Aromtase Inhibitors in Breast Cancer

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INTRODUCTION

Breast cancer is now the second most cancer in women after stomach cancer in Korea, and is increasing continuously. In the year 2000, the crude incidence of breast cancer in Korea was estimated about 23 per 100,000 people.(*1*)

For the process of inducing breast cancer, estrogens appear to play a predominant role. These sex steroids are believed to initiate and to promote the process of the breast carcinogenesis by enhancing the rate of cell division and reducing time available for DNA repair. A new concept is that estrogens can be metabolized to catechol-estrogens and then to quinines that directly damage DNA. These two process-- estrogen receptor mediated, genomic effects on proliferation and receptor independent, genotoxic effects of estrogen metabolites-- can act in an additive or synergistic fashion to cause breast cancer.(*2*)

Breast cancers that arise in patients can be divided into hormone dependent and hormone independent subtypes.(*3*) The role of estrogens as modulators of mitogenesis override the influence of other factors in the hormone dependent subtype. 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.(*4*)

Based upon the concept that estrogen is the proximate regulator of cell proliferation, two general strategies were developed for treatment of hormone dependent breast cancer: blockade of estrogen receptor (ER) action and inhibition of estradiol biosynthesis. Antiestrogens such as tamoxifen bind to ER and interfere with transcription of estrogen induced genes involved in regulating cell proliferation. Clinical trials showed tamoxifen to be effective in inducing objective tumor regressions and to be associated with minimal side effects and toxicity. The second strategy, blockade of estradiol biosynthesis, was demonstrated to be feasible using the steroidogenesis inhibitor, aminoglutethimide, which produced tumor regressions equivalent to those observed with tamoxifen.(*3*) However, side effects from aminoglutethimide were considerable and its effects on several steroidogenic enzymes required concomitant use of a glucocorticoid. Consequently, tamoxifen became the preferred, first line endocrine agent with which to treat ER-positive advanced breast cancer. However, the clinical efficacy of aminoglutethimide focused attention upon the need to develop more potent, better tolerated, and more specific inhibitors of estrogen biosynthesis.

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INHIBITION OF ESTRADIOL BIOSYNTHESIS

Multiple enzymatic steps are involved in the biosynthesis of estradiol and could potentially be used as targets for inhibition. These include cholesterol side chain cleavage, 3-β-ol-dehydrogenase-$\triangle^{4,5}$-isomerase, 17-α hydroxlase, 17-β hydroxysteroid dehydrogenase, estrogen sulfatase, and aromatase. The ideal strategy would be to block the synthesis of estrogen without inhibiting production of other important steroids or the need to use pharmacological amounts of progestins or glucocorticoids. The aromatase enzyme catalyzes the conversion of androstenedione into estrone, and testosterone into estradiol. For this reason, blockade of the terminal step in estradiol biosynthsis 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 glucocoeticoid and mineralcorticoid synthesis (Fig. 1).

PHYSIOLOGY OF AROMATASE

Aromatase is a cytochrome P-450 enzyme catalyzes the rate-limiting step in estrogen biosynthesis, namely the con-

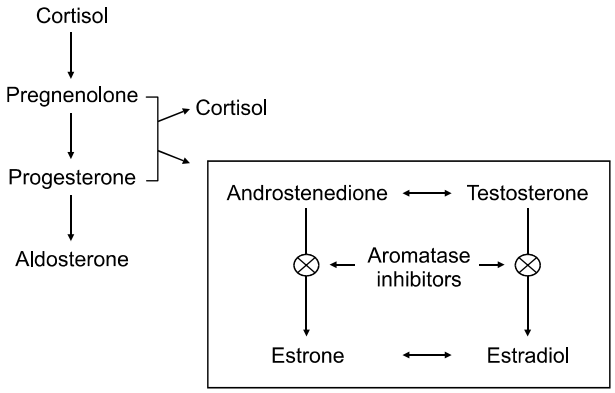

Fig. 1. Mechnism of action of aromatase inhibitors.

version of androgens to estrogens.(*5-8*) 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.(*5*) 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.(*9*)

Aromatase is expressed in many organs including ovary, placenta, hypothalamus liver, muscle, adipose tissue, and breast cancer itself. In the pre-menopausal 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 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, dehydroepiandrosterone and its sulfate are secreted by the adrenal and converted into the aromatase substrates, androstenedione and testosterone, in peripheral tissues. The major source of the aromatase enzyme in postmenopausal women is peripheral tissues, 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.(*10-12*) Proof of local estradiol synthesis includes measurement of tumor aromatase activity by radiometric or product isolation assays, by immunohistochemistry, by demonstration of aromatase messenger RNA in tissue, and by aromatase enzyme assays performed on cells isolated from human tumors and grown in cell culture. The expression of aromatase is highest in the stromal compartment of breast tumors,(*11*) but is present in epithelial cells as well. In breast tissue surrounding the tumors, pre-adipocyte fibroblasts contain aromatase activity that can be detected by biochemical assay or immunohistochemical staining.(*11,12*) Aromatase is also present in normal breast tissue as documented by immunohistochemistry, by demonstration of aromatase message, and by enzyme assays of cultured cells.(*13,14*)

DEVELOPMENT OF AROMATASE INHIBITORS

The first aromatase inhibitors were discovered nearly thirty years ago and included aminoglutethimide and testololactone.(*3*) 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 10,000 fold greater potency than aminoglutethimide and greater specificity. 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.

