therapy according to estrogen receptor and progesterone receptor status of the primary tumor: National Cancer Institute of Canada Clinical Trials Group MA.17, J. Clin. Oncol. 25 (15) (2007) 2006–2011.
[63] E.P. Mamounas, J.H. Jeong, D.L. Wickerham, R.E. Smith, P.A. Ganz, S.R. Land, A. Eisen, L. Fehrenbacher, W.B. Farrar, J.N. Atkins, E.R. Pajon, V.G. Vogel, J.F. Kroener, L.F. Hutchins, A. Robidoux, J.L. Hoehn, J.N. Ingle, C.E. Geyer Jr., J.P. Costantino, N. Wolmark, Benefit from exemestane as extended adjuvant therapy after 5 years of adjuvant tamoxifen: intention-to-treat analysis of the National Surgical Adjuvant Breast And Bowel Project B-33 trial, J. Clin. Oncol. 26 (12) (2008) 1965–1971.
[64] I.E. Smith, L. Lipton, Preoperative/neoadjuvant medical therapy for early breast cancer, Lancet Oncol. 2 (9) (2001) 561–570.
[65] A.P. Forrest, P.A. Levack, U. Chetty, R.A. Hawkins, W.R. Miller, J.F. Smyth, T.J. Anderson, A human tumour model, Lancet 2 (8511) (1986) 840–842.
[66] I.E. Smith, M. Dowsett, S.R. Ebbs, J.M. Dixon, A. Skene, J.U. Blohmer, S.E. Ashley, S. Francis, I. Boeddinghaus, G. Walsh, Neoadjuvant treatment of postmenopausal breast cancer with anastrozole, tamoxifen, or both in combination: the Immediate Preoperative Anastrozole, Tamoxifen, or Combined with Tamoxifen (IMPACT) multicenter double-blind randomized trial, J. Clin. Oncol. 23 (22) (2005) 5108–5116.
[67] L. Cataliotti, A.U. Buzdar, S. Noguchi, J. Bines, Y. Takatsuka, K. Petrakova, P. Dube, C.T. de Oliveira, Comparison of anastrozole versus tamoxifen as preoperative therapy in postmenopausal women with hormone receptor-positive breast cancer: the Pre-Operative "Arimidex" compared to Tamoxifen (PROACT) trial, Cancer 106 (10) (2006) 2095–2103.
[68] W. Eiermann, S. Paepke, J. Appfelstaedt, A. Llombart-Cussac, J. Eremin, J. Vinholes, L. Mauriac, M. Ellis, M. Lassus, H.A. Chaudri-Ross, M. Dugan, M. Borgs, Preoperative treatment of postmenopausal breast cancer patients with letrozole: a randomized double-blind multicenter study, Ann. Oncol. 12 (11) (2001) 1527–1532.
[69] B. Thurlimann, R. Paridaens, D. Serin, J. Bonneterre, H. Roche, R. Murray, E. di Salle, S. Lanzalone, M.G. Zurlo, G. Piscitelli, Third-line hormonal treatment with exemestane in postmenopausal patients with advanced breast cancer progressing on aminoglutethimide: a phase II multicentre multinational study. Exemestane Study Group, Eur. J. Cancer 33 (11) (1997) 1767–1773.
[70] P. Carlini, A. Frassoldati, S. De Marco, A. Casali, E.M. Ruggeri, M. Nardi, P. Papaldo, A. Fabi, F. Paoloni, F. Cognetti, Formestane, a steroidal aromatase inhibitor after failure of non-steroidal aromatase inhibitors (anastrozole and letrozole): is a clinical benefit still achievable? Ann. Oncol. 12 (11) (2001) 1539–1543.
[71] W.R. Miller, J. Bartlett, A.M. Brodie, R.W. Brueggemeier, E. di Salle, P.E. Lonning, A. Llombart, N. Maass, T. Maudelonde, H. Sasano, P.E. Goss, Aromatase inhibitors: are there differences between steroidal and nonsteroidal aromatase inhibitors and do they matter? Oncologist 13 (8) (2008) 829–837.
[72] J. Geisler, D.C. Johannessen, G. Anker, P.E. Lonning, Treatment with formestane alone and in combination with aminoglutethimide in heavily pretreated breast cancer patients: clinical and endocrine effects, Eur. J. Cancer 32A (5) (1996) 789–792.
