Use of aromatase inhibitors in breast carcinoma

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Abstract

Aromatase, a cytochrome P-450 enzyme that catalyzes the conversion of androgens to estrogens, is the major mechanism of estrogen synthesis in the post-menopausal woman. We review some of the recent scientific advances which shed light on the biologic significance, physiology, expression and regulation of aromatase in breast tissue. Inhibition of aromatase, the terminal step in estrogen biosynthesis, provides a way of treating hormone-dependent breast cancer in older patients. Aminoglutethimide was the first widely used aromatase inhibitor but had several clinical drawbacks. Newer agents are considerably more selective, more potent, less toxic and easier to use in the clinical setting. This article reviews the clinical data supporting the use of the potent, oral competitive aromatase inhibitors anastrozole, letrozole and vorozole and the irreversible inhibitors 4-OH androstenedione and exemestane. The more potent compounds inhibit both peripheral and intra-tumoral aromatase. We discuss the evidence supporting the notion that aromatase inhibitors lack cross-resistance with antiestrogens and suggest that the newer, more potent compounds may have a particular application in breast cancer treatment in a setting of adaptive hypersensitivity to estrogens. Currently available aromatase inhibitors are safe and effective in the management of hormone-dependent breast cancer in post-menopausal women failing antiestrogen therapy and should now be used before progesterational agents. There is abundant evidence to support testing these compounds as first-line hormonal therapy for metastatic breast cancer as well as part of adjuvant regimens in older patients and quite possibly in chemoprevention trials of breast cancer.

Introduction

Epithelial cells of the normal breast undergo dramatic changes during various events in a woman’s life such as puberty, the follicular and luteal phases of the menstrual cycle, pregnancy and menopause. The co-ordinated interaction of growth factors and steroid hormones regulate the proliferation and differentiated function of epithelial and stromal cells in the normal mammary gland. The key growth factors are insulin-like growth factor-I, prolactin, insulin, the fibroblast growth factor family of growth factors and growth hormone, and major steroid hormones are estradiol, progesterone and testosterone (Frantz & Wilson 1998).

For the process of inducing breast cancer, estrogens appear to play a predominant role. These sex steroids are believed to initiate and promote the process of breast carcinogenesis by enhancing the rate of cell division and reducing time available for DNA repair. An emerging new concept is that estrogens can be metabolized to catecholestrogens and then to quinones which directly damage DNA. These two processes - the estrogen receptor-mediated, genomic effects on proliferation and the receptor-independent, genotoxic effects of estrogen metabolites - can act either in an additive or synergistic fashion to cause breast cancer (Santen et al. 1999).

Breast cancers which arise in patients can be divided into two subtypes: those which are dependent upon hormones for growth and those which grow independently of hormonal stimulation (Santen et al. 1990). In the hormone-dependent subtype, the role of estrogens as modulators of mitogenesis overrides the influence of other factors. These sex steroids stimulate cell proliferation directly by increasing the rate of transcription of early response genes such as c-myc and indirectly through stimulation of growth factors which are produced largely in response to estrogenic regulation (Dickson & Lippman 1995).
Inhibition of estradiol biosynthesis

Multiple strategies could be used to inhibit estradiol biosynthesis as a treatment for estrogen-dependent breast cancer. Inhibition of several enzymes in the steroidogenic pathway, including cholesterol side-chain cleavage, 3 beta-ol-dehydrogenase-delta 4-5 isomerase, 17-alpha hydroxylase, 17-beta hydroxysteroid dehydrogenase, estrone sulfatase, and aromatase, could be used to reduce the biosynthesis of estradiol and potentially cause hormone-dependent breast tumor regression. An additional strategy is the use of exogenous glucocorticoid to inhibit release of adrenocorticotropic hormone (ACTH) and suppress estrogen production. Finally, synthetic progestins such as megestrol acetate and medroxy-progesterone acetate exert glucocorticoid effects and inhibit estradiol synthesis by suppressing ACTH.

The ideal strategy would be to block the synthesis of estrogen without inhibiting production of other important steroids or giving pharmacological amounts of progestins or glucocorticoids. For this reason, blockade of the terminal step in estradiol biosynthesis catalyzed by the enzyme aromatase is considered a more specific and therefore preferable strategy. Several pharmaceutical companies sought to develop potent aromatase inhibitors designed to specifically block estrogen biosynthesis without altering glucocorticoid and mineralocorticoid synthesis, and without requiring addition of large amounts of progestins or exogenous glucocorticoid.

Physiology and regulation of aromatase

Aromatase is a cytochrome P-450 enzyme which catalyzes the rate-limiting step in estrogen biosynthesis, the conversion of androgens to estrogens (Simpson et al. 1997, Sasano & Harada 1998). Two major androgens, androstenedione and testosterone, serve as substrates for aromatase. The aromatase enzyme consists of a complex containing a cytochrome P-450 protein as well as the flavoprotein NADPH cytochrome P-450 reductase (Simpson et al. 1997). The gene coding for the cytochrome P-450 protein (P-450 AROM) exceeds 70 kb and is the largest of the cytochrome P-450 family (Simpson et al. 1993). The cDNA of the aromatase gene contains 3.4 kb and encodes a polypeptide of 530 amino acids with a molecular weight of 55 kDa. Approximately 30% homology exists with other cytochrome P-450 proteins. Because its overall homology to other members of the P-450 superfamily is low, aromatase belongs to a separate gene family designated CYP19.

Recent studies indicate that the transcription of the aromatase gene is highly regulated (Simpson et al. 1989, 1993, 1997). The first exon of the aromatase gene is transcribed into aromatase message but not translated into protein. There exist nine alternative first exons which can initiate the transcription of aromatase. Each of these alternate exons contains upstream DNA sequences which can either enhance or silence the transcription of aromatase. Different tissues utilize specific alternate exons to initiate transcription. For example, the placenta utilizes alternate exon I.1, the testis alternate exon II, adipose tissue I.3 and I.4 and brain II. Enhancers which react with upstream elements of these alternate exons markedly stimulate the rate of transcription of the aromatase gene. Thus, each tissue can regulate the amount of aromatase transcribed in a highly specific manner (Simpson et al. 1993).

