REVIEW ARTICLE

Aromatase Inhibitors in the Treatment and Prevention of Breast Cancer

By Paul E. Goss and Kathrin Strasser

Purpose: **The purpose of this article is to provide an overview of the current clinical status and possible future applications of aromatase inhibitors in breast cancer.**

Methods: **A review of the literature on the third-generation aromatase inhibitors was conducted. Some data that have been presented but not published are included. In addition, the designs of ongoing trials with aromatase inhibitors are outlined and the implications of possible results discussed.**

Results: **All of the third-generation oral aromatase inhibitors—letrozole, anastrozole, and vorozole (nonsteroidal, type II) and exemestane (steroidal, type I)—have now been tested in phase III trials as second-line treatment of postmenopausal hormone-dependent breast cancer. They have shown clear superiority compared with the conventional therapies and are therefore considered established second-line hormonal agents. Currently, they are being tested as first-line therapy in the metastatic, adjuvant, and neoadjuvant settings. Preliminary results suggest that the inhibitors might displace tamoxifen as first-line treatment, but further studies are needed to determine this.**

Conclusion: **The role of aromatase inhibitors in premenopausal breast cancer and in combination with chemotherapy and other anticancer treatments are areas of future exploration. The ongoing adjuvant trials will provide important data on the long-term safety of aromatase inhibitors, which will help to determine their suitability for use as chemopreventives in healthy women at risk of developing breast cancer.**

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SEVERAL CLASSES OF endocrine agents that antagonize the effects of estrogen are useful in the treatment of estrogen receptor (ER)–positive breast cancer.[1] For example, selective estrogen receptor modulators (SERMs) and pure antiestrogens antagonize ER function by binding competitively to the receptor. Steroidal antiestrogens additionally reduce ER concentration by inducing estrogen receptor degradation.[2] Surgical, medical, and radiation-induced ovarian ablation and aromatase inhibitors antagonize the action of estrogen by reducing its levels both in the circulation and in normal and malignant breast tissue.

Aromatase (estrogen synthetase) inhibitors have become the established second-line treatment for ER-positive metastatic breast cancer after the SERM tamoxifen. The third-generation aromatase inhibitors are currently being compared with tamoxifen in first-line metastatic, adjuvant, and neoadjuvant settings. Should they prove superior to tamoxifen in terms of disease response, toxicity, and, most importantly, patient survival, they might replace tamoxifen as first-line endocrine therapy. Based primarily on a superior side effect profile, anastrozole has recently been approved as first-line therapy of metastatic breast cancer in several countries. The efficacy and excellent tolerability of the newer aromatase inhibitors in the treatment of breast cancer might lead to their use as chemopreventives in healthy women considered at significant risk of developing breast cancer. To this end, studies are underway to investigate their ability to alter surrogate markers of breast cancer risk.

In this article, the rationale for the use of aromatase inhibitors in breast cancer treatment, their mechanism of action, and preclinical test systems used in their evaluation are briefly reviewed. The current clinical status of third-generation aromatase inhibitors is discussed and ongoing clinical trials of these agents are described. Possible future applications of aromatase inhibitors in the treatment and prevention of breast cancer are also outlined.

There may be specific biologic and pharmacologic reasons for giving aromatase inhibitors after tamoxifen. On the

From the Division of Hematology/Oncology, Princess Margaret Hospital, Toronto, Ontario, Canada.

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Over the past 10 years, P.E.G. has received industry funding for investigator-initiated clinical and laboratory studies of aromatase inhibitors as well as honoraria for presenting papers or acting in a scientific advisory capacity. Support of this nature has been received from manufacturers of all of the third-generation inhibitors that have been tested and/or approved for use, including vorozole (Janssen Ortho Inc, North York, Toronto, Ontario), letrozole (Novartis Pharmaceuticals Canada Inc, Dorval, Quebec), exemestane (Pharmacia & Upjohn, Mississauga, Ontario), anastrozole (AstraZeneca, Mississauga, Ontario, Canada), and liarozole (Janssen Ortho). K.S. has not received any financial support from industry.

Address reprint requests to Paul E. Goss, MD, PhD, Division of Hematology/Oncology, Princess Margaret Hospital, 610 University Ave, Toronto, ON M5G 2M9, Canada; email: pegoss@interlog.com.

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other hand, the inhibitors may be more effective than tamoxifen if given as first-line treatment. For these reasons and also because tamoxifen is the current standard of care as first-line hormonal therapy for metastatic disease, as adjuvant therapy and as an approved chemopreventive in the United States, we have structured this review as aromatase inhibitors after tamoxifen, as first-line therapy, and in combination with other agents.