[73] C. Harper-Wynne, J. English, L. Meyer, M. Bower, C. Archer, H.D. Sinnett, C. Lowdell, R.C. Coombes, Randomized trial to compare the efficacy and toxicity of cyclophosphamide, methotrexate and 5-fluorouracil (CMF) with methotrexate mitoxantrone (MM) in advanced carcinoma of the breast, Br. J. Cancer 81 (2) (1999) 316–322.
[74] G. Bertelli, O. Garrone, M. Merlano, M. Occeli, L. Bertolotti, F. Castiglione, F. Pepi, O. Fusco, L. Del Mastro, R.C. Leonard, Sequential treatment with exemestane and non-steroidal aromatase inhibitors in advanced breast cancer, Oncology 69 (6) (2005) 471–477.
[75] N. Zilembo, E. Bajetta, E. Bichisao, A. Martinetti, I. La Torre, P. Bidoli, R. Longarini, T. Portale, E. Seregni, E. Bombardieri, The estrogen suppression after sequential treatment with formestane in advanced breast cancer patients, Biomed. Pharmacother. 58 (4) (2004) 255–259.
[76] S. Chia, W. Gradishar, L. Mauriac, J. Bines, F. Amant, M. Federico, L. Fein, G. Romieu, A. Buzdar, J.F. Robertson, A. Brufsky, K. Possinger, P. Rennie, F. Sapunar, E. Lowe, M. Piccart, Double-blind, randomized placebo controlled trial of fulvestrant compared with exemestane after prior nonsteroidal aromatase inhibitor therapy in postmenopausal women with hormone receptor-positive, advanced breast cancer: results from EFECT, J. Clin. Oncol. 26 (10) (2008) 1664–1670.
[77] M.E. Hammond, D.F. Hayes, M. Dowsett, D.C. Allred, K.L. Hagerty, S. Badve, P.L. Fitzgibbons, G. Francis, N.S. Goldstein, M. Hayes, D.G. Hicks, S. Lester, R. Love, P.B. Mangu, L. McShane, K. Miller, C.K. Osborne, S. Paik, J. Perlmutter, A. Rhodes, H. Sasano, J.N. Schwartz, F.C. Sweep, S. Taube, E.E. Torlakovic, P. Valenstein, G. Viale, D. Visscher, T. Wheeler, R.B. Williams, J.L. Wittliff, A.C. Wolff, American Society of Clinical Oncology/College Of American Pathologists guideline recommendations for immunohistochemical testing of estrogen and progesterone receptors in breast cancer, J. Clin. Oncol. 28(16) 2784–2795.
[78] R.M. Elledge, S. Green, R. Pugh, D.C. Allred, G.M. Clark, J. Hill, P. Ravdin, S. Martino, C.K. Osborne, Estrogen receptor (ER) and progesterone receptor (PgR), by ligand-binding assay compared with ER, PgR and pS2, by immunohistochemistry in predicting response to tamoxifen in metastatic breast cancer: a Southwest Oncology Group Study, Int. J. Cancer 89 (2) (2000) 111–117.
[79] C.A. Lockwood, C. Ricciardelli, W.A. Raymond, R. Seshadri, K. McCaul, D.J. Horsfall, A simple index using video image analysis to predict disease outcome in primary breast cancer, Int. J. Cancer 84 (3) (1999) 203–208.
[80] A.T. Ferguson, R.G. Lapidus, S.B. Baylin, N.E. Davidson, Demethylation of the estrogen receptor gene in estrogen receptor-negative breast cancer cells can reactivate estrogen receptor gene expression, Cancer Res. 55 (11) (1995) 2279–2283.
[81] Y.L. Ottaviano, J.P. Issa, F.F. Parl, H.S. Smith, S.B. Baylin, N.E. Davidson, Methylation of the estrogen receptor gene CpG island marks loss of estrogen receptor expression in human breast cancer cells, Cancer Res. 54 (10) (1994) 2552–2555.
[82] D. Sharma, N.K. Saxena, N.E. Davidson, P.M. Vertino, Restoration of tamoxifen sensitivity in estrogen receptor-negative breast cancer cells: tamoxifen-bound reactivated ER recruits distinctive corepressor complexes, Cancer Res. 66 (12) (2006) 6370–6378.
[83] X. Yang, A.T. Ferguson, S.J. Nass, D.L. Phillips, K.A. Butash, S.M. Wang, J.G. Herman, N.E. Davidson, Transcriptional activation of estrogen receptor alpha in human breast cancer cells by histone deacetylase inhibition, Cancer Res. 60 (24) (2000) 6890–6894.
