Aromatase Inhibitors as Potential Cancer Chemopreventives


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Abstract

Epidemiological and experimental evidence strongly support a role for estrogens in the development and growth of breast tumors. A role for estrogen in prostate neoplasia has also been postulated. Therefore, one chemopreventive strategy for breast and prostate cancers is to decrease estrogen production. This can be accomplished by inhibiting aromatase, the enzyme that catalyzes the final, rate-limiting step in estrogen biosynthesis. The use of aromatase inhibitors is of clinical interest for cancer therapy, and selective, potent aromatase inhibitors have been developed. Several of these agents have demonstrated chemopreventive efficacy in animal models.

The rationale for the use of aromatase inhibitors as chemopreventives and identification of inhibitors to serve as potential chemopreventive agents are the subjects of this review. After background information regarding aromatase is presented, the data for each inhibitor are summarized separately. The discussion focuses on those inhibitors that are clinically available or in clinical trials, including: aminoglutethimide (Cytadren), roglelinide, fadrozole hydrochloride, liarozole hydrochloride, anastrozole (Arimidex), letrozole, vorozole, formestane, exemestane, and tamoxifen. On the basis of results from preclinical studies, aromatase inhibitors may be promising agents for clinical trials in populations at high risk for developing estrogen-dependent cancers.

Total suppression of aromatase may have adverse effects, as is evident in postmenopausal women (increased osteoporosis, cardiovascular disease, and urogenital atrophy). However, on the basis of preclinical studies of chemopreventive efficacy and chemotherapeutic applications of aromatase inhibitors showing dose-response efficacy, it may be possible to obtain chemopreventive effects without total suppression of aromatase and circulating estrogen levels. Suppressing local estrogen production may be an alternative strategy, as suggested by the discovery of a unique transcriptional promoter of aromatase gene expression, I.4, in breast adipose tissue. The development of drugs that target this promoter region may be possible.

Strategies in Development of Cancer Chemopreventive Agents

This paper is the third in a series on strategies used by the Chemoprevention Branch of the National Cancer Institute to develop cancer chemoprevention drugs (1–3). One chemopreventive strategy for hormone-dependent cancers is to interfere with the hormones that stimulate cellular proliferation in these tumors. Among the most important of these targets for intervention are estrogen-responsive tumors. Estrogen production can be decreased by inhibiting aromatase, the enzyme catalyzing the final, rate-limiting step in estrogen biosynthesis. The use of aromatase inhibitors is of clinical interest for cancer therapy, and selective, potent aromatase inhibitors have been developed. The rationale for use of aromatase inhibitors as chemopreventives and identification of inhibitors to serve as potential cancer chemopreventive agents are the subjects of this review.

Association of Estrogen with Carcinogenesis

Breast. Aromatase, the enzyme that catalyzes the rate-limiting step in estrogen formation (4), is expressed in several tissues in women. In premenopausal women, the granulosa cells of ovarian follicles produce the majority of circulating estrogen, primarily in the form of estradiol. Estrogen is also produced extragonadally in liver, muscle, and fat by aromatization of adrenal androgens. After menopause, adipose tissue is the major source of circulating estrogens (5). Extragonadal production of estrogen primarily involves aromatization of adrenal androstenedione, resulting in estrone, a weaker estrogen than estradiol (6). Epidemiological evidence strongly supports a role for estrogens in the development and growth of breast neoplasia. The most consistently documented epidemiological risk factors for breast cancer, early age at menarche, late age at menopause, late age at first full-term pregnancy, and postmenopausal weight gain, all increase cumulative endogenous estrogen exposure (7–9). Experimental evidence also strongly favors a role for estrogens in the development and growth of breast cancers (10, 11). Estrogens promote the development of mammary cancer in rodents and exert both direct and indirect proliferative effects on cultured breast cancer cells (11). Induction of enzymes and proteins involved in nucleic acid synthesis (e.g., DNA polymerase and thymidine kinase) and oncogenes may account for their direct mitogenic effects. Indirect effects of these hormones also occur via induction of pituitary prolactin secretion and expression of various growth factors (e.g., transforming growth factor α and epidermal growth factor) and non-growth factor peptides (e.g., plasminogen activators). It has been estimated that 30% of breast cancers are dependent on estrogen for their proliferation (12, 13). Although the available

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data are not conclusive, there is also evidence that estrogens may be directly genotoxic, for example, by DNA alklylation or oxidation, leading to free radicals, which, in turn, bind to and damage DNA (e.g., Ref. 14).

The estrogen that stimulates tumor growth can be derived from extratumoral or tumor sources, and the relative importance of each is controversial. Within the breast, adipose tissue is the major extratumoral source of aromatase, although aromatase was also detected immunocytochemically in normal breast epithelial cells in one study (15). Aromatase activity is higher in adipose tissue from breast cancer patients than it is from those with benign breast disease (16). In breasts with cancer, aromatase expression (17) and activity (18) are higher in quadrants bearing tumors compared with those without tumors. The exact cellular localization of aromatase expression in breast cancer tissue is also somewhat controversial. Immunocytochemical studies have detected aromatase in breast carcinoma cells (15, 19) and in stromal spindle cells in breast tumors (20). At the present time, it appears that both adipose and breast tumor cells contribute to locally high estrogen production, and determination of their relative importance requires further study.

The effects of estrogens are mediated by the ER. Although only a few studies have been carried out, 100% of precancerous atypical hyperplastic ductal breast lesions that have been studied express ER (21), and ER levels are higher in atypical ductal hyperplasia than they are in normal breast epithelium (22). Changes in ER expression during tumor progression are not well established, but it has been reported that approximately 60% of breast DCISs are ER positive (23). These and other epidemiological and experimental data suggest that all breast cancers are estrogen responsive during some portion of their natural history. Therefore, limiting estrogen exposure should be a feasible chemopreventive approach. Notwithstanding the observation that not all ER-positive breast cancers are responsive to antiestrogen therapy, loss of ER during tumor progression implies that the premalignant stages of the disease may be more sensitive to estrogen deprivation than are later stages, when some tumors have lost estrogen responsiveness.

The prophylactic potential of limiting estrogen exposure has been demonstrated in both clinical and experimental settings. Administration of the ER blocker tamoxifen to women with previous breast cancer significantly decreases the risk of developing a new cancer in the contralateral breast (24). Numerous studies have demonstrated the chemopreventive activity of tamoxifen (e.g., Refs. 25 and 26) and other antiestrogens (27) in experimental breast cancer models. A disadvantage of many antiestrogens is their partial estrogen agonist effects.