CLASSIFICATION OF AROMATASE INHIBITORS

A convenient classification divides inhibitors into mechanism based or 'suicide' inhibitors (Type I) and competitive inhibitors (Type II).(*15*) Mechanism-based 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 of both steroidal and nonsteroidal compounds.(*15*) Structures of the currently available aromatase inhibitors are shown in Fig. 2. In addition, Table 1 showed the features and characteristics of various aromatase inhibitors that can be used for the patients with breast cancer.

FIRST GENERATION AROMATASE INHIBITORS

The early aromatase inhibitor for the treatment of metastatic breast cancer in postmenopausal women was aminoglutethimide.(*3,16*) This drug inhibits the conversion of androgens into estrogens, but also block the conversion of other adrenal hormones into aldosterone and cortisol. Plasma estrone and estradiol levels and urinary estrogens fell by 50～80% in response to this aromatase inhibitor. And, multiple non-selective metabolic effects were demonstrated, including inhibition of 11β-hydroxylase, aldosterone synthase, and thyroxine synthesis, as well as induction of enzyme metabolizing synthetic glucocorticoids and aminoglutethimide itself.(*3*)

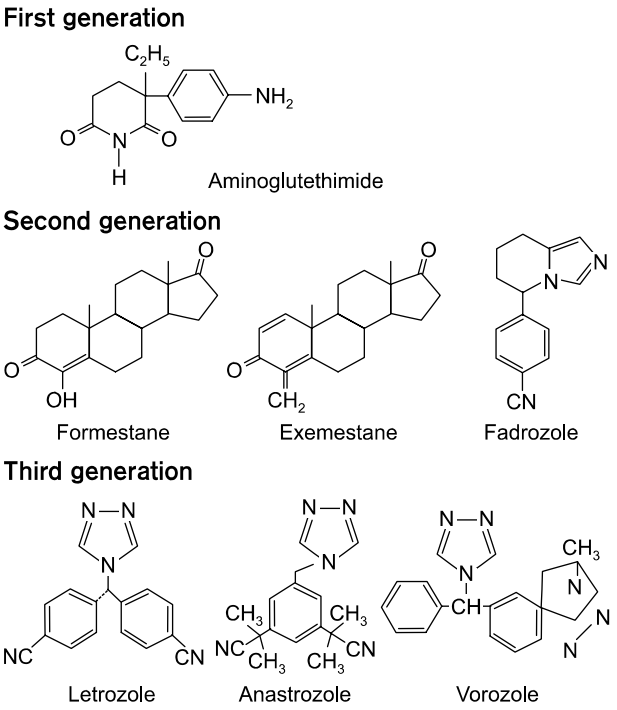

Fig. 2. Structure and classification of aromatase inhibitors. Compounds are represented in approximate order of increasing specificity and potency of aromatase inhibition.

Table 1. Comparison of various features of aromatase inhibitors

Generic name	Fadrozole	Formestane	Exemestane	Anastrozole	Letrozole	Vorozole
Trade name	-	Lentaron	Aromasin	Arimidex	Femara	-
Structure	NS	S	S	NS	NS	NS
Spectrum of action	Inhibition of aromatse, cortisol, and aldosterone	Inhibition of aromatase, androgenic effect	Inhibition of aromatase only	Inhibition of aromatase only	Inhibition of aromatase only	Inhibition of aromatase only
Route of administration	PO	IM	PO	PO	PO	PO
Recommended dosage	1.0 mg bid	250 mg q 2w	25 mg od	1 mg od	2.5 mg od	2.5 mg od
Half-life	About 10 hours	5～10 days	～2 days	50 hours	24 hours	＜8 hours
Side effects	Skin rash, Nausea, Vomiting, Fatigue	Hot flushes, Skin rash, Vaginal bleeding,	Hot flushes Increased sweating, Nausea, Headache, Hair trimming	Diarrhea, Nausea, Weakness, Fatigue, Pain	Hot fushes, Headache, Nausea, Vaginal bleeding Musculoskeletal pain	Dyspnea, Fatigue, Hot flushes, Nausea, Vomiting

S = steroidal; NS = non-steroidal; PO = oral; IM = intramuscular; bid = twice daily; q 2w = every 2 weeks; od = once daily.

When tested in women with advanced breast cancer, aminoglutethimide produced tumor response rates comparable to those observed with tamoxifen.(*17*) However, the nonselective nature of its action often requires concomitant use of corticosteroids to maintain normal levels of cortisol. When aminoglutethimide was combined with a corticosteroid such as hydrocortisone, the regimen produced durable clinical responses in 30～50% of patients. But, patients receiving aminoglutethimide often experience side effects such as lethargy, dizziness, skin rash, orthostatic hypotention, and blockade of thyroxine synthesis.(*18*) The side effect profile of aminoglutethimide and nonselective aromatase inhibitors often makes these drugs less desirable therapeutic choices than currently available aromatase inhibitors.