INHIBITING ESTROGEN SYNTHESIS AS A THERAPEUTIC TARGET

Aromatase is the enzyme complex responsible for the final step in estrogen synthesis, viz the conversion of the androgens androstenedione and testosterone to the estrogens estrone (E_1) and estradiol (E_2). There are substantial data showing that estrogen promotes and probably initiates breast cancer.[3] Inhibiting estrogen at the source of its synthesis is therefore a logical target of breast cancer treatment.

The sites of estrogen synthesis include the ovaries of premenopausal women; extragonadal sites such as fat, muscle, and skin; normal breast stromal cells; and breast tumor tissue. After ovarian failure, estrogen is synthesized in peripheral tissues and circulates at low, relatively nonfluctuating levels.[4,5] This peripheral aromatization in postmenopausal women is almost completely inhibited by single-agent administration of any of the third-generation inhibitors.[6,7] In contrast, there is a barrier to using aromatase inhibitors as monotherapy in premenopausal women. First, high levels of androstenedione compete initially with the inhibitors as substrate for the enzyme complex and consequently estrogen synthesis is not completely blocked.[8-10] Second, suppression of estrogen results in a reflex increase in gonadotrophin levels, provoking an ovarian hyperstimulation syndrome, which causes a steep increase of aromatase in the ovary and in turn overcomes, at least in part, the initial blockade to estrogen synthesis by the inhibitor.[11] However, although both type I (steroidal) and type II (nonsteroidal) inhibitors compete initially with the androgen precursors for the enzyme, the type I inhibitors subsequently inactivate the enzyme irreversibly, thus being referred to as suicide inhibitors. Therefore, with ongoing exposure to type I inhibitors ovarian estrogen synthesis might in principle be more completely suppressed. However, in premenopausal women given the second-generation inhibitor formestane this was not the case and estradiol levels were not significantly suppressed by monotherapy.[12] Thus to date, aromatase inhibitors have been tested predominantly in combination with GnRH-analogs in premenopausal women. However, with the more potent third-generation type I suicide inhibitor exemestane, the possibility of monotherapy in premenopausal women merits further investigation at standard and higher doses.

Increasingly, the female breast has itself been recognized as another important site of estrogen production. Stromal cells in breast adipose tissue produce estrogen that is biologically active in both a paracrine and an autocrine manner.[13] This is probably responsible for the observation that estrogen concentrations in the healthy breasts of postmenopausal women are unexpectedly higher (four- to sixfold) than in serum and similar to those in premenopausal women.[14] In addition up to 70% of breast cancer cells have been shown to synthesize estrogen as a result of intracellular aromatase expression.[15-18] This explains why aromatase expression and activity are higher in breast tumors than in peritumoral fat and in tumor-bearing quadrants of the breast compared with those without tumors.[19-23] There is increasing evidence that this local estrogen production may play a major role in tumor proliferation.[24-27] Intratumoral aromatase has been linked to response to the aromatase inhibitor aminoglutethimide[18,28] but surprisingly not to estrogen receptor expression.[18,29] Despite similar depletion of serum estrogen levels with the current third-generation aromatase inhibitors, variability in patient outcome on these drugs could be attributable to differences in inhibition of local estrogen synthesis.

MODELS FOR EVALUATING AROMATASE INHIBITORS

Potency and Reversibility

For in vitro assessment of aromatase inhibitory capability, microsomal preparations from rat ovaries or from human placenta are used.[30,31] Inhibition of the enzyme and potency of the inhibitor are determined by the amount of tritiated water released in the assay. By washing the microsomal preparations and measuring residual inhibition of aromatase, the inhibitor can be classified as reversible or irreversible.

Depletion of serum estrogen levels has been used as a measure of the potency of aromatase inhibitors in blocking estrogen synthesis in peripheral tissues. However, using traditional assays, suppression below the detection limit has been noted with all of the third-generation inhibitors. This has made differentiating them clinically from one another difficult. In part this has been overcome by using a highly sensitive isotopic kinetic assay that relies on infusing [7^3H]androstenedione and [4^{14}C]estrone and measuring the conversion of androstenedione to E_1 and E_2. This assay has been used in male rhesus monkeys and in both healthy male volunteers and female breast cancer patients.[31,32] Recently, more sensitive antibodies have also been developed. These have allowed differences in serum estrogen suppression to