A second approach to achieve estrogen deprivation involves direct inhibition of estrogen biosynthesis via aromatase inhibitors. As described above, aromatase inhibitors are clinically available for breast cancer therapy. Support for the feasibility of preventing breast cancer by inhibiting aromatase comes from clinical studies in which aromatase inhibitors. As described above, aromatase inhibitors induce regression of established tumors (e.g., 28, 29) and, more importantly for the purposes of this discussion, prevent cancer development (27, 30, 31).

Prostate. Etiological and risk factors for prostate cancer include age of >50 years, family history, high serum testosterone, high-fat diet, prostatitis, and geographical background (prevalence being highest in the United States, Canada, and northwest Europe; Ref. 32). As for breast cancer, significant risk for prostate cancer appears to be associated with exposure to steroid hormones (i.e., the high serum testosterone and high fat consumption). Besides testosterone, estrogens have also been postulated to play a role in prostate cell proliferation and have been implicated in BPH and prostate cancer. BPH is a nonmalignant enlargement of the prostate due to cellular hyperplasia and hypertrophy of both the epithelial and stromal elements of the gland, whereas prostate cancer involves the epithelial tissue. Studies on the role of estrogen in BPH may be informative about the regulation of prostate cell proliferation, but it should be emphasized that prostatic intraepithelial neoplasia, not BPH, is the most likely precursor of prostate carcinoma (33, 34).

In men, 10–25% of estrogen is synthesized locally in the testes, and 75–90% arises from extraglandular aromatization of testosterone and androstenedione (5, 35). The estrogen:androgen ratio increases with age, presumably due to greater estrogen synthesis, accompanied by unchanged or decreased androgen production. Whether the prostate is a source of estrogen is controversial. Some studies have reported an absence of aromatase in normal prostate (36) and BPH (37). Others have reported aromatase activity in normal prostate and BPH (38) and in both BPH and prostate cancer cells (39).

ERs have been found in normal, BPH, and cancerous human prostate tissue (40). Estrogens target the normal prostate stroma, and the involvement of estrogen and stroma-epithelial interaction in BPH has recently been studied (41, 42). In dogs, estrogens synergize with androgens and result in complex stromal and glandular hyperplasia. This effect appears to be due to an estrogen-mediated increase in stromal and epithelial androgen receptor levels (43), although the induction of BPH as a result of injury by estrogen metabolites, followed by 5α-dihydrotestosterone-stimulated growth of altered prostatic cells, has also been postulated (44). Increased levels of estrogen have been found in BPH stroma compared with BPH epithelium and normal prostate epithelium and stroma (45). In both normal prostate and BPH, stromal estrogen levels increase with age, resulting in an increased estrogen:androgen ratio. These results suggest that estrogen is involved in the development of BPH, but the clinical trials of aromatase inhibitors in BPH patients have not been encouraging (41, 46).

Estrogen may also play a role in prostate cancer, as suggested by studies involving rat models. In the Noble rat, prostate dysplasia can be induced by simultaneous treatment with testosterone and estradiol for 16 weeks but not by treatment with testosterone or 5α-dihydrotestosterone alone (47). Long-term treatment of Noble rats with testosterone induces a low incidence of adenocarcinomas in the dorsolateral prostate (48), whereas treatment with testosterone and estrogen significantly increases carcinoma incidence and decreases tumor latency (49, 50). The mechanism of estrogen action on the rat prostate is unknown. No effect of estrogen on prostate androgen receptor levels was observed in estradiol- and testosterone-treated Wistar rats (51). In Wistar rats, estrogen treatment increased nuclear 5α-reductase activity, although microsomal reductase activity was decreased (52). Treatment of Noble rats with

2 The abbreviations used are: ER, estrogen receptor; DCIS, ductal carcinoma in situ; BPH, benign prostatic hyperplasia; AG, aminoglutethimide; 4-OHA, 4-hydroxyandrost-4-ene-3,17-dione; DMBA, 7,12-dimethylbenz(a)anthracene; MNU, N-methyl-N-nitrosourea; FDA, Food and Drug Administration; CNS, central nervous system; PMSG, pregnant mare’s serum gonadotropin; ACTH, adrenocorticotropin; FSH, follicle-stimulating hormone; LH, luteinizing hormone.
estradiol and testosterone was shown to result in a unique DNA adduct in the dorsolateral prostate (the tissue where carcinomas originate in this model), coincident with the appearance of putative preneoplastic lesions, but the structure and mechanism of its formation are unknown (53). This adduct may play a role in carcinogenesis in this model. Interestingly, Spencer et al. (54, 55) also identified DNA adducts in normal human prostate and prostate tumor biopsies. The role of estrogen in human prostate cancer has not yet been fully elucidated. Estradiol has been shown to stimulate the growth of an androgen-responsive human prostate cancer cell line (LNCaP) and to inhibit the growth of an androgen nonresponsive line (PC3) in vitro (56). Treatment with an aromatase inhibitor resulted in pain relief in some patients with prostate cancer; however, there was no correlation between clinical response and estradiol levels (57).

Aromatase Activity and Regulation

Aromatase is an enzyme complex that is localized in the endoplasmic reticulum and consists of a specific cytochrome P450 heme protein and a flavoprotein NADPH cytochrome P450 reductase. It catalyzes the synthesis of estradiol from testosterone and estrone from androstenedione. Three separate hydroxylation steps are catalyzed by the enzyme, which requires 3 mol of molecular oxygen for the conversion of 1 mol of C19 androgen to C18 estrogen (6). Importantly, because aromatization is the last step in steroid biosynthesis, selective inhibition of the enzyme should not disrupt production of other steroids such as adrenal corticoids (Fig. 1).

Although the translated region of the aromatase gene is identical among different tissues, the untranslated regions and regulatory control appear to be tissue specific. At least four major promoter sites have been identified that respond to gonadotropins, glucocorticoids, growth factors, and cytokines. A unique transcriptional promoter of aromatase gene expression, I.4, has been identified in breast adipose tissue (6, 17).

Aromatase Inhibitors

Both nonsteroidal and steroidal aromatase inhibitors have been developed, and the characteristics of each class have been reviewed (58–68). Nonsteroidal inhibitors act by binding to a prosthetic heme group on the enzyme. However, because this heme group is present on all members of the cytochrome P450 superfamily, these inhibitors may lack specificity and, thus, inactivate other steroidogenic enzymes. Unlike steroid inhibitors, nonsteroidal inhibitors lack hormonal agonist or antagonist activity and are more likely to be p.o. absorbed. Structures of the nonsteroidal inhibitors discussed below appear in Figs. 2 (AG, rogletimide, fadrozole, and liarozole) and 3 (anastrozole, letrozole, and vorozole).