Aromatase expression occurs in many organs, including ovary, placenta, hypothalamus, liver, muscle, adipose tissue, and breast cancer itself. Aromatase catalyzes three separate steroid hydroxylations which are involved in the conversion of androstenedione to estrone or testosterone to estradiol. The first two give rise to 19-hydroxy and 19-aldehyde structures and the third, although still controversial, probably also involves the C-19 methyl group with release of formic acid (Fishman & Hahn 1987). This enzymatic action results in the saturation of the A-ring of the steroid molecule to produce an aromatic structure, hence the term aromatization.

In the premenopausal state, the major source of aromatase and of its substrates is the ovary. However, extra-glandular aromatization of adrenal substrates in peripheral sites such as fat, liver and muscle also contributes substantially to the estrogen pool in the early life of the normal woman.
follicular and late luteal phases of the menstrual cycle. In
the postmenopausal state, the ovary loses its complement
of aromatase enzyme although it does continue to secrete
androstenedione. The adrenal subsumes the primary role
of providing substrate for aromatase by directly secreting
testosterone and androstenedione. In addition, dehydro-
epiandrosterone and its sulfate are secreted by the adrenal
and converted into the aromatase substrates, androsten-
dione and testosterone, in peripheral tissues. The
major source of the aromatase enzyme in postmenopausal
women is peripheral tissues and particularly fat and
muscle.

Recent studies identified an additional, important site
of estrogen production, breast tissue itself. Two-thirds of
breast carcinomas contain aromatase and synthesize
biologically significant amounts of estrogen locally in the
tumor (Abul-Hajj et al. 1979, Miller & O’Neil 1987,
Santen et al. 1994). Normal breast tissue also
contains aromatase as documented by immunohisto-
chemistry, by demonstration of aromatase message, and
by enzyme assays of cultured cells (Mor et al. 1998,
Brodie et al. 1999).

The biologic relevance of in situ estrogen production
by aromatase has been demonstrated by xenograft
experiments which compare tumors containing and not
containing aromatase (Yue et al. 1998). Human breast
cancer cells transfected permanently with the aromatase
enzyme are compared with cells transfected with
irrelevant DNA. In these experiments, tumors containing
the transfected aromatase enzyme have higher amounts of
estrogen and grow faster than those with transfection of
irrelevant DNA. Further, these experiments show that
local production of estradiol in the tumor is a greater
source of estrogen than uptake from plasma (Yue et al.
1998). Taken together, these studies support the
importance of in situ estrogen production by breast tumors
and suggest that aromatase inhibitors in patients must be
sufficiently potent to block intra-tumoral aromatase.

Breast tumor tissue aromatase can be regulated by
several enhancers of aromatase transcription (Simpson et
Dexamethasone, phorbol esters, cyclic AMP, interleukin 6, and prostaglandins can all stimulate aromatase transcription in cultured breast cancer cells and specifically in the stromal components. Interestingly, products secreted by epithelial cells in the breast tumors appear to stimulate aromatase in the stroma and provide a means for autoregulation of tumor growth through estrogen production. A rather novel means of regulation of aromatase levels was also recently described - the stabilization of degradation of enzyme (Harada et al. 1999). Aromatase inhibitors bind to the active site of the enzyme and, through mechanisms not completely understood, prevent proteolysis of the aromatase protein. Each of these mechanisms may enhance the amount of aromatase in tumor tissue and increase the need for very potent aromatase inhibitors.

**Development of aromatase inhibitors**

The first aromatase inhibitors were discovered nearly 30 years ago and included aminoglutethimide and testolactone (Santen et al. 1990). Testololactone was not very potent as an inhibitor, and aminoglutethimide blocked several P-450-mediated enzymatic reactions and was associated with troublesome side-effects. On the other hand, aminoglutethimide appeared to be quite effective in causing tumor regressions in patients with breast cancer. For this reason, pharmaceutical companies and individual investigators focused upon developing more potent and specific inhibitors. Second and third generation inhibitors were developed with 10- to 10000-fold greater potency than aminoglutethimide and greater specificity (Figs 1 and 2). The half-lives of the inhibitors increased with synthesis of more potent inhibitors. The third generation aromatase inhibitors are capable of decreasing the levels of circulating estrogens to a greater extent than the first and second generation inhibitors in postmenopausal women with hormone-dependent breast cancer. Hypothetically, these highly potent agents could also reduce levels of intra-tumoral aromatase activity to a greater extent than the earlier inhibitors but this has not yet been examined.

**Pharmacologic classification of aromatase inhibitors**

A convenient classification divides inhibitors into mechanism based or ‘suicide inhibitors’ (Type 1) and competitive inhibitors (Type II) (Brodie 1993). Suicide
inhibitors initially compete with natural substrates (i.e. androstenedione and testosterone) for binding to the active site of the enzyme. The enzyme, then, specifically acts upon the inhibitor to yield reactive alkylating species which form covalent bonds at or near the active site of the enzyme. Through this mechanism, the enzyme is irreversibly inactivated. Competitive inhibitors, on the other hand, bind reversibly to the active site of the enzyme and prevent product formation only as long as the inhibitor occupies the catalytic site. Whereas mechanism-based inhibitors are exclusively steroidal in type, competitive inhibitors consist both of steroidal and non-steroidal compounds (Brodie 1993).

**Methods used to demonstrate aromatase inhibition**

The standard method to study aromatase inhibitors in patients is to measure either plasma or urinary estrogen by RIA. Early studies demonstrated 50-80% inhibition of plasma or urinary estrone or estradiol (Santen et al. 1978, 1981, 1982). Another method involved measurement of each estrogen metabolite in urine with calculation of total aromatized product. This technique provided results similar to those from measurements of urinary estrone or estradiol (Lipton et al. 1995). Using these plasma or urinary methods, each agent appeared to suppress estrogen levels to concentrations approaching the sensitivity of the RIAs used. To gain greater specificity and sensitivity, investigators utilized the isotopic kinetic technique of Siiteri et al. to measure total body aromatase (Grodin et al. 1973, Santen et al. 1978, Jones et al. 1992, Dowsett et al. 1995). This required administration of tritiated androstenedione and $^{14}\text{C}$-estrone to patients under steady-state conditions and measurement of radiochemically pure tritiated estrone and estradiol (Santen et al. 1978). The $^{14}\text{C}$-estrone allowed correction for losses during multiple purification steps. Using this technique, the degree of inhibition with various inhibitors ranged from 90 to 99%.