Table 1. Classification of Aromatase Inhibitors

	First Generation	Second Generation	Third Generation
Nonsteroidal	Aminoglutethimide	Rogletimide	Anastrozole
		Fadrozole	Letrozole
			Vorozole
Steroidal		Formestane	Exemestane

be demonstrated in postmenopausal women given various third-generation inhibitors.[33]

Selectivity

By incubating adult hamster ovarian tissue with luteinizing hormone, the production rates of estrogen, progesterone and testosterone can be determined. Differences in the concentration that inhibits 50% for these steroid hormones are correlated with selectivity of suppression, an important feature of third-generation aromatase inhibitors.[34]

Antitumor Activity and Chemopreventive Potential

The animal models that have been used to demonstrate antitumor efficacy have included the hormone-dependent carcinogen-induced MNU and DMBA rat mammary tumors[35,36] and spontaneous tumors in Sprague-Dawley rats.[37] Several scenarios analogous to the clinical status of patients can be evaluated in these models. For comparability to treatment of breast cancer, reduction of established tumors and inhibition of tumor multiplicity are used. To determine their chemopreventive effects, aromatase inhibitors have been given before or after carcinogen administration. Inhibition of tumor formation in these animals is viewed as a surrogate model for prevention of tumor initiation or promotion in humans.[36]

The recently developed aromatase-transgenic mouse model (*int-5/aromatase*) allows evaluation of the effects of aromatase inhibitors on aromatase-overexpressing breast tissue.[25] In these ovariectomized mice, aromatase overexpression leads to increased estrogenic activity specifically in the mammary glands, resulting in the initiation of various preneoplastic changes such as hyperplasia and dysplasia. The ability of inhibitors to block or reduce these effects has been tested.[26]

A useful model for assessing the effects of inhibitors directly on intratumoral aromatase is the MCF-7_{CA} cell line. This is an MCF-7 cell line transfected with the human placental aromatase gene (*MCF-7_{CA}*), which results in a 10-fold increase in the expression of aromatase. When xenografted in athymic nude mice, which have been ovariectomized, this cell line is able to act directly as an estrogen "pump."[38,39] Inhibition of tumor growth or of uterine hypertrophy can therefore be used as a measure of an inhibitor's effect on intratumoral aromatase activity.

CLASSIFICATION OF AROMATASE INHIBITORS

Aromatase inhibitors have been classified in a number of different ways, including first-, second-, and third-generation; steroidal and nonsteroidal; reversible (ionic binding), and irreversible (suicide inhibitor, covalent binding)[40-42] (Table 1). A figure of the structures of the most important aromatase inhibitors is presented in Fig 1.

The clinical significance of classifying the third-generation inhibitors is uncertain. In the presence of ongoing drug administration, it is arguable whether irreversibility of enzyme inhibition is relevant. On one hand, comparable depletion of circulating estrogen in postmenopausal women to below the level of sensitivity of traditional radio-immunoassays has been reported with either reversible or irreversible third-generation inhibitors. However, as mentioned previously, more sensitive assays recently developed have helped to distinguish the capability of the different inhibitors in suppressing estradiol levels. Furthermore, irreversible inhibition of aromatase may be relevant in suppressing premenopausal ovarian estrogen synthesis as mentioned above, and enzyme-binding characteristics may also be

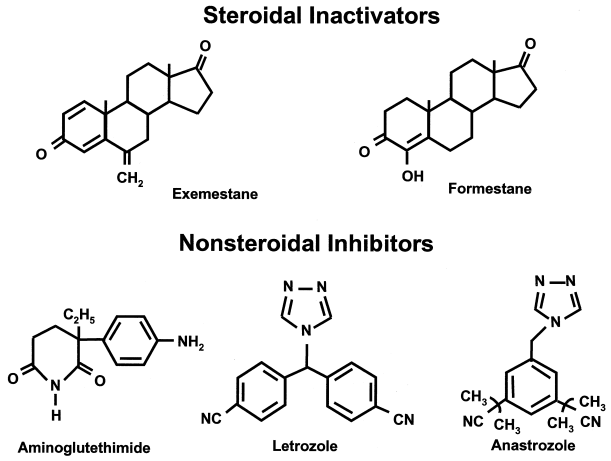

Fig 1. Structures of aromatase inhibitors.

important in the development of clinical resistance to different classes of aromatase inhibitors. Steroids (eg, exemestane) also impart to an inhibitor the potential to affect other steroid levels (eg, androgens), either directly by the parent compound or indirectly by its metabolites. This could be relevant to mechanisms of tumor resistance and also might influence the potential of steroidal inhibitors to act as chemopreventives and to exert effects on other systems such as bone and lipid metabolism. Thus dissimilarities between the two nonsteroidal third-generation reversible inhibitors letrozole and anastrozole and the recently approved steroidal third-generation irreversible inhibitor exemestane may afford different clinical applications and therapeutic indices for these compounds.