Steroidal inhibitors bind either very tightly or irreversibly to the active site of the enzyme and are so-called “mechanism-based” or “suicide” inhibitors; that is, these inhibitors compete with androstenedione and testosterone for the active site of aromatase and are then converted to reactive alkylating species by the enzyme, which form covalent bonds at or near the active site, thereby irreversibly inactivating aromatase. Recovery of
enzymatic activity depends on the rate of de novo enzyme synthesis. The potential advantages of using suicide inhibitors include potent and sustained inhibitory activity, with the possibility of intermittent dosing schedules. Disadvantages include unwanted hormonal agonist (particularly androgen) or antagonist effects. Structures of some of the steroidal inhibitors discussed below appear in Fig. 4 (4-OHA, exemestane, atamestane, and plomestane).

**Representative Agents**

Data pertinent to the potential development of these aromatase inhibitors as chemopreventive drugs are summarized in Table 1.

**Nonsteroidal Inhibitors**

**AG.** AG [Cytadren, 3-(4-aminophenyl)-3-ethyl-2,6-piperidinedione] is structurally related to phenobarbital and was originally developed as an anticonvulsant. AG was the first aromatase inhibitor used clinically. It is available from Ciba-Geigy and is approved for use in breast cancer therapy. AG has demonstrated chemopreventive efficacy in rat models for breast cancer. Administration of diets containing AG decreased DMBA-induced mammary tumor incidence and multiplicity in Holtzman rats (30). Here, tumor incidence was decreased from 70% to 28% and 16% with 0.05% and 0.1% AG in the diet, respectively. Multiplicity was decreased from 3.4 tumors/rat to 2.3 tumors/rat and 2.0 tumors/rat, respectively. In a study sponsored by the Chemoprevention Branch of the National Cancer Institute, a diet containing 400 mg/kg AG also decreased mammary tumor multiplicity from 10.6 tumors/rat to 5.5 tumors/rat and increased latency 30 days in MNU-treated Sprague-Dawley rats (27). The efficacy in the latter study was accomplished in the presence of increased androgen activity, as reflected in a significantly increased body weight in rats treated with AG.

AG is currently approved for the treatment of postmenopausal breast cancer, and objective responses in approximately 33% of patients have been reported (58). However, it also inhibits a number of other steroidogenic cytochrome P450-dependent enzymes, including cholesterol side-chain cleavage enzymes, 11β-hydroxylase, 18-hydroxylase, and 21-hydroxylase, necessitating glucocorticoid replacement therapy. Furthermore, it has significant toxicities, especially CNS effects. Numerous efforts to optimize AG activity toward aromatase while reducing inhibition of other enzymes and diminishing toxic effects have been attempted, including elongation of the ethyl substituent and replacement of the ethyl group by a cycloalkyl moiety (58, 65). Although these modifications improved aromatase inhibitory activity, the AG derivatives are still not very potent compared to other compounds discussed below (58).

**Rogletimide.** Rogletimide [pyridoglutethimide; 3-ethyl-3-(4-pyridyl)piperidine-2,6-dione] is an AG analogue that strongly inhibits aromatase ($K_i = 1.1 \mu M$) but is less potent than AG ($K_i = 0.6 \mu M$; Ref. 66). However, rogletimide is more specific and does not block cholesterol side-chain cleavage. It also has fewer adverse CNS effects. The administration of 50 mg/kg/day rogletimide to rats previously treated with DMBA prevented testosterone-induced increases in mammary tumor size (67).

Several clinical studies in postmenopausal breast cancer patients have been reported. Doses of 200-1200 mg b.i.d. significantly suppressed estradiol levels without having effects on
serum levels of cortisol (68). Rogletimide produced dose-dependent aromatase inhibition, but even at the maximum tolerated dose of 800 mg b.i.d., it was not as effective as AG (69). Side effects of roglitremide include those normally associated with estrogen deprivation: gastrointestinal symptoms, hot flushes, dizziness, and lethargy (70).

Fadrozole. Fadrozole [CGS 16949A; 4-(5,6,7,8-tetrahydromidazol-1,5-yl)benzimidazole], an imidazole, is a potent, competitive aromatase inhibitor being developed by Novartis (Basel, Switzerland). In vitro, fadrozole demonstrated an IC₅₀  of 4.5 nM toward human placental aromatase (71). In MCF-7 cells, fadrozole inhibited aromatase activity and prevented tes-
tosterone-induced MCF-7 growth (72). p.o. administration of 0.260 mg/kg inhibited estrogen synthesis by >90% in immature rats stimulated with PMSG (71).

Fadrozole has also demonstrated chemopreventive activity. In intact Sprague-Dawley rats bearing DMBA-induced mammary tumors, p.o. administration of 2.0 mg/kg/day fadrozole completely suppressed the appearance of new tumors (78). In a 2-year study, treatment of Sprague-Dawley rats with 1.25 mg/kg/day fadrozole completely prevented the appearance of benign and malignant spontaneous mammary neoplasms (73). At 0.25 mg/kg/day, no malignant mammary tumors and a decreased number of benign tumors were seen, whereas at 0.05 mg/kg/day, the incidences of malignant and benign mammary tumors were decreased by 50%.

Therapeutic effects of fadrozole have been demonstrated in preclinical models and in clinical trials, p.o. administration of fadrozole inhibited the growth of established DMBA-induced mammary tumors (28, 74, 75) in Sprague-Dawley rats. The combination of fadrozole and tamoxifen resulted in significantly greater tumor regression than did either treatment alone in intact Sprague-Dawley rats (76). Several Phase I trials have been conducted in postmenopausal breast cancer patients, and a dose of 2 mg b.i.d. resulted in >90% aromatase inhibition (77, 78). In a multiple-dose study, p.o. administration of 0.6–8.0 mg b.i.d. rapidly suppressed blood and urine estrogen levels, but adrenal and cortical responses were also diminished at the highest dose levels (79). Two studies have suggested 1 mg b.i.d. as an optimal dose (80, 81). The main side effects of fadrozole are nausea, hot flushes, and somnolence (82). Fadrozole is currently in Phase III clinical trials as second-line endocrine therapy in postmenopausal breast cancer patients (82).