From these observations, it was recognized that more sensitive plasma assays of estradiol were needed. One
approach was the use of the plasma estrone sulfate assay since basal levels of this conjugate in postmenopausal women are tenfold higher than the levels of unconjugated estrone and estradiol (Samoiljik et al. 1982, Lonning et al. 1997). With this measurement, suppression to 85% of basal values was observed with most inhibitors. Finally, an ultrasensitive bioassay of plasma estradiol which was 50- to 100-fold more sensitive than RIA was developed (Oerter-Klein et al. 1995). Surprisingly, with this assay, one could demonstrate suppression to levels of estradiol of 0.05-0.07 pg/ml, concentrations substantially lower than the 2-5 pg/ml suppressed levels detected by RIA (Fig. 3). As observed with use of other highly sensitive plasma hormone assays, for example for luteinizing hormone (LH), follicle-stimulating hormone (FSH), thyrotropin (TSH), and growth hormone, the levels measured under basal conditions and during suppression with these assays reveals much lower values than with insensitive RIAs. This probably reflects the fact that insensitive assays are measuring a substantial fraction of ‘blank’ or non-specific assay artifact. With the use of highly sensitive assays, this artifactual measurement is eliminated and the actual values measured are much lower. Thus with the ultra-sensitive estradiol bioassay, the basal levels in postmenopausal women average 1-3 pg/ml (vs 5-20 pg/ml with RIA) (Oerter-Klein et al. 1995). During development of the second and third generation aromatase inhibitors, each of these methods has been used to demonstrate the magnitude of suppression of enzymatic activity. For these measurements, the isotopic kinetic technique is considered the ‘gold standard’ since it is highly sensitive and allows comparison among various inhibitors (Fig. 1).

First generation aromatase inhibitors

The first aromatase inhibitor to be widely used in the treatment of metastatic breast cancer in postmenopausal women was the drug aminogluthethimide (Santen et al. 1978, 1981, 1982, 1990). Isotopic kinetic studies demonstrated a 90-95% inhibition of aromatase activity (Santen et al. 1978). Plasma estrone and estradiol levels and urinary estrogens fell by 50-80% in response to this aromatase inhibitor. An additional effect, described by Lonning and colleagues, was the acceleration of cholesterol side-chain cleavage enzymes (Santen et al. 1990). Cross-over responses to aminoglutethimide/hydrocortisone in patients relapsing on tamoxifen were substantial, ranging from 25 to 50% and 36% in the largest randomized study (Gale et al. 1994). In marked contrast, patients initially treated with aminoglutethimide/hydrocortisone responded less frequently when crossed over to tamoxifen (19%) (Gale et al. 1994). This observation reinforced the concept that the antiestrogens be used as first-line agents and the aromatase inhibitors as second- or third-line therapies. With the development of better aromatase inhibitors, aminogluthethimide is now of historical interest only.

Second generation aromatase inhibitors

Fadrozole

Fadrozole (CGS 16949A); 4-(5,6,7,8-tetrahydroimidazo[1,5a]-pyridin-5-yl) benzonitrile monohydrochloride is a fairly potent inhibitor of aromatase with an inhibitory constant ($K_i$) of 0.19 nM (vs 600 nM for aminogluthethimide) (Harvey et al. 1994, Harvey 1996). Cholesterol side-chain cleavage activity is minimal but C-11 hydroxylase inhibitory effects are observed in vitro at high drug concentrations.

Initial dose-seeking studies conducted in patients demonstrated effective aromatase inhibition at doses of 1.8-4.0 mg daily (Harvey et al. 1994). A phase II study then compared doses of 0.6 mg three times daily, 1 mg twice daily, and 2 mg twice daily. Maximal suppression of plasma and urinary estrogens occurred at a dose of 1.0 mg
twice daily and minimal effects on cortisol secretion were observed. Basal cortisol and ACTH levels were unaffected and cortisol levels increased appropriately after exogenous synthetic ACTH (cortrosyn) administration in all patients. Basal levels of aldosterone also remained stable following administration of all three drug doses. There were no changes in urinary or plasma sodium or potassium, nor in standing blood pressure to suggest a clinical state of aldosterone deficiency. However, cortrosyn-stimulated aldosterone levels were significantly blunted at all three doses. (Santen et al. 1991). Based on several phase II trials, toxicity attributed to this agent was mild and consisted mainly of nausea, anorexia, fatigue, and hot flashes. The potency of the compound, its relatively specific effects on aromatase and its lack of toxicity suggested that it might provide a major improvement over aminoglutethimide for treatment of patients with breast cancer.

Two trials compared fadrozole with tamoxifen (Falkson & Falkson 1996, Thurlimann et al. 1996). In the first, 1 mg fadrozole twice daily was compared with 20 mg tamoxifen daily in 212 postmenopausal patients with metastatic breast cancer. Response rates to tamoxifen (27%) and to fadrozole (20%) did not differ significantly nor did response durations (20 months vs 15 months). However, tamoxifen achieved a significantly longer time to treatment failure (8.5 months vs 6 months, \( P < 0.05 \)). In the second study, fadrozole was compared with tamoxifen as first-line therapy in a randomized, controlled trial conducted in South Africa. Response rates to tamoxifen were 48% vs 43% with fadrozole \( (P=\text{not significant}) \). However, response duration was significantly longer with

<table>
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<tr>
<th>Response parameters</th>
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<tr>
<td>Time to progression</td>
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<td>5 months</td>
<td>5.5 months</td>
</tr>
<tr>
<td>Number in study</td>
<td>452</td>
<td>764</td>
<td>551</td>
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NS, not significant.


Megace, megestrol acetate

### Table 1 Comparison of third generation aromatase inhibitors with progestin therapy

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tamoxifen (median duration not reached vs 343 days, \(P<0.009\)) as was overall survival (34 months for tamoxifen vs 26 months for fadrozole, \(P<0.046\)).

Taken together, these studies demonstrated that fadrozole may be inferior to tamoxifen in efficacy and no better tolerated than megestrol acetate. Based upon these findings, the second generation aromatase inhibitor, fadrozole, would likely find its place as third-line therapy. Fadrozole has been approved for the treatment of advanced breast cancer in postmenopausal women in Japan. This agent is not likely to be further developed in the United States since both anastrozole and letrozole appear to be more potent and more selective aromatase inhibitors.