SECOND GENERATION AROMATASE INHIBITORS

1) Fadrozole

Fadrozole is a non-steroidal imidazole aromatase inhibitor that is between 200 and 400 times more potent than amonoglutethimide when tested in microsomal preparations derived from rat ovaries and human placenta.(*19*) This agent is orally active and competitive aromatase inhibitor. While fadrozole is more selective than aminoglutethimide, it interferes with adrenal steroidogenesis to some extent.(*20,21*) Initial dose-seeking studies conducted in patients demonstrated effective aromatase inhibition at dose of 1.8～4.0 mg daily.(*22*) A phase II study demonstrated that 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.

Two large multicenter phase III trials in the USA compared fadrozole hydrochloride to megestrol acetate in 672 patients who had received only tamoxifen as prior hormonal therapy.(*23,24*) Final clinical results show that there were no significant differences between the two treatment arms of the trials with respect to time to progression (TTP), objective response rates, response duration or overall survival. In these two trials, responses to megestrol acetate were somewhat lower than expected from previous studies with objective response rates of 11 and 13% respectively.

Randomized patients receiving fadrozole experienced objective responses of 11 and 16% which did not differ significantly from these with megestrol. Stable disease for more than six months occurred in 25% of patients receiving fadrozole and 20% taking megestrol acetate.

Two trials compared fadrozole with tamoxifen as first-line therapy.(*25,26*) In the first, 1 mg twice daily of fadrozole was compared with 20 mg daily of tamoxifen in 212 postmenopausal patents with metastatic breast cancer. Response rates to tamoxifen (27%) and to fadrozole (20%) did not differ significantly nor did response durations (20 months versus 15 months). However, tamoxifen achieved a significantly longer time to treatment failure (TTF) (8.5 month vs. 6 months; $P < 0.05$). In the second study, fadrozloe was compared with tamoxifen as first line therapy in a randomized, controlled trial conducted in South Africa. Response rates to tamoxifen were 48% versus 43% with fadrozole (P=NS). However response duration was significantly longer with 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 demonstrate 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 treatments 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.

2) 4-Hydrozyandrostenedione (4-OHA, Formestane, Lentaron®)

Formestane (4-hydroxyandrostenedione, 4-OHA, Lentaron) is a structural analog of androstenedione and is thus a highly specific aromatase inhibitor. 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 (Ki=4.1μmM). Extensive studies revealed no estrogenic, antiestrogenic, or antiandrogenic properties.(*27*) However, transformation to 4-hydroxytestosterone occurs and androgenic effects can be demonstrated under certain circumstances.(*28*)

4-Hydroxyandrostenedione 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.(*29*) Höffken and colleagues(*30*) conducted a large trial of 4-OHA in postmenopausal women. Patients initially received 500 mg intramuscularly every two weeks for six weeks and then 250 mg every two weeks thereafter. Of 86 evaluable patients, there were two complete and 19 partial remissions (24%) and 26 with disease stabilization (30%). Studies of the degree of aromatase inhibition using isotopic kinetic techniques demonstrate that 4-hydroxyandrostenedione is not as effective as the third generation inhibitors in blocking estrogen production. For this reason, it is unlikely that this agent will compete successfully with the newer inhibitors.

THIRD GENERATION AROMATASE INHIBITORS

1) Exemestane

Exemestane is an irreversible (Type I, mechanism-based) steroidal aromatase inactivator.(*31-33*) Single dose administration reveals a major reduction of plasma estrogens with this compound.(*31*) A dose of 25 mg daily inhibited aromatase activity as documented by the isotope kinetic technique by 97.9%, with subsequent reduction superior to 85% of circulating estrogen level. Thurlimann et al(*32*) reported an objective response (CR and PR) in 12% and 33% of patients expressing primary or secondary resistance to aminoglutethimide. Two Phase II open label trials examined the effects of 25 mg of exemestane daily by mouth in patients with progressive disease after initial treatment with tamoxifen.(*34,35*) In the US trial (*34*), entry criteria included postmenopausal status and relapse while receiving tamoxifen for metastatic disease or within 12 months of discontinuing adjuvant tamoxifen. Of the 128 women entered, 28% experienced an objective response rate and 47% clinical benefit as defined as CR, PR, or stable disease for greater than 24 weeks. In the Euopean trial of 137 patients, 31% experienced objective responses and 59% clinical benefit.(*35*)

A phase III randomized, double-blind, multicenter study was conducted in 769 postmenopausal women who had progressed on tamoxifen. Kaufmann et al(*36,37*) compared the efficacy and safety of the exemestane with megestrol acetate in women with metastatic breast cancer. The objective response rate (ORR) in women treated with exemestane 25 mg once daily (n=366) was 15% while was 12.4% in women treated with megestrol acetate 40 mg four times daily. Statically significant differences favoring exemestane were seen in terms of time to progression (20.3 vs. 16.6 weeks, P=0.037), TTF (16.3 vs. 15.7 weeks, P=0.042), and overall survival (not reached vs. 123.4 weeks, P=0.039). Because the mechanism of action of exemestane is different from those of letrozole or anastrozole, it may explain its activity after failure of non-steroidal aromatase inhibitors. Although all third generation aromatase inhibitors (letrozole, anastrozole, and exemestane) are now considering as second-line use after failure on tamoxifen, exemestane may be reserved for third-line use after failure of non-steroidal aromatase inhibitors.