**Liarozole Hydrochloride.** Liarozole hydrochloride [R75521, (+/-)-1-5-(m-chloro-α-imidazolyl-1-ylbenzyl)benzimidazole monohydrochloride], an imidazole derivative developed by the Janssen Research Foundation (Beerse, Belgium), is a potent inhibitor of aromatase activity in human placental microsomes and rat granulosa cells in vitro (83). However, it also inhibits other cytochrome P450 enzymes and decreases androgen, progesterone, and cortisol synthesis in vitro. Several studies have investigated the effects of liarozole on the growth of prostate carcinomas in rats. Dietary administration of 80–160 mg/kg liarozole inhibited growth of slow-growing, well-differentiated, androgen-dependent Dunning-H tumors (84, 85), as well as androgen-independent prostate cancers (84, 85).

Liarozole is currently in clinical trials for the treatment of advanced prostate cancer. In male volunteers, administration of a single p.o. dose of 300 mg significantly lowered plasma testosterone and estradiol concentrations for 24 h and blunted the normal cortisol response to ACTH (83). A Phase I dose-escalation trial involving 38 hormone-refractory prostate cancer patients treated with 37.5–300 mg b.i.d. has been conducted. Four patients had a >50% decrease in prostate-specific antigen levels. In patients with measurable soft-tissue disease, two had partial responses, as judged by a >50% decrease in at least one measurable lesion (86). There was no evidence of adrenal insufficiency. Side effects included lethargy, somnolence, and body rash. All patients enrolled in this study were under maximum androgen suppression (orchiectomy or gondadotropin-releasing hormone therapy).

Results from rat model studies, in which liarozole was effective in androgen-independent tumor models, and from the Phase I trial, in which responses were seen in androgen-suppressed patients, have led to the suggestion that some of liarozole’s effects are due to modulation of retinoic acid metabolism. Liarozole has been shown to inhibit cytochrome P450 isozymes that are responsible for retinoic acid catabolism (87), and, in fact, liarozole has been shown to increase endogenous levels of retinoic acid in a number of target tissues. In view of this, it is not surprising that liarozole exerts retinoid-mimetic effects in vivo (88). At the present time, the specific mechanisms responsible for liarozole’s inhibition of prostate tumor growth are unknown and may involve inhibition of aromatase, retinoic acid catabolism, and, in some cases, androgen biosynthesis.

**Anastrozole.** Anastrozole [ICI D1033; ZD1033; α,α,a',α'-tetramethyl-5-(1H-1,2,4-triazole-1-ylmethyl)-1,3-benzenedi-acetonitrile, Arimidex] is a triazole developed by Zeneca (Wilmington, DE), which has been approved by the FDA for treatment of recurrent postmenopausal breast cancer in patients who have failed tamoxifen therapy. In vitro, anastrozole demonstrated an IC₅₀ of 15 nm toward human placental aromatase. In mature rats, the drug is maximally active at p.o. doses of about 0.1 mg/kg and is selective for aromatase.

Several Phase I clinical trials have been conducted in postmenopausal women (some with breast cancer) at p.o. doses of 0.5, 1, 3.5, or 10 mg, once daily, for 8–14 days. Maximal decreases in estradiol levels, below the detection limit of 3 pm were noted at doses ≥1 mg. Dosing with 0.5 or 1.0 mg for 10 days or 3 mg for 14 days reduced serum estradiol levels to 80% of baseline, with no significant effect on serum gonadotropin concentrations (89). No changes in plasma cortisol, aldosterone, androstenedione, dehydroepiandrosterone sulfate, or ACTH were observed, and plasma cortisol levels increased normally subsequent to ACTH challenge. Mild-to-moderate unspecified adverse effects were observed (89, 90).

Two randomized Phase III trials with 1 and 10 mg of anastrozole (daily) or megestrol acetate (40 mg p.o., four times daily) have been conducted in postmenopausal patients with advanced breast cancer who relapsed after tamoxifen (91, 92). After a median follow-up of 6 months, the response rate was approximately one-third of the patients in each group. Gastrointestinal disturbances, such as nausea, vomiting, and diarrhea, occurred in about 30% of patients. Asthenia, headache, hot flushes, and pain (bone and back) occurred in 10–15% of patients. Because anastrozole had fewer side effects than did megestrol acetate and because the higher dose of 10 mg did not result in additional clinical benefit, 1 mg of anastrozole daily was recommended as a therapeutic dose.

**Letrozole.** Like its analogue, fadrozole, and like anastrozole, letrozole [CGS 20267; 4,4'-((1H-1,2,4-triazol-1-ylmethylene)bis-benzonitrile] is a triazole developed by Novartis as a treatment for postmenopausal breast cancer. Although it has the same affinity for aromatase in vitro as fadrozole, it is reportedly 4,4'-((1H-1,2,4-triazol-1-ylmethylene)bis-benzonitrile] is a triazole developed by Novartis as a treatment for postmenopausal breast cancer. Although it has the same affinity for aromatase in vitro as fadrozole, it is reportedly
79%, respectively; estrogen levels remained below baseline, even at 14 days after this single dose (97). In postmenopausal breast cancer patients, a p.o. doses of 0.1, 0.5, or 2.0 mg/day for 6 weeks, followed by 6 weeks of 0.25, 1.0, or 5.0 mg/day decreased serum estradiol, estrone, and estrone sulfate levels by >95% at all doses by 2 weeks of treatment. Estrogen levels remained depressed throughout the 12-week trial. No effects on basal or ACTH-stimulated levels of cortisol or aldosterone were noted (79, 98). In another p.o. multidose study in a similar patient population (96), 0.1, 0.5, or 2.5 mg q.d. for 28 days significantly suppressed serum estrogen levels, with no concomitant changes in serum FSH, LH, thyroid-stimulating hormone, cortisol, 17α-hydroxyprogesterone, androstenedione, or aldosterone. No hematological or biochemical toxicities were reported in either study. Headache, gastrointestinal disturbances, and hot flushes were the most common side effects noted. In a more recent trial involving postmenopausal women previously treated with endocrine and/or chemotherapy of advanced disease, 0.5-mg daily doses of letrozole decreased plasma estrone and estradiol by >86% and ~67%, respectively (99). Letrozole is currently in Phase III clinical trials (70).

