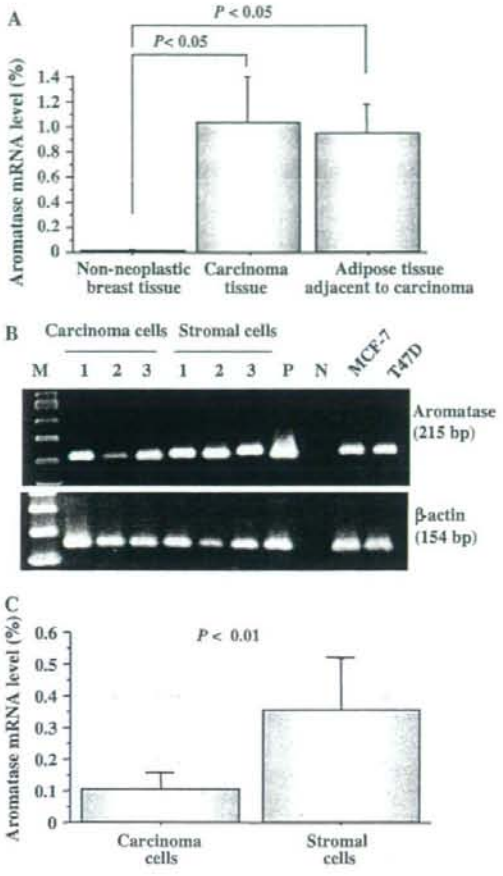


**Fig. 2** Cellular expression of aromatase mRNA in breast carcinoma. **a** Aromatase mRNA levels were significantly higher in breast carcinoma and adipose tissue adjacent to the carcinoma ($P < 0.05$, respectively) than in non-neoplastic breast tissue ($n = 12$ in each group). **b** Localization of aromatase mRNA in breast carcinoma, obtained by LCM/real-time PCR analysis. Aromatase mRNA was detected in both breast carcinoma cells and intratumoral stromal cells. Three representative cases of breast carcinoma (*1–3*) and two breast carcinoma cell lines (MCF-7 and T47D) are represented in this agarose gel photo. *M* molecular marker, *P* positive control (placental tissue), *N* negative control (no cDNA substrate). **c** Cellular expression of aromatase mRNA in breast carcinoma by LCM/real-time PCR analysis. The aromatase mRNA level was significantly ($P < 0.01$) higher in intratumoral stromal cells than in breast carcinoma cells ($n = 12$ in each group). **a**, **c** The aromatase mRNA level was summarized as a ratio with an internal standard ($\beta$-actin) and then evaluated as a ratio (%) with the positive control (placental tissue)

The immunolocalization of aromatase in breast carcinoma was examined by several groups, but the results reported by them were inconsistent. Previously, Sasano et al. [14] showed the immunolocalization of aromatase in stromal cells, such as intratumoral fibroblasts (Fig. 3a) and adipocytes, of breast carcinoma, and Santen et al. [15] also demonstrated that aromatase immunoreactivity occurred predominantly in stromal cells. On the other hand, Esteban et al. [16] and Brodie et al. [17] reported the immunolocalization of aromatase in breast carcinoma cells. Recently, Sasano et al. [18] validated several aromatase antibodies that had been newly developed for immunohistochemistry, and demonstrated that the immunoreactivity of a monoclonal antibody for aromatase (#677) was detected in various types of cells, such as intratumoral stromal cells, carcinoma cells (Fig. 3b) and normal duct epithelial cells, which is in good agreement with the localization of aromatase mRNA described above. In our study, the aromatase immunoreactivity obtained by #677 antibody was significantly associated with the aromatase mRNA level in a carcinoma cell component (Fig. 3c), but not that in an intratumoral stromal cell component (data not shown), and Sasano et al. [18] reported that aromatase activity in breast carcinoma tissue was positively associated with aromatase immunoreactivity (#677) in a carcinoma cell component, but not that in a stromal cell component. However, further examinations are required to clarify the clinical and biological significance of aromatase in relation to cell types in breast carcinoma.

Aromatase immunoreactivity was mainly detected in carcinoma cells in noninvasive breast carcinoma (Fig. 3d), regarded as a precursor lesion to invasive carcinoma [19].

The differences between the results for aromatase immunolocalization obtained in previous studies are possibly due to the different natures of the aromatase antibodies employed. Immunohistochemistry for aromatase is generally expected to be the most attractive method of evaluating aromatase expression, considering the great success that diagnostic laboratories have had in detecting ER, progesterone receptor (PR) and HER2 in breast carcinoma tissues. Therefore, further examinations are required to establish a standardized approach, including the determination of aromatase antibody, the immunohistochemical procedure and the evaluation system.

## Regulatory factors of aromatase expression in breast carcinoma

The mechanism by which mechanism aromatase expression is increased in various types of cells in breast carcinoma remains largely unclear. When we examined the expression of aromatase mRNA in breast carcinoma tissues by real-time PCR analysis, the aromatase mRNA level was highest in invasive breast carcinoma, modest in noninvasive breast carcinoma, and lowest in the non-neoplastic breast tissue (Fig. 4a), and LCM/real-time PCR analysis revealed that the aromatase mRNA level was significantly

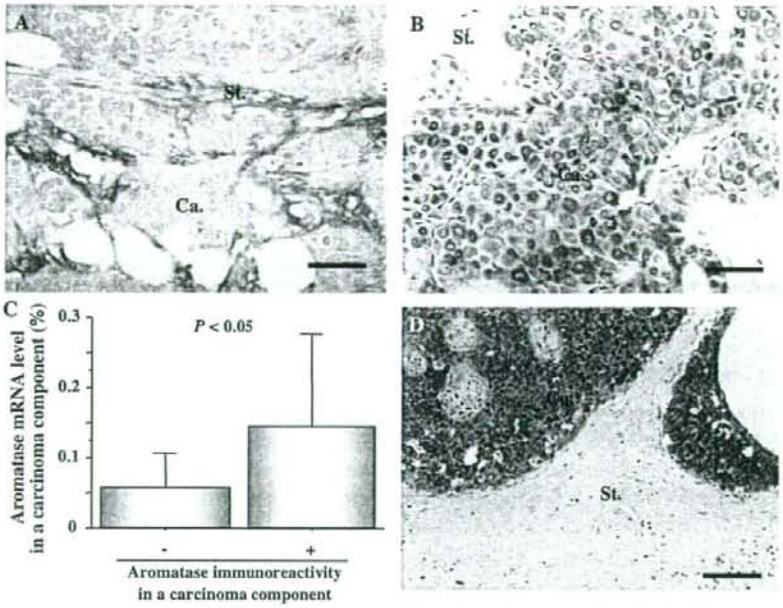


**Fig. 3** Immunohistochemistry for aromatase in breast carcinoma. **a** Aromatase immunoreactivity was detected in intratumoral stromal cells (*St.*), but not in carcinoma cells (*Ca.*), in invasive breast carcinoma, when we used the same rabbit polyclonal antibody as used in [14]. **b** On the other hand, aromatase immunoreactivity was detected in carcinoma cells in invasive breast carcinoma when we used the same mouse monoclonal antibody as used in [18] (#677). **c** Aromatase immunoreactivity (#677) was significantly ($P < 0.05$) correlated with the mRNA level in a carcinoma cell component in invasive breast carcinoma ($n = 18$). The aromatase mRNA level was evaluated by LCM/real-time PCR. **d** Aromatase immunoreactivity was mainly detected in carcinoma cells in noninvasive breast carcinoma. *Bar* represents 50 μm

higher in invasive breast carcinoma than in noninvasive breast carcinoma in both carcinoma cell and intratumoral stromal cell components [19]. Subsequent co-culture experiments demonstrated that aromatase activity was significantly increased when co-culturing with MCF-7 breast carcinoma cells and intratumoral stromal cells isolated from breast carcinoma tissue compared to the aromatase activities observed during each single culture (Fig. 4b, c) [20]. Previous in vitro studies have demonstrated that breast carcinoma cells secrete various factors that induce aromatase expression in adipose fibroblasts, including prostaglandin E2, interleukin (IL)-1, IL-6, IL-11 and tumor necrosis factor α [21, 22]. On the other hand, it has been also reported that exogenous growth factors such as epidermal growth factor, transforming growth factor and keratinocyte growth factor stimulate aromatase activity in MCF-7 cells [20]. Therefore, aromatase expression may be, partially at least, regulated by tumor–stromal interactions in breast carcinoma, which may be promoted by the invasion of carcinoma cells into stroma.