2) Anastrozole (Arimidex®)

Anastrozole is an orally active, non-steroidal competitive aromatase inhibitor. It was the first aromatase inhibitor to be approved in the United States for the management of advanced breast carcinoma on postmenopausal women since 1996. This approval was based of results of two pivotal trials that together accrued a total of 764 patients randomized to receive either anastrozole 1 mg p.o. q.d. or anastrozole 10 mg q.d. or megestrol acetate 40 mg qid.(*38*) 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 mg and the 10 mg daily dosages. Overall responses including complete and partial objective response rates and stabilization of disease of grater 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.(*39-41*) 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 megesterol acetate because it was better tolerated. It was associated with less undesirable weight gain, dyspnea, and fewer thromboembolic events when compared to megestrol acetate.(*38*) Since there were on differences between the two doses of anastrozole, the drug was approved at a dose of 1 mg daily.

With further maturity of this trial, anastrozole 1 or 10 mg daily conferred a survival advantage compared to the progestin (median of 26.7 months vs. 22.5 months).(*42*) The two-year survival was 56.1% for the group of patients receiving anastrozole 1 mg compared to 46.3% for patients treated with

megestrol acetate. The demonstration that anastrozole has superior efficacy with respect to overall survival and reduced side effects versus megestrol acetate would suggest that the aromatase inhibitor be used as second line therapy in preference to megestrol acetate.

3) Letrozole (Femara®)

Letrozole, a potent non-steroidal competitive aromatase inhibitor is now approved for second-line therapy in post-menopausal women with advanced breast cancer.(*44-46*) This agent possesses considerable selectivity for aromatase. In preclinical studies letrozole caused inhibition of aldosterone production in vitro only at concentrations 10,000 times higher than those required for inhibition of estrogen production. Letrozole is a highly potent and selective aromatase inhibition of estrogen production. When administered orally to adult female rats at a dose of 1 mg/kg/d for 14 days, letrozole decreases uterine weight to that observed after a surgical ovariectomy. At doses greater than 1,000 times higher than the concentration required to cause a 50% inhibition of the aromatase enzyme, letrozole dose not significantly suppress either aldosterone or corticosterone in rats. Letrozole also causes significant regression of DMBA-induced rat mammary tumors.(*45*)

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. The levels of estradiol were decreased by 95% to levels of 0.05～0.07 pg/ml as detected by this assay. Additional studies established the fact that letrozole was quite selective for the inhibition of aromatase since, over a wide dose range, there were no significant changes in the levels of gonadotropins, ACTH, cortisol, aldosterone, or TSH.(*46,47*) Early trials of letrozole in heavily pro-treated postmenopausal women with metastatic breast cancer demonstrated both clinical efficacy and lack of significant toxicity.

Dombernowsky et al(*43*) showed the data that letrozole 2.5 mg once daily to be more effective and better tolerated than megestrol acetate in the treatment of postmenopausal women with advanced breast cancer previously treated with antiestrogens. In this pivot trial, 551 postmenopausal women with metastatic breast carcinoma progressing after treatment with tamoxifen were randomized to receive either letrozole 0.5 mg daily, letrozole 2.5 mg daily or standard doses of megestrol acetate (160 mg daily). The women in the three treatment groups were comparable in all respects. The two doses of letrozole caused similar prompt and profound suppression of plasma and urinary estrogens. Letrozole 2.5 mg yielded a significantly higher overall response rate (24%) than either megetrol acetate (16%, P=0.04) or letrozole 0.5 mg (13%, P=0.004). The median duration of response for letrozole 2.5 mg was 33 month compared to 18 months for both megestrol acetate and the lower dose of letrozole. Similarly, there is trend in time to tumor progression and survival that favors the letrozole 2.5 mg dose. Letrozole was better tolerated than megestrol acetate with respect to serious adverse experiences, discontinuation due to poor tolerability, cardiovascular side effects, and weight gain.

In a similar study by Gershanovich et al(*44*) involving 555 postmenopausal patients with advanced breast cancer progressing after tamoxifen therapy, letrozole was compared to aminoglutethimide 250 mg twice daily with corticosteroid support. Letrozole 2.5 mg daily produced an objective response rate of 19.5% vs. 12.4% for aminoglutethimide. The median duration of response was 21 months for letrozole compared to 14 months for aminoglutethimide and 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 and letrozole are superior to megestrol acetate and aminoglutethimide.