(+)-Vorozole. (+)-Vorozole [R83842; (+)-6-((4-chlorophenyl)-1H-1,2,4-triazole-1-$\gamma$-l-ethyl)-(1-methyl-1H-benzotriazoles)], also a triazole, is the (+)-enantiomer of the racemic mixture R76713 and is being developed by Janssen Research Foundation. The Chemoprevention Branch is also evaluating it in preclinical studies as a potential drug for chemoprevention. (+)-Vorozole is a potent competitive inhibitor, demonstrating an IC$_{50}$ of 1.4 nm for the inhibition of aromatase in FSH-stimulated rat granulosa cells and a p.o. ED$_{50}$ of 0.0034 mg/kg for the reduction of plasma estradiol levels in PMSG-primed female rats (100).

(+)-Vorozole exhibited highly significant chemopreventive activity in a MNU-induced Sprague-Dawley rat mammary tumor model. In this test, which was sponsored by the Chemoprevention Branch, daily p.o. doses of 2.5 and 5 mg/kg decreased tumor incidence from 100 to 10% and tumor multiplicity from 5 to 0.1 tumors/animal compared with carcinogen-treated controls; the two doses were equally efficacious (31). These results are consistent with those reported by De Coster et al. (101) in the DMBA Sprague-Dawley rat mammary tumor model, in which (+)-vorozole suppressed the appearance of new tumors and reduced growth of established tumors. However, in both of these studies, chemopreventive and antitumor activity was associated with significant increases in body weight, and the MNU-treated animals appeared bulky and heavily muscled. These effects appear to be due to the weak androgenic activity of the agent in intact rats (101). It should be noted that this androgenic activity was only observed in the presence of functional ovaries and not in ovariectomized rats.

More recent studies sponsored by the Chemoprevention Branch showed that lower doses of vorozole were also highly effective. At 80 μg/kg/day p.o., vorozole decreased MNU-induced mammary tumorigenesis by 50%. Additionally, with ≤17 days of vorozole treatment, DNA synthesis decreased profoundly, and apoptosis increased. The latter results suggest that changes in cell proliferation kinetics could be evaluated as surrogate end points in clinical chemoprevention trials of aromatase inhibitors.

Like the other nonsteroidal inhibitors, (+)-vorozole is currently being examined as a therapy for advanced postmenopausal breast cancer (70). Several Phase I clinical studies have been conducted with vorozole racemate (29, 102, 103); Phase II studies with the (+)-enantiomer have also been carried out in postmenopausal breast cancer patients (104, 105). In one randomized double-blind Phase II study, 1 month of treatment at doses of 1, 2.5, or 5 mg, once daily, reduced serum estradiol levels approximately 90%. Significant suppression of serum estrone (52–55%) and estrone sulfate (64–69%) levels was also observed. After an open-label extension of the treatment period, 33% complete or partial responses and 17% disease stabilizations were reported. Side effects were mild, and no changes in serum levels of aldosterone, testosterone, androstenedione, 17α-hydroxyprogesterone, or thyroid-stimulating hormone were noted. A small decrease in cortisol levels was observed at the high dose; however, the significance of this finding is unclear because 17α-hydroxyprogesterone levels did not rise (104). Results obtained in another Phase II trial with (+)-vorozole were similar (105). Increased levels of serum androgens were not observed in clinical studies in postmenopausal women treated with (+)-vorozole (104). Vorozole is one of the few aromatase inhibitors reported to influence estrogen levels in premenopausal women. A single p.o. dose of 20 mg of racemic vorozole (0.97 μmol/kg) significantly reduced plasma estradiol levels >60% after 8 h (29). The ability of vorozole to cause a sustained decrease in estrogen levels in this population is unknown, but it would be interesting to evaluate for chemoprevention.

Steroidal Inhibitors

Formestane. Formestane (CGP 32349, 4-OHA), a mechanism-based irreversible inhibitor, was the first steroidal compound structurally designed as an aromatase inhibitor (63, 106). Formestane has recently been introduced onto the market for the treatment of postmenopausal breast cancer in the United Kingdom and an i.m. formulation is undergoing Phase III clinical trials in North America (70).

Injection of 4-OHA has demonstrated chemopreventive effects in rat mammary tumor models. Doses of 50 mg/week s.c. decreased MNU-induced tumor incidence and increased survival of tumor-bearing Ludwig/Wistar rats (107). In contrast, in another study using MNU-treated Sprague-Dawley rats, daily intragastric doses of 6 or 15 mg/rat did not affect tumor incidence, and only the higher dose slightly decreased tumor multiplicity (31). However, these results may reflect pharmacokinetic problems associated with intragastric administration of 4-OHA. Therapeutic effects of 4-OHA in rat mammary tumor models have been demonstrated (108).

Phase I and Phase II clinical trials of 4-OHA have been carried out, i.m. injections of 250 mg every other week suppressed estradiol levels about 60% throughout the treatment period in most studies (~6 weeks; Refs. 108 and 109). In a randomized Phase II trial of postmenopausal patients with breast cancer, objective responses were obtained in 8 of 24 patients in the 250-mg group and in 13 of 28 patients in the 500-mg group (110). Serum estradiol levels decreased >40% below baseline in both groups after 15 days of treatment and remained unchanged thereafter, with no effects on adrenal steroids during the 6 months of treatment. p.o. administration of 250 mg/day was also effective in this patient population, resulting in a decrease in estradiol levels to 53% of baseline within 7 days (111).

A trial was also conducted in a small group (n = 5) of premenopausal breast cancer patients (112). In this population, estrogen levels were not consistently suppressed by 8 weeks of treatment with the maximally tolerated dose (500 mg/week,

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3 C. J. Grubbs, unpublished data.
i.m.), but treatment with the combination of 4-OHA and the LH-releasing hormone analogue goserelin resulted in striking reductions in estradiol levels and in objective remissions in four of six patients.

4-OHA has also been evaluated in a few patients with relapsed metastatic prostate cancer following orchiectomy (57). It produced pain relief in 18 of 25 patients, but there were no objective responses, and tumor flare was observed in 17 patients. The cause of the tumor flares is unknown, but may be due to exemestane’s weak androgen effects.

Obstacles to the use of formestane as a chemopreventive include administration by i.m. injection and slight androgenic activity upon p.o. administration (113, 114). i.m. injection is the preferred route of administration because of increased efficacy compared with p.o. dosing and because the quantity of steroid necessary for dosing is difficult to formulate into an acceptable tablet or capsule (114).