Careful analysis of the fadrozole/megestrol acetate trials raises the concern that responses to endocrine therapies appeared to be less frequent than observed in prior studies. For example, the randomized comparison of the first generation aromatase inhibitor, aminoglutethimide, with surgical adrenalectomy demonstrated responses of 40-50% in patents previously treated with tamoxifen (Santen et al. 1981). Other studies with megestrol acetate as second-line therapy demonstrated responses ranging from 30 to 50%. Several possibilities could explain the low response rates. In recent studies, more stringent criteria have been used than in previous trials. For example, recalcification of mixed lytic/blastic metastases were previously considered objective evidence of partial responses. Such lesions are now considered non-assessable, non-measurable disease. External review of cases probably also increases the stringency of assessment. It should be noted that in a previous study comparing tamoxifen alone vs tamoxifen and fluoxymesterone, the objective response rate for tamoxifen alone was only 10% (Swain et al. 1988). These considerations lead to the conclusion that one can only compare new agents with established ones such as tamoxifen and assess the relative differences between them. It is inappropriate to compare the percent of objective responses to those observed in historical controls.

4-Hydroxyandrostenedione (4-OHA)

Formestane (Lentaron; 4-OHA; 4-hydroxyandrost-4-ene-3,17-dione) is a structural analog of androstenedione and is thus a highly specific aromatase inhibitor (Lonning 1998). It was the first steroidal suicide-type (Type I) aromatase inhibitor to enter clinical trials and is now commercially available in Europe. Using the in vitro placental aromatase assay system, 4-OHA was shown to be 60-fold more potent than aminoglutethimide (\(K_i=4.1 \mu M\)). Extensive studies revealed no estrogenic, anti-estrogenic, or antiandrogenic properties (Brodie & Wing 1987). However, transformation to 4-hydroxytestosterone occurs and androgenic effects can be demonstrated under certain circumstances (Brodie et al. 1981).

4-OHA (Lentaron) has been studied extensively in Europe in postmenopausal women with breast cancer. Data from four phase II clinical trials of 4-OHA demonstrated a 33% objective regression rate of breast cancer in postmenopausal patients previously treated with multiple endocrine therapies. Toxicity included six patients with sterile abscesses due to intramuscular injections, two of sufficient severity to warrant discontinuation of therapy. No androgenic effects were observed (Goss et al. 1986).

Hoffken et al. (1990) conducted a large trial of 4-OHA in postmenopausal women. Patients initially received 500 mg intramuscularly every two weeks for 6 weeks and then 250 mg every 2 weeks thereafter. Plasma estradiol levels fell from baseline values of 10-11 pg/ml to levels of approximately 4 pg/ml for up to 7 months of therapy. The drug appeared specific since no reduction of cortisol or symptoms of cortisol deficiency were observed. Of 86 evaluable patients, there were 2 complete and 19 partial remissions (24%) and 26 with disease stabilization (30%). Side-effects included minor systemic symptoms in 11% (hot flashes, constipation, alopecia, and pruritus) and local symptoms in 8% (pruritus, local pain, and erythema). These side-effects resulted in discontinuation of therapy in only 2% of patients. Phase III trials are now ongoing to compare this inhibitor with standard endocrine therapies. In general, 4-OHA is better tolerated than aminoglutethimide. This agent is not available in the USA. Studies of the degree of aromatase inhibition using isotopic kinetic techniques demonstrate that 4-OHA is not as effective as the third generation inhibitors in blocking estrogen production (Fig. 1). For this reason and because it must be injected i.m., it is unlikely that this agent will compete successfully with the newer inhibitors.

Exemestane

Another steroidal aromatase inhibitor under active investigation is exemestane. Exemestane (6-methylene-androsta-1,4-diene-3,17-dione) is an irreversible (Type I or mechanism-based) aromatase inhibitor (Evans et al. 1992, Thurlimann et al. 1997, Lonning 1998). Its \(K_i\) for competitive inhibition is 10.2 nmol/l and for irreversible inactivation is \(K_i\) (intact) of 26 nmol/l. Single dose administration reveals a major reduction of plasma estrogens with this compound (Lonning 1998). A dose of 25 mg daily inhibited aromatase activity as documented by the isotope kinetic technique by 97.9%. Thurlimann et al. (1997) reported an objective response (complete response (CR) and partial response (PR)) in 12% and 33% of patients expressing primary or secondary resistance to aminoglutethimide. Other studies are ongoing but not yet completed.
Third generation aromatase inhibitors

Anastrozole
Anastrozole (Arimidex; ICI-D1033; 2,2’-[5-(1H-1,2,4-triazol-1-ylmethyl)-1,3-phenylene]bis(2-methyl-propionitrile) is a potent and selective benzyltriazole derivative (Buzdar et al. 1996a, 1997, Buzdar 1998). At a concentration of 15 nmol/l this compound inhibits aromatase activity by 50%. In rodents, maximal hormonal suppression is achieved with an oral dose of 0.1 mg/kg. Activity is assessed by examining the degree of inhibition of ovulation and of androstenedione-induced uterine hypertrophy. Studies conducted in monkeys demonstrate similar inhibitory potency when expressed on a mg/m² basis and assessed by measurement of plasma estradiol. Studies in women demonstrated suppression of plasma estrogen to levels approaching assay sensitivity (Kleeburg et al. 1997). Anastrozole produces no effects on aldosterone, cortisol, or thyroxine synthesis (Kleeburg et al. 1997). The estimated elimination half-life in humans is 32.2 h.

Anastrozole was the first aromatase inhibitor to be approved in the USA for the management of advanced breast carcinoma in postmenopausal women. This approval was based on results of two pivotal trials that together accrued a total of 764 patients randomized to receive either anastrozole (1 mg p.o./day) or anastrozole (10 mg/day) or megestrol acetate 40 mg (q/day) (Buzdar et al. 1996a). These patients had metastatic disease that was progressing following therapy with tamoxifen given either in the adjuvant setting or as first-line endocrine therapy for metastatic disease. Patients in the three arms of the trial had similar prognostic characteristics including age, estrogen receptor status, disease-free interval, and sites of metastases. Results from these important trials showed similar overall response rates to either dose of anastrozole or to megestrol acetate. No statistically significant dose-response differences were observed between the 1 and the 10 mg daily dosage. The rates of overall objective response of 10.3% and 8.9% were also surprisingly low, probably for reasons discussed above. Overall responses including complete and partial objective response rates and stabilization of disease of greater than 6 months averaged 35%. It should be noted that recent studies have demonstrated that disease stabilization for greater than 6 months is a meaningful clinical parameter since patients experiencing this response survive equally as long as patients undergoing partial objective response (Howell et al. 1998). Patients with complete or partial objective responses or stable disease survive longer than those with disease progression.