Recently a third multicenter randomized study (US02) of letrozole in patients with advanced breast cancer progressing on tamoxifen has been published.(*48*) This study had a similar design to that of the first study noted above, in that patients were randomized to a standard dosage of megestrol acetate, letrozole 2.5 mg daily, or letrozole 0.5 mg daily. While the designs of the studies were similar, the results are somewhat different. In the US02 study, there was no difference in the objective response rates between megestrol acetate, letrozole 2.5 mg, and letrozole 0.5 mg. The TTP and TTF were significantly better in patients treated with letrozole 0.5 mg compared with those treated with megestrol acetate (P=0.018, respectively), while the time to death (TTD) was of borderline significance (P=0.053). There was no difference between letrozole 2.5 mg and megestrol acetate in terms of TTP, TTF, or TTD. There was a non-significant trend (P=0.073 and P=0.076) in favor of letrozole 0.5 mg versus 2.5 mg in terms of TTP and TTF, respectively. There was no difference between two doses in terms of TTD. The findings of this study are of interest in that letrozole 2.5 mg is currently recommended and

commercially available dose. These results also suggest that letrozole is in fact similar to other aromatase inhibitors in terms of the lack of unequivocal evidence of a dose response in large randomized clinical trials.

4) Vorozole

This agent is another third-generation, non-steroidal, oral aromatase inhibitor which is highly potent and specific for aromatase.(*49*) In preliminary trials, the usual 2.5 mg once-daily dose of this compound was well tolerated and produced good objective response rates, from 21～33%. Clinical efficacy appears to be similar to that of anastrozole and letrozole. 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). 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.

AROMATASE INHIBITORS VERSUS TAMOXIFEN AS FIRST LINE THERAPY

The fourth clinical question asked whether aromatase inhibitors are superior to tamoxifen. Two ongoing trials are designed to compare 1 mg of anastrozole daily with 20 mg of tamoxifen as first line therapy for metastatic breast cancer. A European trial, with entry of 668 patients reported objective response rates of 32.9% with anastrozole and 32.6% with tamoxifen.(*50*) And clinical benefit (i.e. CR, PR, or stable disease for ＞24 weeks) in 56.2% of patients receiving anastrozole and 56.5% on tamoxifen. No statistically significant differences emerged with respect to percent disease progression or median TTP. Survival data are not yet available. The second trial(*51*) entered 171 patients into the anastrozole arm and 182 into the tamoxifen arm. Objective responses occurred in 21.1% of patients receiving anastrozole and 17.0% on tamoxifen. Clinical benefit was observed in 59.1% of women on anastrozole and 45.6% on tamoxifen (P=0.005, two sided). TTP was significantly longer with anastrozole (11.1 months) than with tamoxifen (5.6 months) and the result was statistically significant (P=0.005). These two large trials suggest at least equivalent efficacy of aromatase inhibitors and tamoxifen overall, with a superiority for anastrozole in hormone-receptor positive tumors.(*52*) A longer period of observation is necessary to determine if survival differences will be observed in women receiving one or the other agent.

More recently, another European study has been reported comparing anastrozole with tamoxifen as initial endocrine therapy in patients with advanced breast cancer.(*53*) Patients entering this study had receptor-positive tumors. Anastrozole-treated patients showed a higher objective response and clinical benefit rate than patients who received tamoxifen. Anastrozole was associated with significantly longer TTP than tamoxifen (10.6 months vs. 5.3 months, retrospectively: P＜0.05). This study also reported that there was a survival advantage for the patients treated with anastrozole compared with tamoxifen (P＜0.05). This is the first study to report a survival advantage of a third-generation aromatase inhibitor over tamoxifen as first-line endocrine therapy for advanced cancer.

A potential advantage of the aromatase inhibitors is the lack of estrogenic effects associated with their use. Recent data suggest that the estrogen replacement therapy causes an increase in the rate of thromoembolic events in post-menopausal women. It is of interest then to examine the rate of these events in women receiving tamoxifen versus anastrozole. This is clearly of interest in terms of patients with advanced breast cancer, but it might be particularly significant in terms of adjuvant therapies, where patients tend to continue on endocrine agents for longer periods of time. In the two trials of similar design referred to above, anastrozole was associated with thromboembolic events in 4.8% and 4.1% and with tamoxifen in 7.3% and 8.2%.(*50,51*) These data suggest that the aromatase inhibitors might be preferable for patients with a history of prior thromboembolic events.

A large phase III randomized trial comparing letrozole versus tamoxifen as first-line endocrine therapy in patients with advanced breast cancer has also been reported.(*54*) 907 patients were randomly assigned letrozole 2.5 mg once daily (453 patients) or tamoxifen 20 mg once daily (454 patients). Patients had estrogen receptor- and/or progesterone receptor-positive tumors, or both receptors were unknown in this study. The primary end point was TTP. Secondary end points included overall objective response rate, its duration, rate and duration of clinical benefit, TTF, overall survival, and tolerability. TTP was significantly longer for letrozole than for tamoxifen (median, 41 v 26 weeks). Treatment with letrozole reduced the risk of progression by 30% (hazards ratio, 0.70; 95% CI=0.60 -0.82, P=0.0001). TTP was significantly longer for letrozole irrespective of dominant site of disease, receptor status, or prior adjuvant antiestrogen therapy. Similarly, TTF was significantly

longer for letrozole (median, 40 vs 25 weeks). ORR was higher for letrozole (30% vs 20%; P=0.0006), as was the rate of clinical benefit (49% vs 38%; P=0.001). Survival data are currently immature and not reported. While this is an important finding, it does not negate the superior initial control achieved with an aromatase inhibitor over tamoxifen, which has now been recorded for both letrozole and anastrozole in advanced breast cancer.