AROMATASE INHIBITORS AS MONOTHERAPY

After Tamoxifen

There are at least two preclinical observations suggesting that aromatase inhibitors may be particularly suitable after initial treatment with tamoxifen. First, in vitro hormone-dependent MCF-7 cells develop estrogen hypersensitivity when passaged in estrogen-deprived media.[43] This leads to growth response to estrogen in concentrations four orders of magnitude lower than usually required.[43] In vivo experiments have also shown that MCF-7 cells in nude mice initially regress in response to tamoxifen but are later stimulated by its weak estrogen agonist properties.[44] Second, estrogen-deprived MCF-7 cells develop upregulation of aromatase, which in turn may result in increased autocrine stimulation by estrogen.[43] In principle, tamoxifen might have the same effect.

Thus, theoretically, cessation of tamoxifen in a patient with disease progression and initiation of an aromatase inhibitor might simultaneously withdraw tamoxifen's estrogen agonist effect and deplete both locally produced and circulating estrogen to which the disease may be exquisitely sensitive.[43,45]

These principles have been tested in several trials of aromatase inhibitors as second-line hormonal therapy in patients who experience disease progression while receiving tamoxifen. In this context, first-line endocrine therapy with tamoxifen means both as adjuvant and as first-line treatment for metastatic disease and both types of patients were enrolled in the metastatic second-line trials discussed below. Studies of aromatase inhibitors as third-line therapy are included, because most patients in these trials were also treated with tamoxifen as first-line therapy.

The same strategy of giving an aromatase inhibitor after tamoxifen is being extensively studied in the adjuvant setting, and these trials are also discussed in detail below. Finally, although the potential of aromatase inhibitors as monotherapy and single-agent treatment in chemoprevention is discussed in the next section, it is conceivable that the strategy of tamoxifen followed by an aromatase inhibitor might also be applicable in this setting.

After tamoxifen as second-line therapy of metastatic disease. For many years the progestin megestrol acetate and the first-generation aromatase inhibitor aminoglutethimide were the standard of care as second-line hormonal treatment of postmenopausal metastatic breast cancer after tamoxifen. Because they showed comparable clinical efficacy despite their different mechanisms of action, it was believed that the maximum potential of endocrine therapy had been reached. The side-effect profiles of these drugs, however, are clearly troublesome and frequently lead to toxicity-related withdrawal of treatment.

The third-generation nonsteroidal aromatase inhibitors anastrozole, letrozole, and vorozole and the steroidal inhibitor exemestane have significantly superior toxicity profiles compared with those of these conventional therapies and, to some extent, greater clinical efficacy. They have now all been studied as second-line therapy after tamoxifen against megestrol acetate, and letrozole and vorozole have also been compared with aminoglutethimide.[46-55] Table 2 lists the results of these trials, including those from the recently published exemestane versus megestrol acetate trial. Only the doses that were approved for use are presented. Data from the two trials of anastrozole versus megestrol acetate were combined because the trial designs were identical. Significant efficacy and/or toxicity advantages were demonstrated for all of the inhibitors. Furthermore, none of them were significantly inferior to the comparator in any end point of efficacy. Importantly, in all trials, the third-generation aromatase inhibitors showed a significant advantage over standard treatment in at least one end point of toxicity. In particular, they were all clearly superior to megestrol acetate in terms of weight gain. The toxicity profiles of the third-generation inhibitors are similar, with the most common adverse events being nausea, vomiting, hot flashes, fatigue, and headaches. Importantly, the toxicity profiles reported from these trials are influenced by the fact that the patients were coming off treatment with tamoxifen (with its long half-life), and more accurate assessment will be possible from the first-line metastatic and adjuvant trials.