Previous studies have also demonstrated the regulation of aromatase expression by various transcriptional factors. Transcription of aromatase is activated by steroidogenic factor 1/adrenal 4 binding protein (SF1; designated NR5A1) in the ovaries, which binds to a nuclear receptor half site within their promoter regions to mediate basal transcription and in part cAMP-induced transcription. However, SF1 is not expressed in breast carcinoma. Clyne et al. [23] and Zhou et al. [24] examined various orphan nuclear receptors known to bind to such a nuclear receptor half site in 3T3-L1 preadipocytes, and reported the induction of aromatase expression by liver receptor homolog-1 (LRH-1; NR5A2) in adipose stromal cells in breast carcinoma. LRH-1 was immunolocalized in adipocytes adjacent to the carcinoma and carcinoma cells [25]. LRH-1 expression was positively associated with aromatase in the adipose tissues adjacent to the carcinoma [24], but not in the breast carcinoma cells [25]. Therefore, LRH-1 may regulate aromatase expression mainly in the adipocytes adjacent to the breast carcinoma.

On the other hand, estrogen-related receptor α (ERRα; NR3B1) has a positive regulatory effect on aromatase in SK-BR-3 breast carcinoma cells [26], but not in 3T3-L1 preadipocytes [23]. ERRα was mainly immunolocalized in

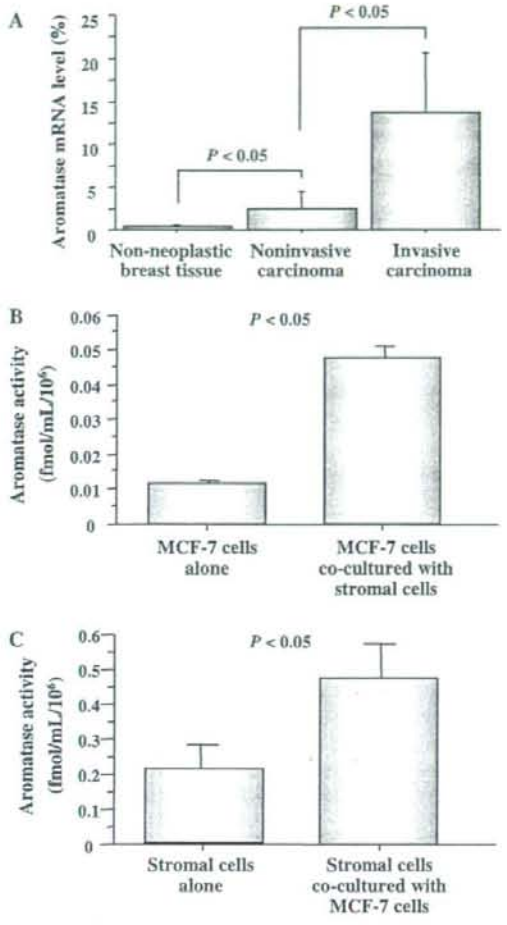


**Fig. 4** Aromatase expression in noninvasive and invasive breast carcinoma. **a** Aromatase mRNA expression was significantly ($P < 0.05$) higher in noninvasive breast carcinoma ($n = 12$) than in non-neoplastic breast tissue ($n = 8$). Aromatase mRNA level was also significantly ($P < 0.05$) higher in invasive breast carcinoma ($n = 12$) than in noninvasive carcinoma ($n = 12$). The aromatase mRNA level was summarized as a ratio (%) with that of an internal standard (ribosomal protein L 13a). **b**, **c** Effects of co-culturing on aromatase activity in breast carcinoma cells and intratumoral stromal cells. **b** Aromatase activity of MCF-7 cells was significantly ($P < 0.05$) increased when co-culturing with intratumoral stromal cells isolated from breast carcinoma tissue compared to the single culture. **c** Similarly, the aromatase activity of the intratumoral stromal cells was also significantly ($P < 0.05$) elevated when these cells were co-cultured with MCF-7 cells

breast carcinoma cells, but not in intratumoral stromal cells or adipocytes [27], and the expression level of ERRα mRNA in carcinoma cells was positively associated with that of aromatase mRNA in breast carcinoma [20]. Thus, aromatase expression is regulated by various factors in breast carcinoma, and key regulators may differ according to the cell types that express the aromatase.

## Expression of other estrogen-producing enzymes in breast carcinoma

### STS

A major circulating form of plasma estrogens is estrone sulfate, a biologically inactive form of estrogen, in postmenopausal women. Estrone sulfate has a long half-life in peripheral blood, and the level of estrone sulfate is approximately ten times higher than that of unconjugated estrogens such as estrone, estradiol and estriol during the menstrual cycle and in postmenopausal women [28]. STS is a single enzyme that hydrolyzes estrone sulfate to estrone (Fig. 1). The enzymatic activity of STS is detected in the great majority of breast carcinomas, and is considerably higher than the aromatase activity in breast tumors [12]. STS immunoreactivity was detected in carcinoma cells in approximately 70% of breast carcinoma cases [29, 30], and STS immunoreactivity was significantly associated with an increased risk of recurrence in breast carcinoma patients [31]. STS mRNA expression was also reported to be higher in breast carcinoma tissue than in the normal tissue, and it was significantly associated with poor clinical patient outcome [31, 32]. STS inhibitors are currently being developed by several groups, and the results of a phase I study suggest that an STS inhibitor may provide effective treatment for hormone-dependent breast carcinomas, including those which progress upon treatment with aromatase inhibitors [33].

### 17βHSD1

17βHSD catalyzes an interconversion of estrogens or androgens. Thirteen isozymes of 17βHSD have been cloned [34], and 17β-reduction (17βHSD1, 3, 5, 7, etc.) or oxidation (17βHSD2, 4, 6, etc.) of estrogens and/or androgens is catalyzed by different 17βHSD isozymes. Among these isozymes, 17βHSD1 enzyme uses NADPH as a cofactor and mainly catalyzes the reduction of estrone to estradiol (Fig. 1). Oxidative 17βHSD activity is the preferential direction in normal breast tissues, but the reductive 17βHSD pathway is dominant in breast carcinoma [12]. Miyoshi et al. [3] reported that 17βHSD1 mRNA levels and the intratumoral estradiol/estrone ratio was significantly higher in postmenopausal than in premenopausal breast carcinoma. 17βHSD1 immunoreactivity was detected in carcinoma cells in approximately 60% of the breast carcinomas, and it was correlated with ER and PR [35]. In

addition, breast carcinoma patients with high levels of 17$\beta$HSD1 mRNA were associated with increased risk of developing late relapses of breast carcinoma [36]. Therefore, 17$\beta$HSD1 is suggested to be responsible for regulating the process leading to the accumulation of estradiol in the breast carcinoma, and the majority of the estradiol synthesized by 17$\beta$HSD1 in breast carcinoma cells may act directly on these cells.

## Aromatase as a negative regulator of intratumoral androgen production in breast carcinoma

In contrast to estrogens, androgens are considered to predominantly exert antiproliferative effects via androgen receptor (AR) in breast carcinoma cells, although some divergent findings have been reported [12]. Tissue concentrations of androgens in breast carcinomas were investigated by several groups [37–39]. A potent androgen, 5$\alpha$-dihydrotestosterone (DHT), was significantly higher in breast carcinoma than in plasma [38], and intratumoral production of DHT in breast carcinoma has also been proposed for the circulating inactive androgen androstenedione, like estrogens (Fig. 1). AR is expressed in the majority of human breast carcinoma tissues [40–42], suggesting important roles for androgens in breast carcinoma, as well as estrogenic actions.

The substrates of aromatase are androstenedione and testosterone, and these are precursors not only to estradiol synthesis but also to DHT production (Fig. 1). DHT itself is nonaromatizable. The intratumoral concentration of DHT was significantly associated with that of testosterone in breast carcinoma tissue [37, 38], which suggests that the DHT level in the breast carcinoma is greatly influenced by the amount of the precursor. Spinola et al. [43] showed that treatment with an aromatase inhibitor markedly elevated intratumoral testosterone concentrations in dimethylbenz(a)anthracene (DMBA)-induced rat mammary tumors, and Sonne-Hansen and Lykkesfeldt [44] reported that aromatase preferred testosterone as a substrate in MCF-7 cells. Very recently, we demonstrated that aromatase expression was inversely associated with intratumoral DHT concentration in breast carcinoma, and that aromatase suppressed DHT production from androstenedione in coculture experiments with MCF-7 cells and intratumoral stromal cells isolated from breast carcinoma [39]. Therefore, aromatase is suggested to act as a negative regulator for intratumoral DHT production in breast carcinoma, possibly by reducing concentrations of the precursor testosterone.