RELATIVE EFFICACY OF THIRD GENERATION INHIBITORS

Table 2 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 (ie. 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. TTP 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). There is no direct comparison of any of these three aromatase inhibitors as first-line therapy. Any comparison, therefore, must be by indirect assessment of how they have each compared versus tamoxifen in the studies detailed above. Such interstudy comparisons are fraught with difficulties (e.g. different entry criteria and different patient populations) and should be interpreted with much caution. In a recent review, Buzdar(*55*) summarized the main clinical outcomes. In this article, the percentage of patients with hormone-receptor-positive tumors is different in each study, and therefore of particular interest is the comparison of the three aromatase inhibitors in the subgroup of patients with receptor-positive tumors. This showed that for the primary objective of the studies (i.e. TTP), the benefit of each aromatase inhibitor over tamoxifen was remarkably similar.

THIRD GENERATION AROMATASE INHIBITORS IN THE ADJUVANT SETTING

Trials are now ongoing to determine the efficacy of aromatase inhibitors versus tamoxifen versus 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) for 5 years and enrolled a total of 9366 patients. The ATAC trial recently reported the first efficacy data from trials of third-generation aromatase inhibitors as adjuvant therapy.(*56,57*) The mean age and mean weight of the patients and the hormone receptor status of the primary tumors were well balanced between three arms, as were primary treatment, tumor size and grade, and nodal status (Table 3). The primary end-points were disease-free survival and tolerability. After a median of 33 month's follow-up and a median duration of treatment of 30.7 months, anastrozole monotherapy was found to be significantly more effective in prolonging disease-free survival than tamoxifen. Only 317 of 3,125 women in the anastrozole group had a relapse of their breast cancer or died compared with 379 of

Table 2. Comparison of third generation aromatase inhibitors

Response parameters	Vorozole[62]	Anastrozole[54]	Letrozole[55,56]
Overall survival	25.7 months	26.7 months	28 months[56] 25.3 months[55]
Objective response rate (CR+PR)	10.5%	10.3%	19.5% 23.6%
Clinical benefit (CR+PR+stable＞6 months)	47%	35%	36.3% 35%
Time to progression	2.7 months	5 months	3.4 months 5.6 months
Number in study	452	764	555 551

CR = complete response; PR = partial response.

Table 3. Study design of the ATAC trial

ATAC Trial: Arimidex, Tamoxifen, Alone or in Combination	
Eligibility	Postmenopausal, stage 1 & 2 operable breast cancer, post-primary treatment
ARM 1 (n=3125)	Anastrozole 1 mg+placebo×5 years
ARM 2 (n=3116)	Tamoxifen 20 mg+placebo×5 years
ARM 3 (n=3125)	Anastrozole 1 mg+Tamoxifen 20 mg×5 years

		ARM 1	ARM 2	ARM 3
Mean age (years)		64.1	64.1	64.3
Mean weight (kg)		70.8	71.1	71.3
Previous HRT (%)		35.6	35.4	35.3
Receptor status (%)	Positive	83.7	83.4	84.0
	Negative	8.3	8.7	7.6
	Other	8.0	7.9	8.4
Primary treatment (%)	Mastectomy	47.8	47.3	48.1
	Padiotherapy	63.3	65.2	62.0
	Chemotherapy	22.3	20.8	20.8
	Tamxifen before surgery	1.6	1.6	1.7

3,116 women in the tamoxifen group (P=0.013; hazard ratio= 0.83, CI=0.71-0.96). This represents a 17 percent reduction in the risk of breast cancer recurring with anastrozole treatment compared to tamoxifen. The anastrozole/tamoxifen combination showed no additional efficacy or tolerability benefits compared with tamoxifen alone (P=0.772). Among women with confirmed hormone-sensitive tumors, the reduction in risk with anastrozole compared to tamoxifen was even more striking, at 22 percent (P=0.005; hazard ratio=0.78, CI=0.65-0.93). The reduction in disease events was seen at all sites of disease further confirming that the results do represent an overall improvement in efficacy of anastrozole over tamoxifen. The reduction was most marked in the reduction of contralateral breast cancers in favor of anastrozole compared with tamoxifen (P=0.007; hazard ratio=0.42, CI=0.22-0.79). These results are summarized in Table 4.