In initial reports, the third generation aromatase inhibitor, anastrozole, was considered superior to megestrol acetate because it was better tolerated. It was associated with less undesirable weight gain, dyspnea, and fewer thromboembolic events when compared with megestrol acetate (Buzdar et al. 1996a, 1997). Since there were no differences between the two doses of anastrozole, the drug was approved at a dose of 1 mg daily.

### Table 2 Dose response studies with third generation aromatase inhibitors

<table>
<thead>
<tr>
<th>Response parameters</th>
<th>Anastrozole 1 mg</th>
<th>Anastrozole 10 mg</th>
<th>P</th>
<th>Letrozole 0.5 mg</th>
<th>Letrozole 2.5 mg</th>
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<tr>
<td>Overall survival</td>
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*Gershanovich et al. (1998); **Dombernowsky et al. (1998).
Surprisingly, with further maturity of this trial, anastrozole (1 or 10 mg daily) conferred a survival advantage compared with the progestin (median of 26.7 months vs 22.5 months) (Buzdar et al. 1997) (Table 1). The 2-year survival was 56.1% for the group of patients receiving anastrozole (1 mg) compared with 46.3% for patients treated with megestrol acetate. The demonstration that anastrozole has superior efficacy with respect to overall survival and reduced side-effects vs megestrol acetate would suggest that the aromatase inhibitor be used as second-line therapy in preference to megestrol acetate.

**Letrozole**

The second aromatase inhibitor to gain approval in the United States with the indication for management of postmenopausal women with metastatic breast cancer was letrozole (Femara) (Dombernowsky et al. 1998, Gershanovich et al. 1998). Letrozole (4,4′-(1H-1,2,4-triazol-1-yl-methylene)-bis-benonitrile) is also a potent non-steroidal competitive aromatase inhibitor. This agent possesses considerable selectivity for aromatase. In preclinical studies, for example, letrozole caused inhibition of aldosterone production *in vitro* only at concentrations 10000 times higher than those required for inhibition of estrogen production. Letrozole is also potent and selective aromatase inhibitor. When administered orally to adult female rats at a dose of 1 mg/kg per day for 14 days, letrozole decreases uterine weight to that observed after a surgical ovariectomy. At doses greater than 1000 times higher than the concentration required to cause a 50% inhibition of the aromatase enzyme, letrozole does not significantly suppress either aldosterone or corticosterone in rats. Letrozole also causes significant regression of

<table>
<thead>
<tr>
<th>Table 3 Comparison of first with third generation aromatase inhibitors</th>
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<th>Table 4 Comparison of third generation aromatase inhibitors</th>
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*Gershanovich et al. (1998), **Dombernowsky et al. (1998).
Dimethylbenzanthracene (DMBA)-induced rat mammary tumors (Schiweck et al. 1993).

Clinical studies in normal healthy volunteers as well as dose-seeking phase I trials in postmenopausal women with advanced breast cancer showed that letrozole in a dose as little as 0.25 mg p.o. daily caused maximal suppression of plasma and urinary estrogens. A highly sensitive recombinant DNA-based estradiol bioassay was used to assess estradiol levels in one of these studies (Oertel-Klein et al. 1995). The levels of estradiol were decreased by 95% to levels of 0.05-0.07 pmol/l as detected by this assay (Fig. 3). This observation underscores the limitation of standard RIA techniques for detection of estradiol levels in patients given highly potent aromatase inhibitors.

The lower dose of letrozole. Similarly, there was a trend in stabilization for greater than 6 months) of 36% and 35% (complete and partial tumor regression and disease control, respectively) compared with 27% and 33% for letrozole (0.5 mg) and megestrol acetate. However, the median duration of response for letrozole (2.5 mg) was 33 months compared with 15 months for aminoglutethimide and 15 months for aminoglutethimide and 32% for megestrol acetate. However, the median duration of response for letrozole (2.5 mg) was 33 months compared with 15 months for aminoglutethimide and 15 months for aminoglutethimide. 20% of tumor regression and disease control, respectively) compared with 27% and 33% for letrozole (0.5 mg) and megestrol acetate. However, the median duration of response for letrozole (2.5 mg) was 33 months compared with 15 months for aminoglutethimide and 15 months for aminoglutethimide.

Vorozole
This agent is another third generation, non-steroidal, oral aromatase inhibitor which is highly potent and specific for aromatase (Goss 1998). Nearly all of the aromatase inhibitor activity resides in the dextroenantiomer (R083842). Clinical efficacy appears to be similar to that of anastrozole and letrozole (Tables 1 and 2). Vorozole appears to be superior to aminoglutethimide/hydrocortisone with respect to clinical benefit (i.e. complete and partial objective regression plus stabilization of disease for greater than 6 months) (Table 3). Its efficacy did not differ significantly from that of megestrol acetate although it was associated with fewer side-effects. Because of the proven efficacy and prior approval of anastrozole and letrozole, further clinical development of vorozole has recently been abandoned and full description of its clinical properties is referenced but not detailed.

Comparison of potency of aromatase inhibitors
The relative potencies of aromatase inhibitors can be determined in vitro and characterized as inhibitory constants (i.e. $K_i$) or as the concentration that inhibits aromatase by 50%. However, these measurements do not provide information which can be extrapolated to patients. In addition to $K_i$, drug half-life and amount of drug that can be given safely contribute substantially to degree of inhibition achievable in patients. Consequently, the most useful comparator of potency among agents is the measurement of degree of aromatase inhibition in women with breast cancer. This requires highly sensitive and specific means of measuring aromatase inhibition. PlasmaRIA techniques are not sufficiently sensitive to precisely quantitate degree of suppression and the ultrasensitive estradiol bioassay has not been used to compare inhibitors.