[84] X. Yang, D.L. Phillips, A.T. Ferguson, W.G. Nelson, J.G. Herman, N.E. Davidson, Synergistic activation of functional estrogen receptor (ER)-alpha by DNA methyltransferase and histone deacetylase inhibition in human ER-alpha-negative breast cancer cells, Cancer Res. 61 (19) (2001) 7025–7029.
[85] G. Sabnis, L. Macedo, L. Gediya, V. Njar, A. Brodie, Upregulation of ERα and aromatase by HDACi MS-275 in an ER negative breast cancer xenograft model, in: 90th Annual Meeting of Endocrine Society, San Francisco, CA, 2008 (abstract #P1-100).
[86] G. Sabnis, O. Goloubeva, L. Macedo, L. Gediya, V. Njar, A. Brodie, Combination of HDACi entinostat (SNDX-275) with letrozole in provides superior control over tumor growth in MDA-MB-231 xenograft model, in: The 31st Annual San Antonio Breast Cancer Symposium, San Antonio, TX, 2008.
[87] G. Sabnis, L. Gediya, V. Njar, A. Brodie, HDAC inhibitors sensitize ER negative breast cancer cells to AIs, in: Breast Cancer Research and Treatment Proceedings of the 30th Annual San Antonio Breast Cancer Symposium, San Antonio, TX, 2007 (abstract # 2096).
[88] L.F. Macedo, G. Sabnis, A. Brodie, Preclinical modeling of endocrine response and resistance: focus on aromatase inhibitors, Cancer 112 (Suppl. 3) (2008) 679–688.
[89] D. Jelovac, G. Sabnis, B.J. Long, L. Macedo, O.G. Goloubeva, A.M. Brodie, Activation of mitogen-activated protein kinase in xenografts and cells during prolonged treatment with aromatase inhibitor letrozole, Cancer Res. 65 (12) (2005) 5380–5389.
[90] R. Clarke, M.C. Liu, K.B. Bouker, Z. Gu, R.Y. Lee, Y. Zhu, T.C. Skaar, B. Gomez, K. O'Brien, Y. Wang, L.A. Hilakivi-Clarke, Antiestrogen resistance in breast cancer and the role of estrogen receptor signaling, Oncogene 22 (47) (2003) 7316–7339.
[91] L. Shi, B. Dong, Z. Li, Y. Lu, T. Ouyang, J. Li, T. Wang, Z. Fan, T. Fan, B. Lin, Z. Wang, Y. Xie, Expression of ER-{alpha}36, a novel variant of estrogen receptor {alpha}, and resistance to tamoxifen treatment in breast cancer, J. Clin. Oncol. 27 (21) (2009) 3423–3429.
[92] R. Schiff, P. Reddy, M. Ahotupa, E. Coronado-Heinsohn, M. Grim, S.G. Hilsenbeck, R. Lawrence, S. Deneke, R. Herrera, G.C. Chamness, S.A. Fuqua, P.H. Brown, C.K. Osborne, Oxidative stress and AP-1 activity in tamoxifen-resistant breast tumors in vivo, J. Natl. Cancer Inst. 92 (23) (2000) 1926–1934.
[93] Y. Zhou, C. Yau, J.W. Gray, K. Chew, S.H. Dairkee, D.H. Moore, U. Eppenberger, S. Eppenberger-Castori, C.C. Benz, Enhanced NF kappa B and AP-1 transcriptional activity associated with antiestrogen resistant breast cancer, BMC Cancer 7 (2007) 59.
[94] A. Ring, M. Dowsett, Mechanisms of tamoxifen resistance, Endocr. Relat. Cancer 11 (4) (2004) 643–658.
[95] G. Sabnis, O. Goloubeva, R. Gilani, L. Macedo, A. Brodie, Sensitivity to the aromatase inhibitor letrozole is prolonged after a "break" in treatment, Mol. Cancer Ther. 9(1) 46–56.
[96] L.F. Macedo, G.J. Sabnis, O.G. Goloubeva, A. Brodie, Combination of anastrozole with fulvestrant in the intratumoral aromatase xenograft model, Cancer Res. 68 (9) (2008) 3516–3522.