Results of large multicenter trials have demonstrated the superior efficacy of aromatase inhibitors compared to the anti-estrogen tamoxifen [8–11]. Although this might be due to agonistic effects of tamoxifen in an estrogen-deprived environment [8], we can also speculate that aromatase inhibitors exert additional antiproliferative effects by increasing the local DHT concentration upon estrogen deprivation. Further examinations are required to clarify the clinical importance of androgenic actions in association with the response to aromatase inhibitors in breast cancer patients.

## Intratumoral production of sex steroids in noninvasive breast carcinoma

Noninvasive breast carcinoma is regarded as a precursor lesion to invasive breast carcinoma. The great majority of noninvasive breast carcinomas are histologically diagnosed as ductal carcinomas in situ (DCIS), and the risk of invasive ductal carcinoma developing after a diagnosis of DCIS was reported to be 4–10 times higher than in normal women [45, 46]. The incidence of noninvasive breast carcinoma has markedly increased over the past two decades with advances in mammographic screening [47, 48], and it now comprises approximately 10–20% of all breast carcinomas diagnosed [49–51].

It is well known that sex-steroid receptors, such as ER, PR and AR, are frequently positive in noninvasive breast carcinoma [50, 52–54], suggesting important roles for sex steroids in noninvasive breast carcinoma, just as in invasive carcinoma. Tamoxifen was reported to inhibit the growth of premalignant mammary lesions and the progression to invasive carcinoma in a transplantable mouse model of noninvasive breast carcinoma [55]. The National Surgical Adjuvant Breast Project (NSABP) P-1 trial demonstrated that tamoxifen significantly reduced the risk of noninvasive breast carcinoma by 50% [56], and the results of the NSABP B-24 trial indicated that adjuvant tamoxifen therapy reduced the recurrence of noninvasive breast carcinoma by 30% [57]. However, information on sex steroids in noninvasive breast carcinoma is currently very limited compared to that on sex steroids in invasive carcinoma, as described above, and the clinical and/or biological significance of sex steroids in noninvasive carcinoma remains largely unclear.

When we examined intratumoral concentrations of sex steroids in noninvasive breast carcinoma, both estradiol and DHT levels were significantly ($P < 0.05$, respectively) higher in noninvasive breast carcinoma than in non-neoplastic breast tissue (Fig. 5a, b) [19]. The results of the study also demonstrated that estrogen (aromatase, STS, and 17$\beta$HSD1) and androgen (17$\beta$HSD5 and 5$\alpha$-reductase type 1) producing enzymes were abundantly expressed in noninvasive carcinoma tissues [19]. Therefore, it is suggested that both estrogens and androgens are locally produced in

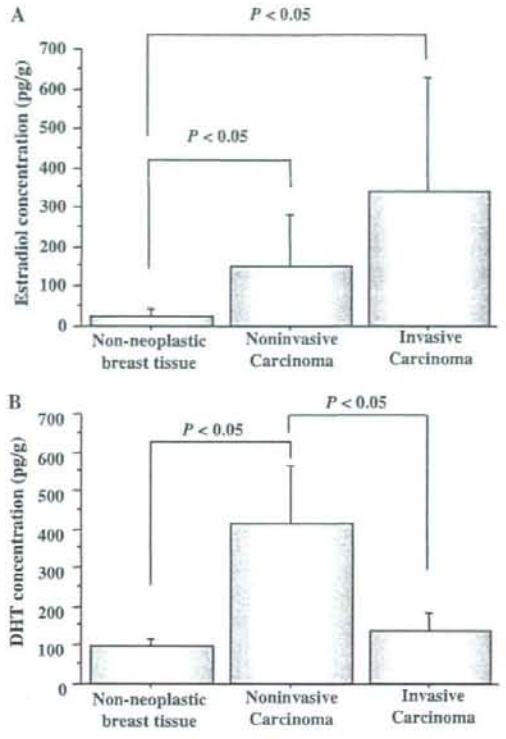


**Fig. 5** Tissue concentrations of estradiol (**a**) and DHT (**b**) in noninvasive breast carcinoma. Both estradiol and DHT levels were significantly ($P < 0.05$, respectively) higher in noninvasive breast carcinoma ($n = 12$) than in non-neoplastic breast tissue ($n = 8$). In addition, the intratumoral concentration of DHT was significantly ($P < 0.05$) higher in noninvasive breast carcinoma than in invasive carcinoma ($n = 12$) in our study

noninvasive breast carcinoma, as in invasive carcinoma, and that endocrine therapies may be clinically effective for a select group of noninvasive breast carcinoma patients. Further examinations are required to clarify the significance of sex steroids in noninvasive breast carcinoma.

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# Aromatase Inhibitors: Are There Differences Between Steroidal and Nonsteroidal Aromatase Inhibitors and Do They Matter?

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**Disclosure:** W.R.M. discloses that this article discusses letrozole (Novartis), anastrozole (AstraZeneca), and exemestane (Pfizer) for validation of mechanism of action. He has also been on the advisory board for Pfizer and received speaker's honoraria. J.B. has received honoraria and research funding from Pfizer (exemestane). A.M.H.B. has received honoraria and research support from AstraZeneca (anastrozole research). R.W.B. discloses that this article discusses exemestane (Pfizer), anastrozole (AstraZeneca), and letrozole (Novartis) for use in breast cancer therapy. He also received an honorarium for participating in an expert panel meeting from Pfizer/CC Ford Healthcare. E.d.S. is an employee and has stock options in Pfizer (exemestane). P.E.L. has received speaker's honoraria from Novartis, AstraZeneca, and Pfizer. A.L. discloses no conflict of interest. N.M. discloses that the article discusses exemestane (Pfizer), anastrozole (AstraZeneca), and letrozole (Novartis) for breast cancer treatment, and he has been on an advisory board and received honoraria from Pfizer (exemestane). T.M. has research funding from Novartis (letrozole) and has received a consulting fee from Pfizer (exemestane). H.S. has research funding and has received an honorarium from Pfizer (exemestane). P.E.G. discloses that this article discusses letrozole (Novartis), anastrozole (AstraZeneca), and exemestane (Pfizer) for validation of mechanism of action. He also discloses that the article discusses aromatase inhibitors for prevention (Novartis, AstraZeneca, Pfizer), and that he has received speaker's honoraria from Novartis, AstraZeneca, and Pfizer. The content of this manuscript has been reviewed by independent peer reviewers to ensure that it is balanced, objective, and free from any commercial bias; they have disclosed no financial relationships relevant to this content.

**Key Words.** Aromatase inhibitor • Exemestane • Letrozole • Anastrozole • Mechanism of action

ABSTRACT

**Aromatase inhibitors (AIs) are approved for use in both early- and advanced-stage breast cancer in postmenopausal women. Although the currently approved "third-generation" AIs all powerfully inhibit estrogen synthesis, they may be subdivided into steroidal and nonsteroidal inhibitors, which interact with the aromatase enzyme differently. Nonsteroidal AIs bind noncovalently and reversibly to the aromatase protein, whereas steroidal AIs may bind covalently and irreversibly to the aromatase enzyme. The steroidal AI exemestane may exert androgenic effects, but the clinical relevance of this has yet to be determined. Switching between steroidal and nonsteroidal AIs produces modest additional clinical benefits, suggesting partial noncross-resistance between the classes of inhibitor. In these circumstances, the response rates to the second AI have**

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1083-7159/2008/$30.00/0 doi: 10.1634/theoncologist.2008-0055

**generally been low; additional research is needed regarding the optimal sequence of AIs. To date, clinical studies suggest that combining an estrogen-receptor blocker with a nonsteroidal AI does not improve efficacy, while combination with a steroidal AI has not been evaluated. Results from head-to-head trials comparing steroidal and nonsteroidal AIs will determine whether meaningful clinical differences in efficacy or adverse events exist between the classes of AI. This review summarizes the available evidence regarding known differences and evaluates their potential clinical impact.** *The Oncologist* 2008;13:829–837

## Introduction

Breast cancer is the most common cancer among women worldwide [1]. Most breast cancers in postmenopausal women are estrogen receptor positive ($ER^+$) [2, 3]. In this population, endocrine therapies designed to prevent estrogen-driven proliferation can induce tumor regression. There are currently two major treatment modalities to prevent the effects of estrogen: "antiestrogens," which target and antagonize ER-mediated action, and aromatase inhibitors (AIs), which inhibit estrogen biosynthesis. Aromatase is the final enzymatic step in catalyzing the biosynthesis of estrogens (Fig. 1), thereby making the enzyme an attractive therapeutic target.

The evolution of AIs has seen the transition from prototype agents that were either not particularly potent or selective to third-generation drugs that are considerably more potent and specific. Third-generation inhibitors represent a significant advance in the therapy of $ER^+$ breast cancer in postmenopausal women, and are approved as treatments for both early- and late-stage $ER^+$ breast cancer [4–6].