The isotopic kinetic technique for quantitating total body aromatase activity serves then as the best method to compare the potency of various inhibitors in patients (Grodin et al. 1978, Dowsett et al. 1985, Jones et al. 1992). Lomning and others (Jones et al. 1992) have compared a number of these agents and reviewed published studies of others. With this methodology, 4-OHA inhibits aromatase by 92%, fadrozole by 93%, exemestane by 97.9%, anastrozole by 93%, vorozole by 98%, and letrozole by 99%. (Fig. 1). It is not clear why there was a statistically significant improvement in overall survival for the patients receiving letrozole. Moreover, letrozole produced less somnolence and skin rash. The results of these large, well-done, randomized trials suggest that the side-effect profile and the dosing schedules of both anastrozole and letrozole are superior to megestrol acetate and aminoglutethimide.
whether the aromatase activity remaining during therapy is biologically important. Most biologic systems operate on a log dose-response basis. Since residual aromatase activity is 8% with 4-OHA and only 1% with letrozole, these differences could have biologic relevance.

**Summary of conclusions from large clinical trials with third generation aromatase inhibitors**

These studies allow answers to three important questions. (1) Do higher doses of third generation aromatase inhibitors produce greater clinical effects than do lower doses? (2) Do third generation inhibitors produce greater clinical benefit than the first generation aromatase inhibitor aminoglutethimide? (3) Do the third generation inhibitors produce greater clinical benefit than does megestrol acetate? A fourth question, ‘Which is the most effective third generation aromatase inhibitor?’ cannot be answered until head to head comparisons between agents are made. Relative efficacy based upon results among very large, but non-randomized trials cannot be validly interpreted but provide trends to be tested in future studies.

With respect to the dose-response question (question 1), two large studies clearly demonstrated that 2.5 mg letrozole is more effective than the 0.5 mg dose. In contrast, no differences were demonstrated when comparing 1 with 10 mg anastrozole daily (Table 2). With respect to the superiority of third over first generation inhibitors (question 2), letrozole, at the 2.5 mg dosage produced significantly better responses than did aminoglutethimide with respect to duration of response, time to progression and time to treatment failure (Table 3). The percent objective response rates was also greater with letrozole than with aminoglutethimide and the rate of side-effects was less than with the first generation aromatase inhibitor. Finally, with respect to the superiority of third generation inhibitors to other agents (question 3), anastrozole was clearly superior to megestrol with respect to overall patient survival (Table 1). Letrozole was also superior in clinical efficacy to megestrol with respect to percent objective responses, time to progression and time to treatment failure. The overall duration of survival was not significantly different but the trend favored 2.5 mg letrozole (P=0.10). With greater maturity of these studies, differences in overall survival could emerge. It should be noted that earlier studies (as cited above) suggested equal efficacy of anastrozole and megestrol but updated data demonstrate a clear enhancement of overall survival imparted by anastrozole when compared with megestrol. Vorozole, on the other hand, did not differ from megestrol with respect to any parameter reflecting efficacy.

Each of these trials demonstrated that the third generation aromatase inhibitors were better tolerated than megestrol acetate. Side-effects reported for letrozole and anastrozole were low grade in severity including mild headache, nausea, diarrhea, and hot flashes and were infrequent. Significantly, letrozole and anastrozole were associated with less weight gain, dyspnea, thromboembolic events, and vaginal bleeding when compared with megestrol acetate (Buzdar et al. 1996a, 1997, Buzdar 1998, Dombernowsky et al. 1998).

Both of these aromatase inhibitors, anastrozole and letrozole, are highly potent, specific, and well tolerated. It is probable that in clinical practice, either of these agents will now replace megestrol acetate or other progestins as second-line therapy after tamoxifen in postmenopausal women with metastatic breast carcinoma.

**Relative efficacy of third generation inhibitors**

Table 4 compares several parameters observed with the various third generation inhibitors. Overall survival is quite similar with each agent and ranges from 25.3 months to 28 months. Objective response rates on the other hand appeared somewhat higher with letrozole (19.5 and 23.6%) than with vorozole (10.5%) and anastrozole (10.3%). The percent of patients experiencing clinical benefit (i.e. objective response plus stabilization of disease for greater than 6 months) appeared similar for each therapeutic modality and ranged from 47% with vorozole to 35% with anastrozole to 36.3 and 35% with letrozole. Time to progression appeared the shortest with vorozole (2.7 months) and somewhat longer but similar with anastrozole (5 months) as with letrozole (3.4-5.6 months). Head to head comparisons are now required to determine if the somewhat longer durations of response and objective response rates observed with letrozole represent statistically significant differences.

**Comparisons of aromatase inhibitors with antiestrogens as first-line endocrine therapy**

Prior studies demonstrated that tamoxifen and aminoglutethimide/hydrocortisone when compared in four randomized, controlled, double-blind studies showed similar efficacy in patients with advanced breast cancer (Smith et al. 1981, Lipton et al. 1982, Alonso-Munoz et al. 1988, Gale et al. 1994). Since letrozole has been shown superior to aminoglutethimide in a direct comparative trial, it appears logical to determine whether the third generation aromatase inhibitors are superior to tamoxifen as first-line therapy. These trials are now ongoing but no results are as yet available.
Comparison of antiestrogens with third generation aromatase inhibitors in the adjuvant setting

Trials are now ongoing to determine the efficacy of aromatase inhibitors vs tamoxifen vs the combination of antiestrogen and aromatase inhibitor. The largest trial is termed the ATAC trial (anastrozole alone versus tamoxifen alone and in combination - i.e. anastrozole plus tamoxifen). An anticipated 20,000 patients will be enrolled in this trial and currently 500 per month are being entered in 200 centers worldwide. This and other similar trials should establish the relative efficacies of these two therapeutic strategies.

Secondary issues in these trials are the differential actions of the antiestrogens and aromatase inhibitors on non-breast tissues. Tamoxifen acts as an estrogen agonist on uterus and increases the incidence of uterine cancer whereas the aromatase inhibitors would be expected to reduce estrogenic stimulation on the uterus. The beneficial effects of tamoxifen on bone and potentially on the cardiovascular system differ from the potential of the aromatase inhibitors to accelerate the process of bone resorption and the incidence of cardiovascular disease. Subprojects within the ATAC trial are examining these issues in detail.