Table 4. Summary of results from the ATAC trial

A. Disease-free survival (all patients)

	Hazard ratio	95.2% CI	P-value
AN vs TAM	0.83	0.71-0.96	0.013
Comb vs TAM	1.02	0.89-1.18	0.772

B. Disease-free survival (receptor-positive patients)

	Hazard ratio	95.2% CI	P-value
AN vs TAM	0.78	0.65-0.93	0.005
Comb vs TAM	1.02	0.87-1.21	0.8

C. Contrlateral breast cancer

	Hazard ratio	95.2% CI	P-value
AN vs TAM	0.42	0.22-0.79	0.007
Comb vs TAM	0.84	0.51-1.40	0.5

AN = anastrozole; TAM = tamoxifen; Comb = combination.

Anastrozole was also found to have many important tolerability advantages over tamoxifen. Most strikingly, anastrozole was associated with significantly fewer reports of endometrial cancer when compared with tamoxifen. This finding was supported by a significantly lower incidence of vaginal bleeding and vaginal discharge among anastrozole-treated patients compared to those taking tamoxifen. Another known risk associated with tamoxifen is thromboembolic events. In the ATAC trial, both the overall incidence of thromboembolic events and that of deep vein thromboses were significantly reduced in the anastrozole group. Important from the patient's perspective, the incidence of hot flushes and weight gain were also significantly reduced. However, as expected, women taking tamoxifen did have a lower risk of experiencing musculo-skeletal disorders or the types of fractures common in this age group compared with those taking

anastrozole.

Recently, the ATAC trials group reported the more updated results, based on a mean follow-up of 47 months for disease-free survival.(*58*) The updated hazard ratios, 95.2% CIs, and *p*-values for the anastrozole vs tamoxifen comparison are as follows: disease-free survival, 0.86 [0.76-0.99], P=0.030; disease-free survival in hormone-receptor positive population, 0.82 [0.70-0.96], P=0.014; time to recurrence (TTR), 0.83 [0.71-0.96], P=0.015; and TTR in hormone-receptor positive population, 0.78 [0.65-0.93], P=0.007. Reductions in contralateral breast cancer rates remained in favor of anastrozole (objective response=0.62 [0.38-1.02], P=0.062), with statistical significance achieved in the hormone-receptor positive sub-group (objective response=0.56 [0.32-0.98], P=0.042).

Secondary issues in these trials are the differential actions of the anti-estrogens and aromatase inhibitors on non-breast tissues. Tamoxifen acts as an estrogen agonist on uterus and increase 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. Long-term tolerability is a key issue in the adjuvant setting as women usually take treatment for at least five years.

AROMATASE INHIBITORS AS NEOADJUVANT ENDOCRINE THERAPY

Neoadjuvant therapy, administered prior to surgery in an attempt to shrink the primary tumor has been used to treat large operable and locally advanced breast cancers. Dixon et al(*59*) conducted a trial comparing the use of aromatase inhibitors and tamoxifen in postmenopausal patients with estrogen receptor-positive locally advanced T4b, N0-1, M0 and large operable breast cancers T2＞3 cm, T3, T4, N0-1 and M0. Patients have been treated with 2.5 mg letrozole (12 patients), 10 mg letrozole (12 patients), 1 or 10 mg anastrozole (24 patients) and 20 mg tamoxifen (65 patients). There was no apparent difference in response rate between 2.5 and 10 mg letrozole. Median clinical, mammographic and ultrasound reductions in tumor volumes for patients treated with letrozole were 81% (95% confidence interval (CI) 66-88), 77% (95% CI=64-82) and 81% (95% CI=69-86) respectively and for anastrozole, values were 87% (95% CI=59-97), 73% (95% CI=58-82) and 64% (95% CI=52-76) respectively. This compares with a median reduction in tumor volume for tamoxifen-treated patients as assessed by ultrasound of 48% (95% CI=27-48). The authors suggested from this study that the aromatase inhibitors, letrozole and anastrozole, might be effective agents in the neoadjuvant setting. Further studies will be required to document this possibility.

ADAPTIVE HYPERSENSITIVITY HYPOTHESIS

One of the possible explanations 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 than 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 pg/ml. 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.

Santen laboratory tested the estradiol hypersensitivity hypothesis directly in an in vitro cell culture system.(*60*) 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 for 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.

Shim et al(*61*) also confirmed the hypersensitivity to estradiol in the in vivo study using a nude mouse model. Ovariectomized animals were inoculated with wild type MCF-7 cells on one flank of the body and long term estrogen deprived (LTED) cells on the other flank. Plasma E_2 levels of the animals were clamped to 1.25, 2.5, 5, 10, and 20 pg/ml by silastic implants containing different doses of E_2. Tumor growth was monitored for a period of two months. Growth of LTED cells was stimulated by very low doses of estradiol which did

not affect he growth rate of wild type MCF-7 xenogrfts. At higher doses of E_2, however, growth rate of wild type cells exceeded that of LTED cells (Fig. 3). This observation was consistent with in vitro data that long term estrogen deprivation enhanced sensitivity of MCF-7 cells to both stimulatory and inhibitory effect of estrogen.

Long term exposure to tamoxifen might also result in development of hypersensitivity to estradiol. Under these circumstances, a maked reduction of estradiol synthesis with an aromatase inhibitor would result in tumor regression. Take 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.