AIs can be classified either as steroidal (type I inhibitors) or nonsteroidal (type II inhibitors), based on their chemical structure. In terms of the current inhibitors, anastrozole and letrozole are nonsteroidal, whereas exemestane is a steroidal inhibitor.

All AIs are similar in that they inhibit estrogen synthesis by blocking aromatase activity, thereby reducing endogenously synthesized estrogen. However, there are distinct differences among them. These are either class-independent differences in terms of potency [7] or class-specific differences in terms of the mechanism of binding to aromatase. There may also be clinically relevant differences among AIs related to the androgenic properties of steroidal AIs. This review summarizes the available evidence relating to class differences and evaluates how these may translate into clinical effects.

## Interactions with Aromatase

Aromatase is a member of the cytochrome P450 (CYP)19 family of enzymes [8–10]. Nonsteroidal type II AIs, such as anastrozole and letrozole, interact noncovalently with the heme moiety of aromatase and occupy its substrate-binding site, thereby preventing binding of androgens to the catalytic site [11] (Fig. 2). This antagonism is reversible, and the type II AIs can be competitively displaced from the active site by endogenous substrate. In contrast, the steroidal type I inhibitor exemestane is an analogue of the natural aromatase substrate androstenedione. Exemestane is recognized by the active site of aromatase as an alternate substrate [8, 10, 12] (Fig. 2). However, it appears to be converted by aromatase into a reactive intermediate that binds irreversibly and covalently to the substrate-binding site of aromatase, permanently inactivating the enzyme [12]. Irreversible AIs are also known as inactivators or "suicide" inhibitors because aromatase is inactivated because of its own mechanism of action [12].

Differences in binding between nonsteroidal and steroidal AIs lead to divergent effects on aromatase in experimental systems. In the MCF-7aro breast cancer cell line, which has been transfected with aromatase, exemestane destabilizes the enzyme so that aromatase protein exposed to exemestane is degraded at a greater rate by proteosomic enzymes than the nonexposed enzyme [13]. In contrast, nonsteroidal inhibitors may increase aromatase protein levels because they stabilize the structure of the enzyme [13] and/or induce transcription of aromatase mRNA [14, 15]. The consequence of this upregulation, together with the reversible nature of type II inhibitors, is that it is possible to demonstrate increases in enzyme activity following exposure to type II inhibitors. Thus, both Miller and Dixon (using human breast fibroblasts) [16] and Soudon (using the choriocarcinoma cell line JEG-3) [17] have shown that exposure to type II inhibitors followed by assaying in the absence of inhibitor results in an increase in aromatase activity, an effect not seen with exemestane. This is consistent with data from ex vivo assays for aromatase activity following neoadjuvant treatment with aminoglutethimide [18]; aminoglutethimide is a potent inducer of multiple CYP enzymes [19].

Theoretically, irreversible steroidal AIs such as exemestane are expected to have a longer duration of inhibition than reversible AIs because estrogen synthesis can only resume following de novo synthesis of aromatase. How-

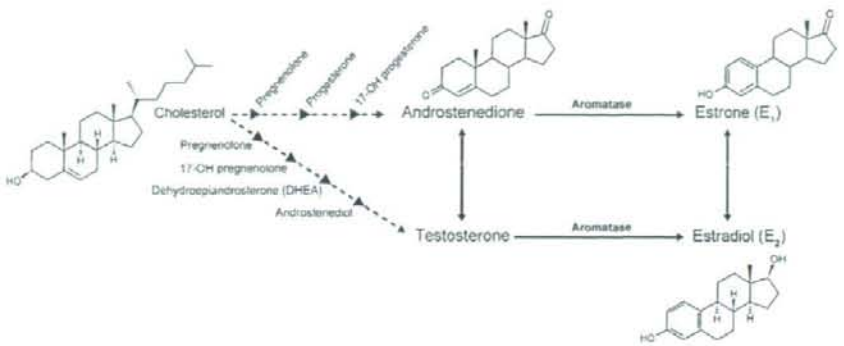


**Figure 1.** The estrogen synthesis pathway.

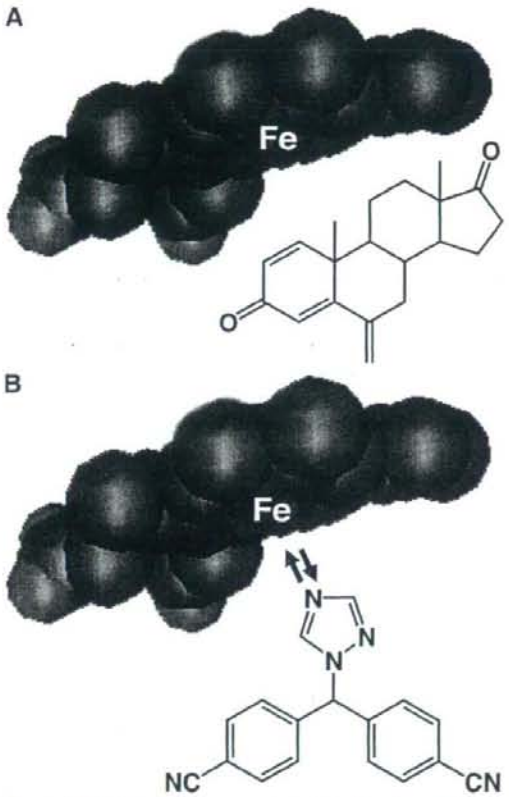


**Figure 2.** Schematic representation of the binding of (**A**) steroidal (type I [exemestane]) and (**B**) nonsteroidal (type II [letrozole]) aromatase inhibitors to the aromatase enzyme.

ever, based on in vivo data, this occurs relatively quickly (i.e., within 1–2 days) [20]. In addition, the pharmacokinetic properties of each specific AI affect the duration of estrogen suppression. In any event, the clinical scheduling for exemestane, as with the nonsteroidal inhibitors, is daily dosing [6]. The inductive/stabilizing effects of nonsteroidal AIs on aromatase protein do not seem to affect their ability to lower estrogen levels or whole body aromatase activity in the short term [21, 22]. When given daily in therapeutic doses, both letrozole and anastrozole profoundly inhibit aromatase activity and effectively suppress estrogen levels in postmenopausal women at 3 months. However, it is possible (although currently unproven) that prolonged treatment with nonsteroidal AIs could result in high levels of aromatase and resumption of estrogen biosynthesis, which may contribute to the development of resistance. This hypothesis could be tested by collecting tissues from patients recurring on nonsteroidal AIs and measuring levels of aromatase mRNA, protein, and activity. Although there are few data exploring the long-term endocrine effects of an AI, a study by Dowsett et al. [23] reported that plasma estrone levels of aminoglutethimide-treated patients increased slightly shortly before relapse, which is consistent with the concept.

Although aromatase in normal tissues is differentially regulated through a variety of promoters [24], there appears to be only one human aromatase gene and a single protein translate. However, it is possible that in tumors there may be mutations that could affect aromatase protein structure or sensitivity to inhibitors. In this respect, structural functional studies on aromatase have produced proteins that appear resistant to a steroidal AI (formestane) while maintaining sensitivity to nonsteroidal AIs [25]. Such a phenotype has been reported in some breast cancers, for which causative mutations have not been identified [26]. The possibility exists, therefore, that aromatase in individual breast cancers may be more susceptible to one class of AI than another. This may be important because intratumoral aromatase may be responsible for the higher levels of estrogen generally seen

**Table 1.** Relative binding affinity (RBA) of exemestane and 17-hydroexemestane to the androgen receptor (AR) as compared with the specific ligand 5α-dihydrotestosterone (DHT) or metribolone (R1881)

| Androgen receptor | Ligand (RBA = 100%) | RBA versus the specific ligand (%) | | Reference |
|---|---|---|---|---|
| | | Exemestane | 17-Hydroexemestane | |
| Human[a] | DHT | <0.1 | ~40 | Chang et al. (2003) [30] |
| Rat[b] | DHT | 0.28 | 27 | Pharmacia & Upjohn Co. (2005) [6] |
| Rat[c] | R1881 | 0.66 | 34 | Ariazi et al. (2007) [31] |

[a]Human breast cancer cell line (MDA453) that expresses endogenous AR and is transfected with an MMTV-luciferase reporter gene.
[b]Cytoplasmic rat prostate receptor.
[c]Baculovirus-produced rat AR ligand-binding domain tagged with a His-glutathione S-transferase epitope.

in postmenopausal tumor tissue, relative to those in the circulation [16, 27–29].