Selection of patients for aromatase inhibition therapy

Endocrine therapy is usually offered to patients with metastatic disease who have receptor positive (estrogen receptor positive and progesterone receptor positive) or receptor unknown disease (Santen et al. 1990). In addition to the level of receptors, clinical features that might suggest a favorable response include a long disease-free interval after initial surgery or the presence of nodal, soft tissue, bone, pleural or nodular lung metastases. Patients with central nervous system involvement, extensive liver disease, lymphangitic spread of tumor in the lungs or rapidly progressing and life-threatening disease are not ordinarily considered candidates for hormonal therapy.

Considerable clinical experience and data from the literature suggest that most endocrine therapies with the possible exceptions of androgens and glucocorticoids are equally effective.

The decision to choose one endocrine therapy over another depends upon the menopausal status of the patient, considerations of efficacy, ease of administration, cost, and side-effects. Historically, of all the endocrine therapies, tamoxifen was associated with the fewest side-effects. This aspect still favors tamoxifen as the endocrine therapy of first choice. Aromatase inhibitors are then considered either as second- or third-line endocrine approaches. More recently, however, the widespread use of tamoxifen as adjuvant therapy, frequently administered for long periods of time, presents the clinician with a new therapeutic dilemma. A practical approach is to rely on tamoxifen as first-line therapy for patients with metastatic disease who have not received this agent in the adjuvant setting or have discontinued tamoxifen for a period of greater than 1 year. For other patients who are still candidates for hormone therapy, the major choices at present are between progestins such as megestrol acetate or medroxyprogesterone acetate and aromatase inhibitors. Given their equal or greater efficacy and better tolerability, aromatase inhibitors should now replace progestins as second-line hormonal therapy for metastatic breast cancer.

It has been speculated that the determination of aromatase content of a particular tumor by either biochemical measurement or immunohistochemistry might aid in selecting patients who are likely to respond to therapy with aromatase inhibitors (Bezwoda et al. 1987). Preliminary evidence also suggests that breast cancers that overexpress the Her-2/neu protein may be relatively resistant to hormonal therapies including tamoxifen and aromatase inhibitors (Leltzel et al. 1995, Yamaguchi et al. 1997). If confirmed, this information together with other considerations might well assist the clinician in better selecting patients for these forms of therapy.

Premenopausal patients

Considerations of efficacy, cost, toxicity, and ease of administration also dictate the choice of endocrine therapy in premenopausal patients. Based on these considerations, first-line therapy would include either tamoxifen or oophorectomy. Effective castration can be accomplished either by surgery, pelvic irradiation, or the use of luteinizing hormone-releasing hormone analogs. In humans, the premenopausal ovary has generally been considered to be resistant to blockade of estrogen production by aromatase inhibitors since any lowering of plasma estrogens would lead to reflex increases in both FSH and LH (Santen et al. 1980). These increased gonadotropin levels would then induce increased ovarian production of estradiol and of androstenedione, the major substrate for aromatase action. On the other hand, the activity of the very potent third generation inhibitors makes it possible that these compounds might also inhibit ovarian steroidogenesis and therefore may be of value in the treatment of breast cancer in premenopausal women. However, there are no clinical studies demonstrating complete ovarian blockade with aromatase inhibitors. One preliminary study showed no effective inhibition (Yamaguchi 1999), consequently, the use of aromatase inhibitors should be restricted to the treatment of breast cancer either in postmenopausal patients or in premeno-
pausal women whose ovaries have been rendered non-functional by use of gonadotropin-releasing hormone agonists or by surgical or radiation ablation.

Mechanisms for lack of cross resistance of aromatase inhibitors and antiestrogens

Logic would suggest that inhibitors of estrogen action, such as tamoxifen, would be completely cross resistant with agents designed to block estrogen synthesis. However, early studies demonstrated that sequential responses to inhibitors of estrogen biosynthesis commonly occurred in patients initially responding to and then relapsing after treatment with the antiestrogen, tamoxifen. For example, 25-50% of patients initially responding to tamoxifen and then relapsing experienced secondary tumor regressions in response to the aromatase inhibitor aminoglutethimide in combination with hydrocortisone.

Potential explanations for lack of cross resistance among hormonal therapies

One potential explanation for the lack of cross resistance between antiestrogens and aromatase inhibitors was raised by observations made during further study of the actions of the antiestrogens. A variety of data examining the effects of antiestrogens on various organs and in various species demonstrated that estrogen receptor antagonists exert both hormone agonistic and antagonistic actions, depending upon the tissue studied (Santen 1997). For example, tamoxifen acts as a potent estrogen on bone, liver, pituitary, and uterus while exerting antiestrogenic effects on breast. The various responses to antiestrogens could be modulated by adaptive mechanisms such as, for example, increased production of cyclic AMP or activation of the protein kinases A and C pathways (Santen 1997). Observations in xenograft models of human breast cancer were particularly striking with respect to this adaptive process. Initial exposure to tamoxifen caused tumor regression but prolonged exposure allowed the tumor to adapt such that tamoxifen shifted from exerting estrogen-antagonistic to estrogen-agonistic effects. Re-transplantation of the xenografts into additional animals allowed demonstration that tamoxifen stimulated these tumors to grow and that the pure antiestrogen, ICI 182,782, could antagonize this estrogenic effect (Gottardis & Jordan 1988).

These observations led to the hypothesis that, in patients, breast tumors initially responding to tamoxifen but then regrowing had also undergone adaptation. Such tumors might then respond secondarily to agents such as the aromatase inhibitors which would lower estrogen levels but not be expected to exert estrogen-agonistic actions. The hypothesis of adaptation has also been used to explain why women appear to be benefited to a greater extent with 5 than with 10 years of tamoxifen in the adjuvant setting. Adaptation to tamoxifen, occurring between 5 and 10 years of exposure to this agent, might allow tamoxifen to ultimately become a stimulator of growth of the remaining micrometastases (Santen 1997).

Adaptive hypersensitivity hypothesis

Another possible explanation for secondary responses to aromatase inhibitors following exposure to tamoxifen is the development of adaptive hypersensitivity to estradiol. This phenomenon was initially suggested by clinical observations demonstrating sequential tumor regressions in women undergoing oophorectomy followed by exposure to an aromatase inhibitor. Oophorectomy reduces estradiol levels from approximately 200 pg/ml (premenopausal levels) to 5-10 pg/ml (post-oophorectomy concentrations) resulting in tumor regression. The cancer then begins to regrow in the presence of these low estradiol levels but undergoes further regression when aromatase inhibitors lower levels further to 0.05-0.07 pmol/l. These observations are best explained by the hypothesis that long-term deprivation of estradiol can induce an adaptive sensitization of the tumor to estradiol.