[97] S. Kato, H. Endoh, Y. Masuhiro, T. Kitamoto, S. Uchiyama, H. Sasaki, S. Masushige, Y. Gotoh, E. Nishida, H. Kawashima, D. Metzger, P. Chambon, Activation of the estrogen receptor through phosphorylation by mitogen-activated protein kinase, Science 270 (5241) (1995) 1491–1494.
[98] P.B. Joel, A.M. Traish, D.A. Lannigan, Estradiol-induced phosphorylation of serine 118 in the estrogen receptor is independent of p42/p44 mitogen-activated protein kinase, J. Biol. Chem. 273 (21) (1998) 13317–13323.
[99] D. Chen, E. Washbrook, N. Sarwar, G.J. Bates, P.E. Pace, V. Thirunuvakkarasu, J. Taylor, R.J. Epstein, F.V. Fuller-Pace, J.M. Egly, R.C. Coombes, S. Ali, Phosphorylation of human estrogen receptor alpha at serine 118 by two distinct signal transduction pathways revealed by phosphorylation-specific antisera, Oncogene 21 (32) (2002) 4921–4931.
[100] S.R. Johnston, Enhancing the efficacy of hormonal agents with selected targeted agents, Clin. Breast Cancer 9 (Suppl. 1) (2009) S28–S36.
[101] R.C. Wu, J. Qin, P. Yi, J. Wong, S.Y. Tsai, M.J. Tsai, B.W. O'Malley, Selective phosphorylations of the SRC-3/AIB1 coactivator integrate genomic reponses to multiple cellular signaling pathways, Mol. Cell 15 (6) (2004) 937–949.
[102] J. Font de Mora, M. Brown, AIB1 is a conduit for kinase-mediated growth factor signaling to the estrogen receptor, Mol. Cell Biol. 20 (14) (2000) 5041–5047.
[103] G.J. Sabnis, L.F. Macedo, O. Goloubeva, A. Schayowitz, A.M. Brodie, Stopping treatment can reverse acquired resistance to letrozole, Cancer Res. 68 (12) (2008) 4518–4524.
[104] G. Sabnis, A. Schayowitz, O. Goloubeva, L. Macedo, A. Brodie, Trastuzumab reverses letrozole resistance and amplifies the sensitivity of breast cancer cells to estrogen, Cancer Res. 69 (4) (2009) 1416–1428.
[105] G. Sabnis, O. Goloubeva, D. Jelovac, A. Schayowitz, A. Brodie, Inhibition of the phosphatidylinositol 3-kinase/Akt pathway improves response of long-term estrogen-deprived breast cancer xenografts to antiestrogens, Clin. Cancer Res. 13 (9) (2007) 2751–2757.
[106] A. Lipton, K. Leitzel, S.M. Ali, L. Demers, H.A. Harvey, H.A. Chaudri-Ross, D. Evans, R. Lang, W. Hackl, P. Hamer, W. Carney, Serum HER-2/neu conversion to positive at the time of disease progression in patients with breast carcinoma on hormone therapy, Cancer 104 (2) (2005) 257–263.
[107] A. Lipton, S. Ali, K. Leitzel, HER-2 protein expression predicts response to trastuzumab in FISH-positive patients with metastatic breast cancer, in: 31st AACR-CTRC San Antonio Breast Cancer Symposium, San Antonio, TX, 2008.
[108] P.K. Marcom, C. Isaacs, L. Harris, Z.W. Wong, A. Kommarreddy, N. Novielli, G. Mann, Y. Tao, M.J. Ellis, The combination of letrozole and trastuzumab as first or second-line biological therapy produces durable responses in a subset of HER2 positive and ER positive advanced breast cancers, Breast Cancer Res. Treat. 102 (1) (2007) 43–49.
[109] J. Mackey, B. Kaufman, M. Clemens, Trastuzumab prolongs progression-free survival in hormone-dependent and HER2-positive metastatic breast cancer, Breast Cancer Res. Treat. 100 (Suppl. 1) (2006) (abstract 3).
[110] S. Johnston, J. Pippen Jr., X. Pivot, M. Lichinitser, S. Sadeghi, V. Dieras, H.L. Gomez, G. Romieu, A. Manikhas, M.J. Kennedy, M.F. Press, J. Maltzman, A. Florance, L. O'Rourke, C. Oliva, S. Stein, M. Pegram, Lapatinib combined with letrozole versus letrozole and placebo as first-line therapy for post-menopausal hormone receptor-positive metastatic breast cancer, J. Clin. Oncol. 27 (33) (2009) 5538–5546.