## Androgenic Versus Nonandrogenic Properties

The androgenic structure of type I steroidal AIs may give rise to hormonal effects apart from the decrease in estrogen production caused by inhibition of aromatase. The androgenic properties of exemestane make it distinct from the nonsteroidal AIs letrozole and anastrozole. Exemestane is structurally related to androstenedione. The principal metabolite of exemestane, 17-hydroexemestane (17β-hydroxy-6-methylenandrosta-1,4-diene-3,17-dione), binds with high affinity to the androgen receptor (AR) (Table 1) [6, 30, 31]. When exemestane is given to postmenopausal women at the approved dose of 25 mg daily, the circulating levels of 17-hydroexemestane are approximately 15% that of unchanged exemestane [32].

Because >60% of breast tumors express ARs, a role for androgens in the natural history of breast cancer has been suggested [33]. Androgens have been used in the past to treat breast cancer, albeit with modest response rates (e.g., ~20%) that were further limited by severe adverse effects, such as masculinization [34–37]. Some preclinical evidence has demonstrated that estrogen depletion induced by AIs further sensitizes breast cancer cells to the antiproliferative effects of androgens [38, 39], potentially suggesting an additional and complementary anticancer mechanism for steroidal AIs. However, research is needed to determine whether steroidal AIs, such as exemestane, are capable of exerting any androgenic effect in breast cancer that is clinically relevant.

When considering the androgenic properties of AIs, differential effects on healthy tissue may also affect clinical benefit for breast cancer patients. Work in model systems suggests that exemestane treatment may lead to fewer adverse effects related to bone loss than nonsteroidal AIs because of its androgenicity [40–42]. Preclinical studies have demonstrated that these effects are related to activation of the AR by exemestane in osteoblasts [31, 40]. Several clinical findings support a lower degree of bone loss with exemestane [43–46]. One might expect that the steroidal structure of exemestane may lend itself to an androgenic effect on bone that would be evident by a rise in bone formation markers (such as procollagen type 1 N-propeptide) not present with the nonsteroidal AIs. However, two studies of similar design in human volunteers, comparing the effects of steroidal AIs with those of nonsteroidal AIs on markers of bone turnover, have produced conflicting results—one showing a significant rise in bone formation markers and the other not [46, 47]. Further evidence comes from the Bone Substudy of the Intergroup Exemestane Study, which demonstrated a smaller magnitude of bone mineral density loss following 1–2 years of exemestane treatment compared with historical reports of bone density loss following treatment with anastrozole or letrozole in postmenopausal women with breast cancer [43]. Long-term and head-to-head clinical trials monitoring the incidence of bone fractures are needed to determine the extent of the relative effects of exemestane compared with nonsteroidal AIs.

## Clinical Implications

### Crossresistance and Sequential Therapy

Observations from many clinical studies have suggested that crossresistance between steroidal and nonsteroidal AIs does not always occur (Table 2) [48–58]. These efforts have followed the seminal study by Murray and Pitt showing that breast cancer patients previously treated with the first-generation nonsteroidal AI aminoglutethimide subsequently responded to 4-hydroxyandrostenedione [50]. In general, objective response rates with a second-line AI are not high (0%–26%), but clinical benefit (which includes stable disease of ≥6 months' duration) is observed in 20%–

**Table 2.** Response to third-line sequential treatment with aromatase inhibitors/inactivators in postmenopausal women with metastatic breast cancer

| Study | *n* | Study type | First drug | Second drug | ORR (%) | ORR + SD ≥6 mos (%) |
|---|---|---|---|---|---|---|
| Murray and Pitt (1995) [50] | 112 | Prospective, single center | AG | Formestane | 21 | 43 |
| Thürlimann et al. (1997) [51] | 78 | Prospective, multicenter | AG | Exemestane | 26 | 39 |
| Lønning et al. (2000) [52] | 136 | Prospective, multinational | AG | Exemestane | 8 | 27 |
| Carlini et al. (2001) [53] | 20 | Retrospective, single center | Nonsteroidal AI | Formestane | 0 | 55 |
| Zilembo et al. (2004) [54] | 22 | Prospective, single center | Nonsteroidal AI | Formestane | 0 | 50 |
| Lønning et al. (2000) [52] | 105 | Prospective, multinational | Nonsteroidal AI | Exemestane | 5 | 20 |
| Bertelli et al. (2005) [55] | 23 | Prospective, multicenter | Nonsteroidal AI | Exemestane | 9 | 44 |
| Gennatas et al. (2006) [56] | 60 | Prospective, single center | Nonsteroidal AI | Exemestane | 20 | 38 |
| Iaffaioli et al. (2005) [48] | 50 | Prospective, multicenter | Anastrozole | Exemestane | 8 | 44 |
| Carlini et al. (2003) [49] | 21 | Prospective, single center | Anastrozole | Formestane | 0 | 57 |
| Harper-Wynne et al. (1999) [57] | 21 | Prospective, single center | Formestane | Anastrozole | 0 | 62 |
| Bertelli et al. (2005) [55] | 23 | Prospective, multicenter | Exemestane | Nonsteroidal AI | 22 | 56 |
| Geisler et al. (1996) [58] | 10 | Single center | AG | Formestane | 20 | 50 |

Abbreviations: AG, aminoglutethimide; AI, aromatase inhibitor; ORR, objective response rate to second drug; SD, stable disease.

62% of patients. This effect is observed regardless of the treatment sequence: nonsteroidal AI followed by steroidal AI [48–56] or steroidal AI followed by nonsteroidal AI [55, 57]. It must be noted that these were open-label trials, many of which were single-center studies with a small number of enrolled patients, receiving a range of prior and/or concomitant therapies (e.g., chemotherapy), and at least one study used a higher dose of the second AI than is recommended [51].

Recently, results from the phase III Evaluation of Fulvestrant versus Exemestane Clinical Trial have become available [59], in which patients with advanced hormone-responsive breast cancer refractory to a nonsteroidal AI were randomized to receive either fulvestrant or exemestane. As in earlier sequential therapy studies, patients receiving second-line exemestane treatment had a low response rate (6.7%), although the clinical benefit rate was 31%. However, the aggregate of clinical evidence indicates that patients whose disease becomes resistant to one AI may still respond to a different class of AI.

Interestingly, corresponding data on the crossresistance of AI classes in experimental systems are lacking. However, a recent study using MCF-7aro cells made resistant to AIs indicates that differential patterns of gene expression are found according to different AIs, again suggesting that mechanisms of resistance are different [60, 61].

Potential explanations for the lack of crossresistance between steroidal and nonsteroidal AIs include (a) the nature of the interaction with the enzyme's active site, (b) differential sensitivities of aromatase variants to specific compounds, (c) androgen-agonistic effects, and (d) inherent differences in potencies among AIs. The validity of these explanations, as well as any optimal treatment sequence for resistant disease (e.g., nonsteroidal followed by steroidal versus steroidal followed by nonsteroidal), remains to be determined.

## Combination Therapy with Selective ER Modulators

In experimental models, combination therapy with the selective estrogen receptor modulator (SERM) tamoxifen and the nonsteroidal AI anastrozole or letrozole did not improve the antitumor efficacy of the nonsteroidal AI alone [62, 63]. These results contrast with those of the steroidal AI exemestane and tamoxifen, wherein the combination was more effective in reducing tumor growth than either agent alone in an experimental model (Figure 3) [64, 65]. A mechanism explaining the additive effects of the steroidal AI exemestane in combination with tamoxifen has not been established and warrants further evaluation.

Clinical findings from the Arimidex, Tamoxifen, Alone or in Combination trial demonstrated that the combination of anastrozole and tamoxifen was not more efficacious than tamoxifen alone [66]. Previous studies combining aminoglutethimide and tamoxifen in patients with metastatic disease also revealed no superiority compared with either

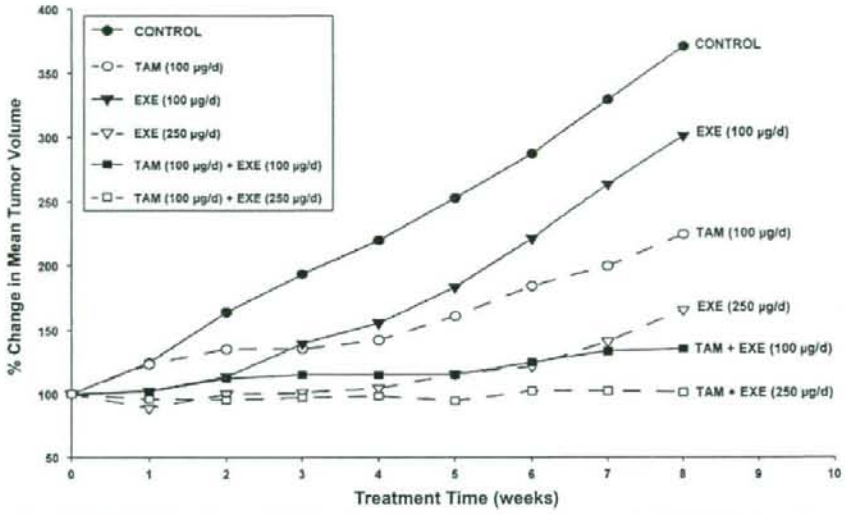


**Figure 3.** The effect of exemestane alone or in combination with tamoxifen on the growth of MCF-7Ca breast tumor xenografts in ovariectomized athymic nude mice.