One could consider this analogous to Cannon’s law of denervation hypersensitivity whereby estradiol deprivation causes hypersensitivity to estradiol.

We tested the estradiol hypersensitivity hypothesis directly in an in vitro cell culture system (Masamura et al. 1995). Breast cancer cells were deprived of estradiol over several months in culture by growing them in media stripped of estradiol by treatment with charcoal. This period of estrogen deprivation induced a four log enhancement in sensitivity to the cell proliferative effects of estradiol. The hypersensitivity phenomenon could be reversed by re-exposure of cells to estradiol, suggesting adaptive mechanisms rather than selection of hypersensitive clones of cells.

Long-term exposure to tamoxifen might also result in development of hypersensitivity to estradiol. Under these circumstances, a marked reduction of estradiol synthesis with an aromatase inhibitor would result in tumor regression. Taken together, these observations suggest that breast cancer cells adapt to the conditions of ambient hormonal exposure, either to tamoxifen or to estrogen deprivation. This adaptive process provides a plausible explanation for the sequential responses to various hormonal therapies observed clinically in women with breast cancer.

Development of adaptive hypersensitivity has practical implications for the use of aromatase inhibitors. If cells in culture can respond to 10 fM concentration of estradiol, nearly complete inhibition of aromatase may be
necessary to produce most effective anti-tumor therapy. Even the most potent inhibitors available now allow 1% residual aromatase activity. It is not clear whether the inhibitors block aromatase in breast tumor tissue itself to the same degree. These concepts are of interest when considering the dose-response differences between 0.5 and 2.5 mg letrozole daily. Perhaps even more potent aromatase inhibitors could produce even greater clinical effects. This possibility is not supported by the lack of dose-response differences detected between 1 and 10 mg anastrazole per day but perhaps deserves further exploration.

Future perspectives
As discussed above, several new potent and highly specific aromatase inhibitors are now available for the treatment of breast cancer. They offer several distinct advantages over some older forms of endocrine therapy including a well-understood mechanism of action, good toxicity profile, convenient dosing schedules, and the absence of estrogen effects on the endometrium. On the other hand, their long-term effects on bone mineral density and serum lipids are unknown (Harvey 1996).

New clinical trials with these promising agents are either underway or are planned in order to address several questions including their role in the treatment of premenopausal women as discussed above. Although presently approved only as second-line therapies after tamoxifen failure, aromatase inhibitors are now being tested as first-line endocrine treatment for metastatic breast cancer in direct comparison to antiestrogens. Moreover, non-steroidal aromatase inhibitors would not be expected to induce endometrial carcinoma in women and so could be investigated both as adjuvant hormonal therapy as well as in the chemoprevention of human breast cancer. A few clinical studies have attempted to combine different classes of endocrine agents but there are few clinical data to support this approach as being superior to using these agents in sequence to treat metastatic breast cancer, e.g. tamoxifen followed by an aromatase inhibitor, followed by a progestin. In clinical practice, the sequential use of hormonal agents can produce long-term palliation of hormone-dependent breast cancer. Eventually, however, the problem of hormone resistance is encountered. The mechanism by which tumors become resistant to hormones in general are only partially understood. Refractoriness to therapy with aromatase inhibitors is related not to the failure of these agents to suppress estradiol levels as might be seen if there were up-regulation of aromatase, but rather is likely due to some other mechanism of hormone resistance.

The paracrine production of aromatase-specific growth factors and cytokines within the microenvironment of a breast tumor requires further study. Greater understanding of the biologic interaction of these factors could lead, for example, to the development of new therapeutic strategies.

Use of aromatase inhibitors for breast cancer prevention
Estrogens are considered carcinogenic for the breast through the ability to increase the rate of cellular proliferation and consequently to increase the number of genetic mutations which are proportional to the number of cell divisions (Santen et al. 1999). In addition, the increased rate of cell proliferation could reduce the time required for DNA repair. This is the commonly accepted mechanism of estradiol-induced carcinogenesis. An additional mechanism has been proposed which involves the metabolism of estradiol to 4-hydroxyestradiol and then to the 3,4 estradiol quinone. This compound can bind covalently to guanine or adenine and result in depurination of that segment of DNA. Upon replication, these depurinated sites preferentially undergo point mutations. This process could act in an additive or synergistic fashion with the effect of estrogen to increase cell proliferation.

It has been postulated that antiestrogens might prevent breast cancer by blocking the cell-proliferative effects of estrogens. The aromatase inhibitors might prevent breast cancer by two mechanisms: reduction of cell proliferation by inhibition of estrogen levels and prevention of genotoxic metabolite formation by lowering tissue levels of estrogen. Coombes et al. (1991) have reported that 4-OHA prevents Nitrosomethylureal (NMU)-induced rat mammary carcinoma and Steele and colleagues have shown that fadrozole completely inhibits the development of spontaneous breast tumors in aging Sprague-Dawley rats (Gunson et al. 1995).

To assess whether aromatase inhibitors are superior to antiestrogens in the prevention of breast cancer, the optimal study would include patients at high risk of developing breast cancer. Women with a single breast cancer are at high risk of developing a contralateral second cancer. Estimates range from rates of 0.5 to 1.0% of women per year for development of a contralateral breast cancer. For a 60-year-old woman, this rate is 1.5- to 3.0-fold higher than the average incidence of 1:243 women per year who develop their first primary tumor. Thus the ATAC trial with assessment of diagnosis of second primary tumors provides a powerful means of determining whether the aromatase inhibitors will prevent breast cancer. It is known that tamoxifen reduces the incidence of second primaries by 45% under these circumstances. While trials of primary prevention of breast cancer with aromatase inhibitors are being planned, one would expect results from the adjuvant trials to be forthcoming sooner.
In summary, recently reported clinical studies of highly potent aromatase inhibitors have shown that it is possible to develop specific, non-toxic compounds which reduce serum estradiol concentrations to undetectable levels in postmenopausal patients with advanced breast cancer. Some of these compounds may also, in fact, effectively target intratumoral synthesis of estrogen by aromatase. These compounds are emerging as a valuable approach to the treatment of hormone-dependent breast cancer.

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