Exemestane. Exemestane (FCE 24304; 6-methyleneandrost-1,4-diene-3,17-dione) is a specific mechanism-based, irreversible aromatase inhibitor being developed by Pharmacia and Upjohn. It demonstrated an IC_{50} of 43 nm toward human placental aromatase in vitro (115). In female PMSG-stimulated rats, ovarian aromatase was inhibited with ED_{50}s of 3.7 mg/kg and 1.8 mg/kg after single p.o. and s.c. administrations, respectively. In vitro, it did not inhibit desmolase; however, it bound weakly to the androgen receptor, and in orchietomized rats, 3 and 10 mg/kg/day for 7 days s.c. caused a significant increase in prostate weight (115).

In intact Sprague-Dawley rats bearing DMBA-induced mammary tumors, s.c. exemestane (10 or 50 mg/kg/day) inhibited ovarian aromatase, caused tumor regression, and prevented the appearance of new tumors (116). However, p.o. exemestane at doses of 100 and 200 mg/kg/day did not affect tumor growth or development, although it did reduce ovarian aromatase activity. Interestingly, the higher dose of exemestane, which was ineffective therapeutically, did inhibit the outgrowth of new tumors, supporting the view that lower doses than those required for chemotherapy of an aromatase inhibitor may be required for chemoprevention of subpalpable lesions. The significant increase in body weight observed in the groups in which exemestane was efficacious has been suggested to result from the drug’s androgenic activity (116). Using the same animal model, the combination of p.o. tamoxifen (1 mg/kg/day) and s.c. exemestane (20 mg/kg/day) inhibited the appearance of new tumors more effectively than did either agent alone (117). In the combination experiment, body weight was increased by exemestane alone but not by the combination. Tamoxifen alone decreased body weight gain compared with control animals.

Results from a Phase I trial examining the effects of single p.o. doses (0.5–800 mg) in healthy postmenopausal women have been published (115, 118). Maximal suppression of plasma estrone, estradiol, and estrone sulfate to 35, 28, and 39% of basal levels, respectively, was observed with doses ≥25 mg at 3 days; estrone levels were still suppressed 5 days after dosing. An initial increase in plasma estrogen levels observed after doses of ≥200 mg raised the possibility that estrogenic metabolites were formed; nonetheless, by 8 h, serum estrogens decreased at these doses. No significant changes in plasma levels of cortisol, aldosterone, dehydroepiandrosterone sulfate, 17α-hydroxyprogesterone, FSH, or LH were observed, and no clinically significant adverse effects were noted except transient eosinophilia in three patients at doses of ≥200 mg. The significance of increased plasma androstenedione and testosterone levels noted at the 400-mg dose is questionable because of interference from exemestane metabolites with the RIAs used to measure hormone levels. In one European trial, doses of 2.5, 5, 12.5, and 25 mg/day were administered p.o. to 56 postmenopausal patients with advanced breast cancer, previously treated with tamoxifen. All doses tested produced similar reductions in serum estrogen levels (approximately 60%), and the overall objective response rate was 18% (119). Exemestane was well tolerated, with nausea and dyspepsia reported in 16% of patients. Gonadotropin levels were significantly elevated, but no changes in other serum hormone levels and no interference with adrenal synthesis were detected. Thus, exemestane may provide an improved therapeutic index over currently approved steroidal aromatase inhibitors. Additional multidose Phase II trials in postmenopausal women with locally advanced or metastatic breast cancer are underway (70).

Exemestane is also entering a short-term Chemoprevention Branch-sponsored Phase II clinical trial in breast. The cohort for this randomized, double-blinded trial will be approximately 100 (50 per treatment arm) postmenopausal women with a mammogram or breast examination demonstrating an abnormality of ≤4 cm in its greatest dimension and a histopathological diagnosis of either atypical ductal hyperplasia or DCIS. Patients will receive exemestane or placebo for the 1–4-week interval between diagnostic core biopsy and definitive surgery. The objective will be to evaluate exemestane’s modulation of tissue-based and molecular intermediate biomarkers of cancer during the treatment period. Tissue from the diagnostic biopsy and surgery will be compared for changes in severity of the hyperplasia or DCIS, nuclear morphology, DNA ploidy, proliferation (e.g., proliferating cell nuclear antigen, Ki-67, and S-phase fraction), genetic/regulatory effects (e.g., epidermal growth factor receptor expression, p53, apoptosis, and angiogenesis), and evidence of chromosomal damage (e.g., loss of heterozygosity).

Atamestane. Atamestane (SH 489; ZK 95639; 1-methylandrost-1,4-diene-3,17-dione) is being developed by Berlex Laboratories (Wayne, NJ). It is an irreversible inhibitor (120) with an IC_{50} of 20 nm toward human placental aromatase (121). Single s.c. doses of 10 mg/kg atamestane reduced serum estradiol levels in PMSG-primed juvenile rats by about 50% but did not influence aromatase activity in ovarian micromasses isolated from these animals or in adult mammary tumor-bearing rats (116). Atamestane efficacy has been investigated in DMBA-treated female Sprague-Dawley rats. Treatment with 10 or 50 mg/kg/day s.c. did not affect growth of established tumors or influence the appearance of new tumors (116).

In contrast to the other aromatase inhibitors, which were evaluated in treatment of breast cancer, atamestane has been investigated primarily for treatment of BPH (41). In an open-multicenter clinical study, 49 men with obstructive BPH were treated with atamestane 200 mg t.i.d. for 3 months. Serum estrone and estradiol levels were suppressed 69 and 24%, respectively. In prostatic tissue estrone levels were depressed 32%, but intraprostatic estradiol levels remained approximately the same. The drug was well tolerated, and clinical chemistry parameters were normal. Treatment resulted in improvement of BPH-related symptoms and reduced prostate volume (122).

The beneficial effects on BPH were not confirmed in a large controlled multicenter trial with 1200 symptomatic BPH patients treated with p.o. doses of atamestane. Treatment with 25, 100, 300, 400, and 600 mg once daily for up to 12 months resulted in a significant reduction in serum estrogens, and slight increases in serum FSH and LH were found. A slight elevation of serum androgens, which remained within normal physiolog-
ical range, and a dose-dependent but significant increase in the serum androgen:estrogen ratios were also observed. These hormonal changes may, in turn, explain the significant increase in serum prostate-specific antigen levels noted. No changes in overall prostate weight were found, but a clear decrease in the stromal component was noted. Although intraprostatic hormone levels were not measured, these findings, together with the observed serum hormonal changes, suggest stimulation of the activity and/or an increase of the epithelial (glandular) component of the prostate as a result of “androgen dominance.” After 6 and 12 months, no change in peak urinary flow or prostate volume was seen (41).