Abbreviations: EXE, exemestane; TAM, tamoxifen.

Adapted with permission from Brodie A, Jelovac D, Macedo L et al. Therapeutic observations in MCF-7 aromatase xenografts. Clin Cancer Res 2005;11:884–888.

monotherapy [67]. The corresponding randomized study comparing a tamoxifen and steroidal AI combination with tamoxifen alone has not yet been performed.

Based on an initial finding that the first clinically used AI, aminoglutethimide, enhances tamoxifen clearance in humans [67], a number of studies have evaluated potential interactions between third-generation AIs and tamoxifen. Pharmacokinetic analyses have shown that coadministration of tamoxifen and nonsteroidal AIs reduces plasma levels of the AI by approximately 30%–40% compared with administration of the nonsteroidal AI alone [68, 69], although this was not considered to be the cause of the lower efficacy of the combination. In contrast, coadministration of exemestane and tamoxifen showed no evidence of pharmacokinetic interaction relative to either agent alone [70, 71]. More recently, no pharmacokinetic interaction was found by giving exemestane in combination with the SERM raloxifene [32].

Taken together, these results suggest that combination therapy with tamoxifen or another SERM and the steroidal AI exemestane should be investigated further.

## Ongoing Clinical Trials

It is not currently possible to draw meaningful conclusions regarding the differential clinical effects on efficacy between steroidal and nonsteroidal AIs because of a lack of head-to-head comparisons between AIs in large randomized trials. Issues of endocrine effectiveness, safety, sequence, and combination treatment still remain. In this respect, the National Cancer Institute of Canada Clinical Trials Group (NCIC CTG) MA-27 trial is directly comparing a steroidal AI (exemestane) with a nonsteroidal AI (anastrozole) in postmenopausal women with hormone-sensitive primary breast cancer. The primary endpoint of the 5-year study is event-free survival; two bone substudies are evaluating the effects of the respective AIs on bone metabolism in women with or without osteoporosis pretreatment. In addition, two ongoing, placebo-controlled, international breast cancer prevention trials are separately evaluating exemestane (ExCel [NCIC CTG MAP3]) and the nonsteroidal AI anastrozole (International Breast Cancer Intervention Study II, IBIS-II) using similar study designs in women at increased risk for breast cancer. A crosscomparison of the efficacy and end-organ effects of these two very similarly designed trials will be informative.

## Summary

Although both steroidal and nonsteroidal AIs block aromatase activity, there are distinct differences between the classes. The differences relate to the type of binding to the aromatase enzyme (irreversible or reversible binding for steroidal or nonsteroidal AIs, respectively) and potential androgenic effects of exemestane, which may exert additional effects. Based on preclinical and clinical studies,

there does not appear to be an advantage in combining the SERM tamoxifen with a nonsteroidal AI as initial therapy. However, preclinical findings suggest that there may be an advantage in combining tamoxifen with steroidal AIs. This type of combination should be investigated further in controlled clinical trials. Steroidal and nonsteroidal AIs are being directly compared in an ongoing head-to-head randomized controlled trial. Consequently, future results will help determine whether the different classes of AI have distinct efficacy or safety effects in the treatment or prevention of breast cancer.

## Conclusions

The answer to the question "are there differences between steroidal and nonsteroidal AIs?" is undoubtedly "yes," but more evidence is needed to determine the mechanism and clinical implications. Preclinical studies are needed to determine whether differences in the interaction with aromatase, the existence of mutant tumor aromatase, variations in molecular pathways leading to resistance, and androgenic effects might mean that particular tumors and circumstances are more optimally treated by one class of AI than another. At present, the differential properties of the AI classes are not used to plan patient management, which is still largely based on results from clinical trials of individual AIs against tamoxifen or physician preferences and experience. Thus, there are still many unanswered questions that can only be addressed by head-to-head clinical trials of the AI classes.

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The authors were responsible for the substance of the paper. Concept and content were provided by the authors as a group. Editorial support was provided by Vicki M. Houle, Ph.D., and Chris A. Kirk, Ph.D., of Complete Healthcare Communications, Inc., and was funded by Pfizer Inc. They provided assistance in researching and ordering reference articles and rationalizing authors' input into an initial manuscript draft, including development of the EndNote library and copyediting. They also provided administrative assistance in terms of collating and incorporating edits from all authors, coordinating review by all authors, and ensuring their participation according to ICMJE guidelines, and formatting the manuscript and preparing the submission packet per ***The Oncologist*** guidelines.

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Breast Cancer Res Treat
DOI 10.1007/s10549-008-0161-8

CLINICAL TRIAL

# Aromatase expression and outcomes in the P024 neoadjuvant endocrine therapy trial

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Received: 7 August 2008 / Accepted: 7 August 2008


**Abstract** *Background* Expression of aromatase by malignant breast epithelial cells and/or the surrounding stroma implies local estrogen production that could influence the outcome of endocrine therapy for breast cancer. *Methods* A validated immunohistochemical assay for aromatase was applied to samples from the P024 neoadjuvant endocrine therapy trial that compared tamoxifen and letrozole. The presence of aromatase expression by tumor or stromal cells was correlated with tumor response, treatment induced changes in proliferation index (Ki67), relapse-free survival (RFS) and breast cancer-specific survival (BCSS). *Results* Tumor and stromal aromatase expression were highly correlated ($P = 0.0001$). Tumor cell aromatase, as a semi-continuous score, also correlated with smaller tumor size at presentation ($P = 0.01$) higher baseline ER Allred score ($P = 0.006$) and lower Ki67 levels ($P = 0.003$). There was no significant relationship with clinical response or treatment-induced changes in Ki67. However, in a Cox multivariable model that incorporated a post-treatment tumor profile (pathological T stage, N stage, Ki67 and ER status of the surgical specimen), the presence of tumor aromatase expression at baseline sample remained a favorable independent prognostic biomarker for both RFS ($P = 0.01$, HR 2.3, 95% CI 1.2–4.6 for absent expression) and BCSS ($P = 0.008$, HR 3.76, 95% CI 1.4–10.0). *Conclusions* Autocrine estrogen synthesis may be most characteristic of smaller, more indolent and ER-rich breast cancers with lower baseline growth rates. However, response to endocrine treatment may not depend on whether the estrogenic stimulus has a local versus systemic source.

**Keywords** Aromatase · Letrozole · Tamoxifen · Neoadjuvant endocrine therapy

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## Introduction

After the menopause, estrogen continues to be synthesized through peripheral conversion of androgenic precursors to estrone and estradiol by the CYP P450 enzyme aromatase (CYP19). Since this enzyme is widely expressed, sources

of estrogen for breast cancers can therefore be through the circulation (endocrine), from within the breast stroma (paracrine) or through synthesis by the tumor cell (autocrine) [1, 2]. Intra-tumoral estrogen production has been directly demonstrated by measuring the conversion of radio-labeled androgen to estrogen in breast cancer biopsy material [1–3]. However correlations between biochemical measurements of intra-tumoral estrogen synthesis and clinical outcomes have not been firmly established, largely because in vivo assays of aromatase activity are difficult to execute in a large numbers of patients [4–6]. As alternative approaches, aromatase immunohistochemistry (IHC) and measurements of aromatase mRNA levels have been explored [7, 8]. However, most investigators have not validated their IHC assays against the "gold standard" of a biochemical assay for intra-tumoral aromatase activity. Our group has recently developed and characterized a monoclonal antibody against aromatase. The antibody has been utilized in IHC studies which demonstrated positive correlations between aromatase IHC scores and intra-tumoral aromatase activity [9] and aromatase mRNA expression measurements [10] in breast cancer specimens.

In this investigation we applied the aromatase IHC assay to formalin-fixed paraffin-embedded biopsy samples accrued from patients enrolled onto the P024 neoadjuvant endocrine therapy study, a Phase III double blind randomized trial that compared four months neoadjuvant tamoxifen with an equivalent period of letrozole treatment [11–13]. The design of this study provided a valuable opportunity to evaluate simultaneously the relationship between tumor aromatase expression and response to neoadjuvant endocrine therapy as well as the long-term outcomes for patients receiving adjuvant tamoxifen treatment.