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

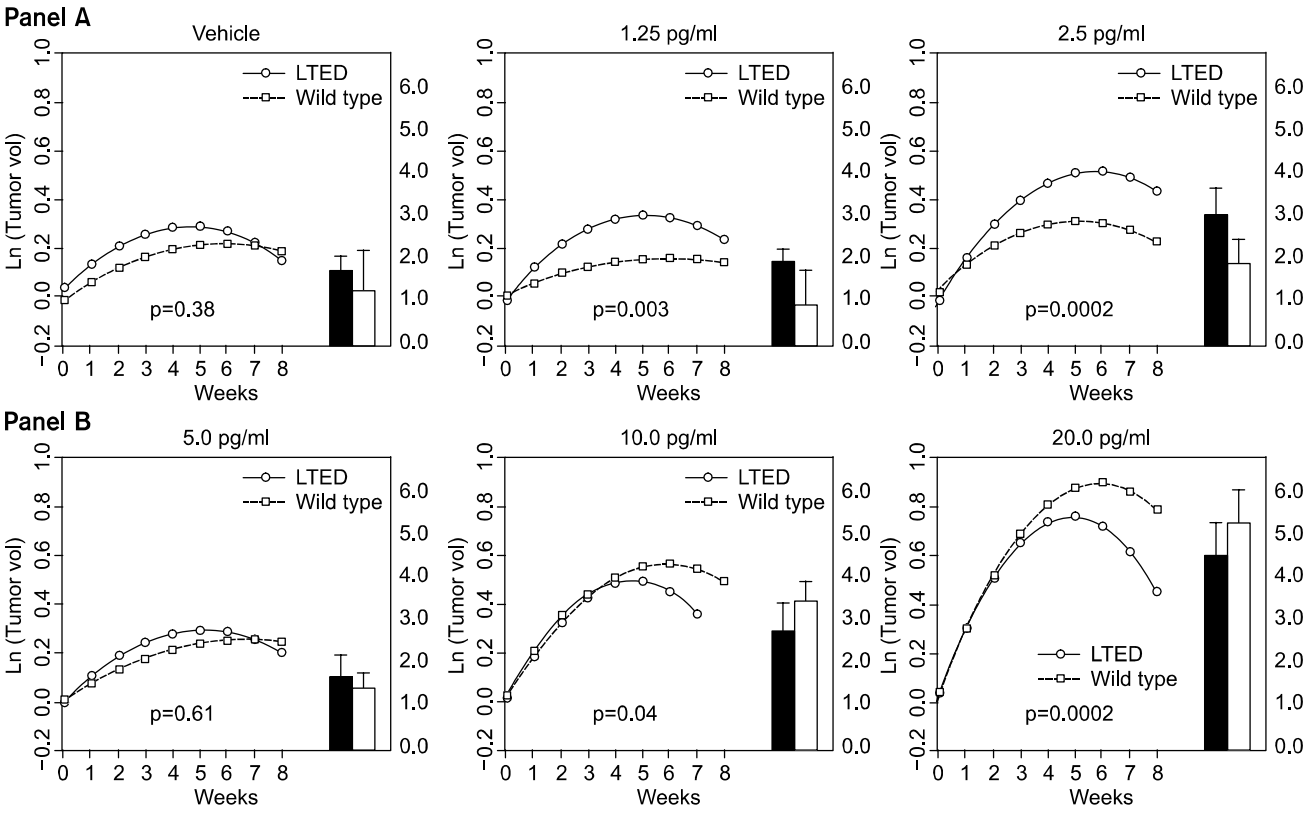

Fig. 3. Panel A. Growth curves in wild-type and long-term estrogen-deprived (LTED) tumors in oophorectomized nude mice receiving only cholesterol-containing Silastic implants (vehicle control) and implants maintaining plasma estradiol levels at 1.25 and 2.5 pg/ml. The statistical significance of the differences between the wild-type and LTED tumors is indicated on each panel. The accompanying bars standard error of the mean represent mean area under the curve for each group, and are shown to illustrate variance among groups. The black bars are representative of volumes of LTED tumors and the crosshatched bars of wild-type tumors. The statistical significances indicated represent paired comparisons between integrated tumor volumes and not between mean areas under the various curves. Integrated tumor volumes were significantly higher in the LTED than the wild-type tumors in response to 1.25 and 2.5 pg/ml, but not in oophorectomized animals. Panel B. Growth curves in wild-type and LTED tumors with plasma estradiol clamped at 5, 10, and 20 pg/ml.

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.(*62*)

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. 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.

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 the number of cell divisions. 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 estradiol-3, 4-quinone. The compound can bind co-valently to guanine 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 (*63*) have reported that 4-OHA prevents NMU-induced rat mammary carcinoma and Steele and colleagues(*64*) have shown that fadrozole completely inhibits the development of spontaneous breast tumors in aging Sparague-Dawley rats.

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 60-year old women, 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 know 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 intra-tumoral synthesis of estrogen by aromatase. Theses compounds are emerging as a valuable approach to the treatment of hormone dependent breast cancer.

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