Similarly, in a study of 160 patients with BPH, treatment with 400 mg daily for 48 weeks decreased mean estradiol and estrone levels by approximately 40 and 60%, respectively, and increased testosterone and dihydrotestosterone by 40 and 30%, respectively (46). However, no effect on clinical parameters was observed, possibly because the increase in androgens counteracts any positive effect of the decrease in estrogens. No clinical studies of atamestane in women were available for review.

**Additional Aromatase Inhibitors**

Several additional aromatase inhibitors are in earlier stages of drug development; these are described briefly below. Plomestane is an inhibitor that has been tested in clinical trials and has demonstrated chemopreventive efficacy in preclinical models, but it is no longer being developed by Hoechst Marion Roussel.

**Nonsteroidal Inhibitors**

ORG 33201. ORG 33201 [(3αR)-trans-1-[(3α-ethyl-9-(ethylthio)-2,3,4,5,6-hexahydro-1H-phenalen-2-yl)(methyl)-1H-imidazole][HCl] 0 was developed by Organon International (Rotterdam, the Netherlands). It is a potent aromatase inhibitor, demonstrating an IC₅₀ of 2.2 nm in vitro toward human placental aromatase, and single p.o. doses of 1 mg/kg decreased plasma estradiol levels in FSH/human chorionic gonadotropin-treated beagle dogs by 70%. Although less potent than fadrozole in the model systems examined, it was more selective and did not demonstrate any additional unwanted hormonal activity (123).

CGP 47645. Structure-activity studies have identified CGP 47645 [4,4’-(fluoro-1H-1,2,4-triazolylmetheny|bis-benzo|nitrile], a fluorinated derivative of letrozole, which is equipotent with letrozole toward aromatase in vitro but is 10 times more active in vivo (124). CGP 47645 induced regression of established mammary tumors in DMBA-treated Sprague-Dawley rats, as well as inhibition of new tumors (94).

**Steroidal Inhibitors**

Minamestane. Minamestane (FCE24928; 4-aminandrosta-1,4,6-triene-3,17-dione) was developed by Pharmacia and Upjohn in an attempt to minimize the androgenic activity of exemestane. It has no inherent androgenic activity (when administered up to 100 mg/kg s.c.), does not bind significantly to androgen or ERs, and is inactive against 5α-reductase or 3α-hydroxysteroid dehydrogenase (125, 126). It is about 12-fold less potent than exemestane (IC₅₀ of 369 nm) toward human placental aromatase in vitro; in PMSG-stimulated rats, ED₅₀ values for inhibition of ovarian aromatase were 1.2 and 14.1 mg/kg after s.c. and p.o. administration, respectively.

Plomestane. Plomestane [MDL 18962, PED, 10-propargylestr-4-ene-3,17-dione] is a potent, irreversible steroidal aromatase inhibitor that was developed initially by Hoechst Marion Roussel; however, the company has stopped production of this compound. Johnston *et al.* (127) reported an IC₅₀ of 4 nm for inhibition of human placental aromatase. In mature rats, ovarian aromatase activity was inhibited in a dose-dependent fashion by 0.1–34 mg s.c.; inhibitory activity ranged from 60% at the lowest to 90% at the highest dose tested (128). A p.o. ED₅₀ value of 18 mg/kg in PMSG-stimulated rats has also been reported (121). Plomestane is an effective inhibitor of peripheral aromatization in baboons after i.v. (ED₅₀ of 0.01 mg/kg) or p.o. (ED₅₀ of <4 mg/kg) administration (129) and, in vitro, is selective for aromatase when compared with other cytochrome P450s (127). Plomestane binds weakly to sex hormone receptors and has weak androgenic activity in preclinical studies (127, 130).

Plomestane has demonstrated chemopreventive efficacy in a study using DMBA-treated female rats, in which it inhibited the appearance of new tumors in a dose-dependent fashion after s.c. injections of 1, 5, or 50 mg/kg/day (131). However, Zaccho *et al.* (116) found no effect in a similar experimental model when 10 or 50 mg/kg/day was administered 6 days/week s.c. for 4 weeks, although ovarian aromatase was inhibited by the high dose. Plomestane has also demonstrated therapeutic efficacy. In animal studies, s.c. doses of ≥1 mg/kg/day caused regression of DMBA-induced mammary tumors in intact rats (128, 131, 132).

Single-dose (0.1–20 mg/kg by p.o. and i.v. administration) Phase I clinical studies have been conducted in normal males. The maximally effective p.o. dose (6.4 mg/kg) decreased serum estradiol and estrone levels approximately 70%; however, this may be an underestimate because of interference with the RIA used to measure estrogen levels. No adverse events or changes in clinical parameters were noted relative to controls (133).

**Assay Systems for Evaluating Aromatase Inhibitors**

Goss and Gwyn (70) have reviewed the model systems used for testing the efficacy of aromatase inhibitors. *In vitro* cell-free studies can be carried out with microsomal aromatase preparations from human placenta or PMSG-stimulated rat ovaries. Tritiated androgens are added in the presence of inhibitor and an NADPH-generating system; estrogen synthesis is measured indirectly by the amount of tritiated water released. Characterization of inhibitory activity as reversible or irreversible can be accomplished by subsequent washing of the microsomal preparation with dextran-coated charcoal, reincubation with androgen precursor, and measurement of residual enzyme inhibition. Mechanism-based irreversible inhibitors exhibit time-dependent inactivation only in the presence of catalytically active enzyme; lack of a cofactor, such as NADPH, prevents inactivation (64).

*In vitro* cell culture systems for screening aromatase inhibitors include the hormone-dependent human breast cancer cell line, MCF-7 (134), and human genital skin fibroblasts (135). Two models using MCF-7 cells that were transfected with the aromatase gene have also been reported (136, 137).

*In vivo* models for screening inhibitors are similar to *in vitro* assays; however, compounds are administered to animals rather than incubated with microsomal preparations. Ovarian microsomes isolated from PMSG-primed female rats treated with the drug are generally used to measure radiolabeled water released after *in vitro* incubation with tritiated androgens (121). Serum estrogen levels of animals treated with aromatase inhibitors can be ascertained using RIAs (116).