## Methods

### Study population and tumor bank

The P024 protocol compared four months neoadjuvant letrozole with tamoxifen in post-menopausal women with clinical stage II and III hormone receptor positive (classified as at least 10% nuclear staining for ER and/or PgR) breast cancers that were ineligible for breast conservative surgery [11]. The tumor bank characteristics, ER and Ki67 measurements have been described previously [12, 13]. Tumor grade, tumor histological subtype, pathological staging information and long-term outcomes were collated from case report forms. The long-term outcomes and the development of the preoperative endocrine prognostic index (PEPI) based on pathological stage, and the ER status and Ki67 expression level of the surgical specimen has also been published [14].

### Aromatase immunohistochemistry

The aromatase monoclonal antibody #677 was raised against native recombinant human aromatase protein. Details of its characterization and utilization for IHC have been previously reported [9]. Tissue sections were immunostained by a biotin-streptavidin method using a Histofine kit (Nichirei Co. Ltd., Tokyo, Japan). The antigen-antibody complex was visualized with 3.3′-diaminobenzidine solution (DAB) and counterstained with hematoxylin. Evaluation of aromatase IHC was performed by assessing the approximate percentage of cells staining (proportion score) and classifying the level into four groups: 0 = <1%, 1 = 1–25%, 2 = 26–50%, and 3 = >50% immuno-positive cells. The relative intensity of aromatase immune-positive cells was classified as follows: 0 = no immunoreactivity, 1 = weak, 2 = moderate and 3 = intense immunoreactivity. When aromatase immunoreactivity was evaluated as a semi-continuous variable, a total score was applied that was composed of the proportion score + relative immunointensity score (SIP score). For contingency table analysis, aromatase staining was classified as any staining present versus absent staining. Immunohistochemical staining patterns of normal ducts, stromal cells, adipose cells and carcinoma cells were evaluated separately.

### Statistics

All $P$ values reported were two sided; $P \leq 0.05$ were considered to be statistically significant. There was no adjustment for multiple testing. The median and interquartile range of the aromatase SIP score was calculated to show the distribution of scores. Kendall's rank correlation coefficients were used to assess relationship between aromatase SIP values and Ki67, ER, and tumor size since aromatase SIP values were ordinal variables and not normal distributed. Fisher's exact and Chi squared tests were used to define associations between aromatase expression status and clinical and cell cycle responses. The nonparametric Mann–Whitney test was applied to compare differences in Ki67 changes between aromatase expression positive and aromatase expression negative tumors. The 95% confidence interval of the geometric Ki67 mean was calculated to show the size of effects in pair-wise comparisons. Relapse-free survival (RFS) was defined as the interval between randomization and the earliest subsequent breast cancer event (all local or systemic recurrences, there were no new breast primaries recorded in this data set). Breast cancer-specific survival (BCSS) was defined as the

interval between randomization and the date of death after breast cancer relapse. For univariable analysis, survival curves were estimated by the Kaplan–Meier product-limit method, with a two-sided log-rank to assess statistically significant differences. We subsequently applied a multivariate Cox proportional hazards regression model to evaluate the independent prognostic relevance of aromatase expression within the context of other independently prognostic variables that were obtained upon analysis of the surgical specimen obtained after completion of neoadjuvant endocrine therapy: i.e. pathological tumor size, lymph node status, ER and Ki67 levels [14]. The REMARK analysis for the multivariable analysis has also been reported [14]. All statistical analyses were performed using SAS 9.1.2 (SAS Institute Inc., Cary NC USA).

## Results

### Aromatase expression and correlation with baseline pathological and clinical variables

Initially four cellular components were scored for aromatase expression (fibroblast cells, adipose cells, benign breast duct cells and invasive cancer cells). However benign ducts and adipose tissue were very inconsistently present in the slides available. Thus, only stromal cell scores and invasive cancer cell scores could be adequately studied in terms of correlations with clinical parameters. Ultimately aromatase analysis was conducted on 197 cases in which central analysis confirmed ER+ status and 23 cases in which the ER status was known to be ER negative in the central laboratory (with a cut point of Allred score of 0 or 2 as the definition of negative). Of these 197 ER+ cases, 192 (96 on letrozole, 96 on tamoxifen) had sufficient tumor cells on specimens to qualify for the analysis presented in this report. Aromatase expression SIP score in the stomal cell and tumor cell compartments were highly correlated (Kendall's Tau 0.46, $P = 0.0001$, Fig. 1a) Tumor cell aromatase SIP score was positively correlated with ER levels as a continuous score (Kendall's Tau $P = 0.006$, Fig. 1b), however there was no significant correlation with progesterone receptor (PgR) level (data not shown). Finally the aromatase SIP score in the cancer compartment was inversely associated with Ki67 level (Kendall's Tau $P = 0.003$ Fig. 1c). To examine correlations between aromatase expression and dichotomized clinical variables the aromatase staining score was reduced to simple present or absent categories. Of the variables examined, both stroma and tumor epithelial aromatase expression were associated with smaller clinical tumor size at baseline and ER positive status as a dichotomous variable (Allred 0–2 vs. Allred 3–8) but aromatase status (present vs. absent) did not interact with the other factors examined (patient age, tumor grade, lymph node status, PgR and HER2 status) (Table 1).

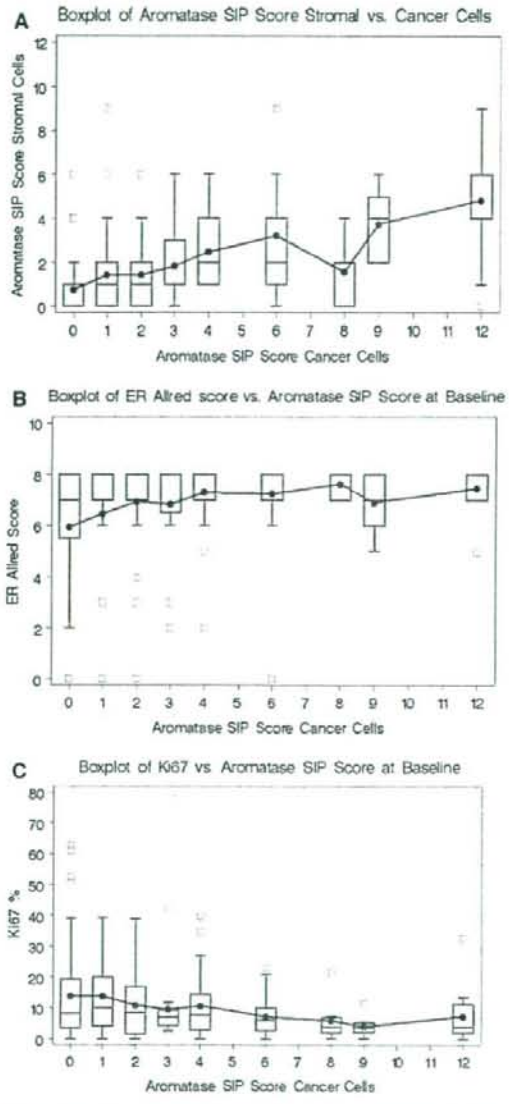


**Fig. 1** Correlations between the site of aromatase expression, ER and Ki67 as semi-continuous variables. Box plots comparing the distributions of aromatase SIP scores in stromal cells and cancer cells (**a**), aromatase SIP scores in cancer cells and ER Allred-scores (**b**) and Ki67 percentage and aromatase SIP scores in cancer cells (**c**) at baseline. The large boxes stretch from the 25th to 75th percentile, the lines crossing the boxes are medians, the dots are means and the small boxes are outliers.

**Table 1** Patients and tumors characteristics by location of aromatase protein expression status at baseline

| Characteristics | Aromatase protein expression [*n* (%)][b] | | | |
|---|---|---|---|---|
| | Tumor epithelial aromatase | | Stromal aromatase | |
| | Negative | Positive | Negative | Positive |
| Treatment | | | | |
| Tamoxifen | 24 (53%) | 72 (49%) | 29 (54) | 63 (48) |
| Letrozole | 21 (47%) | 75 (51%) | 25 (46) | 69 (52) |
| *P*-value | 0.6102 | | 0.4605 | |
| Age (year) | 66.8 | 67.6 | 67.6 | 67 |
| *P*-value[c] | 0.6214 | | 0.6969 | |
| Clinical tumor size (cm) | 5.7 | 4.8 | 5.5 | 4.7 |
| *P*-value[c] | 0.0144 | | 0.0398 | |
| Pre treatment grade | | | | |
| I | 4 (10%) | 16 (13%) | 4 (8%) | 16 (15%) |
| II/III | 38 (90%) | 103 (87%) | 47 (92%) | 90 (85%) |
| *P*-value | 0.5971 | | 0.3064 | |
| Pathological tumor size | | | | |
| ≤20 mm | 11 (27%) | 44 (32%) | 11 (27%) | 44 (32%) |
| >20 mm | 30 (73%) | 95 (68%) | 30 (73%) | 95 (68%) |
| *P*-value | 0.5567 | | 0.5567 | |
| Pathological node status | | | | |
| Negative | 16 (41%) | 55 (43%) | 21 (47%) | 50 (43%) |
| Positive | 23 (59%) | 72 (57%) | 24 (53%) | 65 (57%) |
| *P*-value | 0.8017 | | 0.7160 | |
| HER2 status[a] | | | | |
| Negative | 40 (91%) | 140 (95%) | 50 (93%) | 125 (95%) |
| Positive | 4 (9%) | 7 (5%) | 4 (7%) | 6 (5%) |
| *P*-value | 0.2806 | | 0.4810 | |
| ER status[c] | | | | |
| Negative | 11 (20%) | 12 (8%) | 14 (20%) | 9 (6%) |
| Positive | 44 (80%) | 148 (93%) | 55 (80%) | 131 (94%) |
| *P*-value | 0.0098 | | 0.0027 | |
| PgR status[c] | | | | |
| Negative | 16 (36%) | 48 (33%) | 20 (37%) | 43 (33%) |
| Positive | 28 (64%) | 98 (67%) | 34 (63%) | 87 (67%) |
| *P*-value | 0.6688 | | 0.6072 | |

[a] HER2 IHC with fluorescence in situ hybridization confirmation and IHC for ER and PgR were performed as previously described [12]

[b] Aromatase protein expression considered positive if any aromatase IHC staining was present

[c] For age and clinical tumor size the student's *t* test was used to compare the aromatase positive and negative groups. For binary variables the $X^2$ test was applied with Fisher's exact test if a count in any cell was less than 5

### Aromatase expression and clinical or radiological response to neoadjuvant letrozole or tamoxifen

A series of contingency tables were examined to identify interactions between aromatase expression status and response (Table 2). In the P024 study, response was recorded according to clinical measurements, ultrasound and mammography. There was no evidence of interactions with any of the response definitions, whether the stroma or the tumor cell aromatase status was examined as the interacting factor or whether letrozole or tamoxifen treated cases were considered separately. Consistent with a lack of an influence on endocrine therapy responsiveness, there was no interaction with treatment-induced changes in Ki67 or absolute post-treatment Ki67 levels in either tamoxifen or letrozole-treated tumor samples (Table 3).

### Aromatase expression and relapse-free survival and breast cancer-specific survival

Although there was no association with neoadjuvant response or Ki67 changes, the baseline interactions between aromatase expression, higher ER levels and lower Ki67 levels suggested the possibility that aromatase expression could be a favorable prognostic biomarker for patients undergoing adjuvant endocrine therapy. We therefore examined the impact of aromatase expression on RFS and BCSS (Fig. 2). Tumor aromatase expression was

**Table 2** Analysis of clinical, mammogram and ultrasound response data according to aromatase protein expression status in tamoxifen or letrozole treated patients

| Responses | Aromatase protein expression [*n* (%)] | | | |
|---|---|---|---|---|
| | Tumor epithelial aromatase | | Stromal aromatase | |
| | Negative | Positive | Negative | Positive |
| *Clinical response*[a] | | | | |
| Letrozole only | | | | |
| No | 6 (29%) | 24 (32%) | 11 (44%) | 18(26) |
| Yes | 15 (71%) | 51 (68%) | 14 (56%) | 51(74) |
| *P*-value | 0.7657 | | 0.0984 | |
| Tamoxifen only | | | | |
| No | 12 (50%) | 37 (51%) | 13 (45%) | 34 (54%) |
| Yes | 12 (50%) | 35 (49%) | 16 (55%) | 29 (46%) |
| *P*-value | 0.9067 | | 0.4177 | |
| Fused | | | | |
| No | 18 (40%) | 61 (41%) | 24 (44%) | 52 (39%) |
| Yes | 27 (60%) | 86 (59%) | 30 (56%) | 80 (61%) |
| *P*-value | 0.8587 | | 0.5259 | |
| *Mammo response*[a] | | | | |
| Letrozole only | | | | |
| No | 13 (62%) | 47 (63%) | 17 (68%) | 42 (61%) |
| Yes | 8 (38%) | 28 (37%) | 8 (32%) | 27 (39%) |
| *P*-value | 0.9494 | | 0.5297 | |
| Tamoxifen only | | | | |
| No | 19 (79%) | 54 (75%) | 23 (79%) | 48 (76%) |
| Yes | 5 (21%) | 18 (25%) | 6 (21%) | 15 (24%) |
| *P*-value | 0.6803 | | 0.7418 | |
| Fused | | | | |
| No | 32 (71%) | 101 (69%) | 40 (74%) | 90 (68%) |
| Yes | 13 (29%) | 46 (31%) | 14 (26%) | 42 (32%) |
| *P*-value | 0.7604 | | 0.4278 | |
| *Ultrasound response*[a] | | | | |
| Letrozole only | | | | |
| No | 12 (67%) | 39 (56%) | 16 (73%) | 34 (52%) |
| Yes | 6 (33%) | 31 (44%) | 6 (27%) | 31 (48%) |
| *P*-value | 0.4038 | | 0.0959 | |
| Tamoxifen only | | | | |
| No | 13 (68%) | 44 (64%) | 15 (60%) | 41 (69%) |
| Yes | 6 (32%) | 25 (36%) | 10 (60%) | 18 (31%) |
| *P*-value | 0.7085 | | 0.4016 | |
| Fused | | | | |
| No | 25 (68%) | 83 (60%) | 31 (66%) | 75 (60%) |
| Yes | 12 (32%) | 56 (40%) | 16 (34%) | 49 (40%) |
| *P*-value | 0.3845 | | 0.5116 | |

Response rate refers to the percentage of patients with a complete or partial response

[a] Response definitions by WHO criteria have been previously reported [11]. The $X^2$ test was applied with Fisher's exact test if a count in any cell was less than 5

confirmed to have a modest association with a more favorable disease course, with fewer relapse events over time and a significant univariable log rank test $P = 0.04$ (Fig. 2a) and more prolonged breast cancer survival (Fig. 2b $P = 0.01$). To determine the independence of baseline aromatase expression as a prognostic marker in our established multivariable models based on the post-treatment surgical sample, the baseline aromatase status was analyzed in the context of the preoperative endocrine relapse index (PEPI) (Table 4) [14]. In the PEPI model pathologic tumor size (T1/2 vs. T3/4), pathological nodal status (negative vs. positive), Ki67 per natural log interval and ER status post therapy (Allred 0–2 vs. Allred 3–8) have been found to be independent factors for RFS and BCSS [14]. When tumor aromatase status was entered into a multivariable Cox model containing these four factors, the presence of aromatase expression in the baseline specimen behaved as an independent favorable prognostic biomarker for both RFS ($P = 0.01$, HR 2.3 95% 1.2–4.6 for absent expression) (Table 4A) and BCSS ($P = 0.008$, HR 3.76 95% CI 1.4–10.0 for absent expression) (Table 4B).

## Discussion

The clinical significance of intra-tumoral estrogen production has been debated ever since the phenomenon was first documented by Miller et al., in 1974 [15] through the detection of the conversion of radio-labeled androgen to estradiol within breast cancers in vitro. This potential exists in about 60–70% of breast cancers [1–3]. Subsequently infusion studies with radioactive androgens showed that estrogen biosynthesis occurred in situ within the breast [16, 17] and the presence of mRNA for aromatase, the key enzyme in estrogen production, was also demonstrated in breast cancers and adipose tissue [8]. Because aromatase is the last step in the biosynthetic pathway for estradiol, the enzyme has become a critical target for pharmacological inhibitors that achieve endocrine deprivation for post-menopausal patients requiring endocrine treatment for ER+ breast cancer. Consequently third-generation aromatase inhibitors have evolved as the new standard of care for breast cancer treatment for all stages of the disease. It was therefore logical to address the possibility that the presence of aromatase within breast cancers is associated with a particular requirement for estrogen for growth and therefore whether aromatase expressing tumors are more likely to respond to endocrine therapy in general, and to aromatase inhibitors in particular.

The number of studies examining these relationships is few, have utilized small numbers of tumors and come to limited (often conflicting) conclusions [4, 18, 19]. The