Testing in chemoprevention models *in vivo* will be impor-
tant for evaluating this class of compounds. Models for hormone-dependent premenopausal breast cancer include the MNU- and DMBA-induced Sprague-Dawley rat mammary models. Both systems have been used to screen aromatase inhibitors, but MNU-induced tumors are believed to better model human breast cancer (138).

Models for prostate cancer are the testosterone- and estradiol-treated Noble rat (48, 47) and the male Wistar rats that were treated with cyproterone acetate, MNU, and testosterone (139, 140). A third model that is being developed is the Rao model, in which Noble rats are treated with testosterone prior to MNU and then with testosterone and estradiol for the duration of the study. The biology and histology of this new model is less well defined than those of the other two models, but the induction of tumors in accessory sex glands is more rapid. These three prostate models are currently being used in Chemoprevention Branch-sponsored studies.

Discussion and Conclusions
Several aromatase inhibitors that are clinically available or in clinical trials have demonstrated chemopreventive efficacy in preclinical animal models. These include: AG, fadrozole, letrozole, vorozole, 4-OHA, and exemestane. Current clinical testing of these compounds has focused on postmenopausal breast cancer patients, and these agents may also be useful in a preventive setting in this patient population. Aromatase inhibitors may also be useful as chemopreventives for prostate cancer. Atamestanse and liarozole have been tested in clinical trials for BPH and prostate cancer.

Total suppression of aromatase may have adverse effects, as is evident in postmenopausal women. Menopause is associated with several clinical concerns, including osteoporosis, cardiovascular disease, and urogenital atrophy, which are due, at least in part, to decreased estrogen levels (e.g., see Ref. 141). Osteoporosis affects more than 20 million women and is a major cause of morbidity and mortality in postmenopausal women (e.g., see Ref. 142). Estrogen replacement has been shown to decrease the bone loss associated with osteoporosis. The role of estrogen in the prevention of cardiovascular disease is more controversial, but epidemiological studies indicate that estrogen reduces cardiovascular disease risk. The beneficial effects appear to be linked to changes in plasma lipoproteins and vasodilatory effects on coronary arteries (e.g., see Refs. 143 and 144). Controlled clinical studies are underway that should clarify the role of estrogens in cardiovascular disease.

However, it may be possible to obtain chemopreventive effects without total suppression of aromatase and circulating estrogen levels. Results of preclinical studies in animal models have demonstrated a dose-dependent effect of the aromatase inhibitors on estrogen suppression and chemopreventive efficacy and support the view that chemoprevention requires lower levels of the drugs than does chemotherapy. Clinical studies of aromatase inhibitors in cancer therapy have demonstrated that objective antitumor responses can be obtained without total suppression of aromatase.

Suppressing local estrogen production may be an alternative strategy to decreasing exposure of breast tissues to estrogens. A rationale for this strategy has been put forth by Simpson, Bulun, and their colleagues (6, 145), as follows. In breast cancer patients, O’Neill et al. (146) reported increased aromatase activity in the adipose tissue of the quadrant containing the tumor compared with the rest of the breast. Simpson et al. (6, 147) also found aromatase mRNA levels to be highest in tumor-containing breast quadrants, and they (148) have suggested that these results show cross-talk between the tumors and the surrounding adipose tissue to induce aromatase activity. Further, they proposed a model for this interaction (6, 149). This model has the stimulated estrogen synthesis in adipose cells that produce growth factors that stimulate the cancer cells to further growth. The growth factors may, in turn, stimulate estrogen synthesis in the stromal adipose cells. Other recent studies have shown that concentrations of the aromatase substrate androstenedione are higher in tumors than in blood and that tumor aromatase activity in vivo appears to be increased by cytokines and growth factors (150). The feasibility of inhibiting localized aromatization is suggested by the discovery of a unique transcriptional promoter of aromatase gene expression, 1.4, in breast adipose tissue. Development of drugs that target this promoter region may be possible (6, 17). Provided that diminished local estrogen production is sufficient to inhibit breast tumor development, such a targeted approach could significantly reduce neoplastic growth while maintaining the beneficial effects of estrogens produced in other tissues.

The use of first-generation (AG) and second-generation (4-OHA) aromatase inhibitors to reduce estrogen levels in premenopausal patients has been unsuccessful. These drugs are not potent enough to overcome reflex gonadotropin release (151). Because these hormones stimulate ovarian steroidogenesis, the effect of aromatase inhibitors is counteracted, which results in plasma estrogen levels that are similar to pretreatment levels. High levels of LH and FSH may also lead to ovarian hyperstimulation syndrome (70). Because of the early failure of aromatase inhibitors in premenopausal women, only one report on the ability of the newer drugs to act in this capacity has appeared. Single p.o. doses of (+)-vorozole suppressed serum estradiol levels in premenopausal women by >50%, even 24 h after dosing (29). Vorozole’s ability to maintain estrogen suppression for prolonged time periods in this population is unknown. The observation that aromatase inhibitors are able to inhibit tumor development in rats with intact ovarian function, despite increases in serum gonadotropins, lends support to the possible use of aromatase inhibitors as chemopreventive agents in premenopausal women.

Some experimental evidence implies that androgenic activity accompanies tumor inhibition in premenopausal rodent models. In one study comparing the effects of three aromatase inhibitors on mammary tumor suppression was only observed concomitantly with increased body weight and was suggested to result from androgenic activity (116). In the initial Chemoprevention Branch-sponsored studies, in which high doses of (+)-vorozole demonstrated virtually complete chemopreventive activity, increased body weight and masculinity were evident. Subsequent dose-titration studies demonstrated that doses that decreased tumor multiplicity by >50% were accompanied by lower increases in body weight; normal estrus cycles were observed in these animals. Before clinical studies in premenopausal women are initiated, careful dose titration studies may be needed to determine whether chemopreventive activity can be achieved in the absence of androgenic activity in intact rodent tumor models. If a therapeutic window can be established, partial lowering of estrogen levels in premenopausal women at high risk for breast cancer using aromatase inhibitors might be sufficient to prevent hormonal stimulation of precancerous lesion growth, while maintaining the beneficial effects of estrogens.

Aromatase inhibitors may also be useful chemopreventive agents in the prostate, although, as described above, this area requires further study. Studies in the rat have suggested that estrogens play a role in the development of prostate neoplasia because combining estrogen with testosterone is more effective
than testosterone alone in inducing prostate tumors. Limiting estrogen exposure using aromatase inhibitors, particularly in combination with other chemopreventives such as 5α-reductase inhibitors or antiandrogens, may be a useful chemopreventive strategy in the prostate.

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