In the studies that evaluated and reported quality of life, significant improvements compared with the conventional therapies were seen. None of the third-generation aromatase inhibitors have been compared head-to-head, and because of clear differences in trial designs and patient populations, the present studies are not comparable, either in terms of toxicity or efficacy. This has been reviewed in detail by

Table 2. Second-Line Therapy With Aromatase Inhibitors

	ANA *v* MA[47, 48] (1 mg)	LET *v* MA[51] (2.5 mg)	VOR *v* MA[54] (2.5 mg)	FAD *v* MA[57] (2 mg)	FAD *v* MA[57] (2 mg)	EXE *v* MA[55] (25 mg)	FOR *v* MA[58] (250 mg IM)	LET *v* AG[50] (2.5/500 mg)	VOR *v* AG[53] (2.5/500 mg)
No. of Patients	263/253	174/189	225/227	196/184	152/151	336/403	91/86	185/178	277/279
Response rate (complete + partial response), %	12.6/12.2	**24/16**	11/8	11.3/16.3	13.4/11.5	15/12.4	16.7/16.9	19.5/12.3	23/18
Complete response + partial response + stable disease > 24 weeks, %	42.2/40.3	35/32		35.9/35.9	37.4/41.2	37.4/34.6	42.2/38.6	36.3/29.3	**47/37**
Median TTP, months		5.6/5.5	2.7/3.6	3.9/3.8	5.3/5.8	**4.7/3.8**		**3.4/3.2**	7/6
Median TTF, months		**5.1/3.9**				**3.8/3.7**	4/3.7	**3/3**	**5.3/4.4**
Median OS, months	**27/23**	25/22	26/29	27.1/23.1	25.8/27.9	**NR/28.4**		**28/20**	25.7/21.7
Increased weight/appetite, %	3/13	2/9	1.3/13.7			2.8/5.8	20/32§		
Edema, %	8/13		7/10/2011	12.2/21.2	11.8/18.8				
Hot flashes, %	14/11		19.6/7	11.7/9.2	14.5/11.4	12.6/5.0	15/9†	4.9/3.4	
Thromboembolic disease, %	3/5	0/8					3/9		
Sweating, %	2/6						20/32*		
Dyspnea, %	11/23		14.2/25.1	7.7/23.4	14.5/28.2	0.3/3.0			
Nausea, %	18/23		20.4/11	21.9/13	36.2/11.4	9.2/5.0		10.3/9.6	
Vomiting, %	10/7			9.2/4.9	18.4/7.4	2.8/0.8		3.8/5.6	
Anorexia, %	7/5			9.2/3.8	19.7/6				
Skin rash, %						2.0/0			
Quality of life		LET > MA	VOR > MA			EXE > MA‡			VOR > AG

NOTE. The two FAD *v* MA trials were of similar design; significant results are printed bold.

Abbreviations: ANA, anastrozole; MA, megestrol acetate; LET, letrozole; VOR, vorozole; FAD, fadrozole; EXE, exemestane; AG, aminoglutethimide; NR, not reached.

*More than 3 kg.

†Moderate and severe.

‡In general, but not on all subscales.

§More than 3 kg.

Hamilton and Piccart[56] for the trials with anastrozole, vorozole, and letrozole. Thus although letrozole and exemestane seem to have performed particularly well compared with the other inhibitors in terms of efficacy, further studies will be needed to confirm this. For example, a trial of letrozole versus anastrozole as second-line therapy after tamoxifen is ongoing.

There are two second-generation inhibitors that although not widely used are on the market. Fadrozole, a nonsteroidal inhibitor, is currently marketed in Japan. It was also tested in second-line as treatment of postmenopausal metastatic breast cancer after tamoxifen and showed efficacy and toxicity comparable to that of megestrol acetate[57] (Table 2). The steroidal inhibitor formestane (4-OH-androstenedione) showed advantages over megestrol acetate as second-line treatment of metastatic breast cancer in terms of efficacy and tolerability but is administered intramuscularly, which is associated with injection-site reactions[58] (Table 2).

Liarozole, a novel agent with a dual mechanism of action viz potent inhibition of aromatase and of retinoic acid catabolism (a retinoic acid metabolism–blocking agent), has been withdrawn from clinical development for reasons of predominantly retinomimetic toxicities. Nevertheless, in phase II studies in postmenopausal patients, liarozole showed promising activity in both ER-positive disease after tamoxifen and in ER-negative breast cancer.[59,60]

In summary, the third-generation aromatase inhibitors have now become standard second-line treatment of advanced breast cancer because of their better toxicity profile and improved clinical efficacy compared with conventional therapies. Ongoing and future trials will allow comparisons in terms of efficacy and tolerability between the different agents. In the near future they might also partially supplant tamoxifen as first-line treatment as outlined below.

After tamoxifen as third-line therapy of metastatic disease. Exemestane is the only aromatase inhibitor that has been tested in phase II trials as third-line therapy, after tamoxifen and then megestrol acetate had been given.[61,62] Thirty percent of patients experienced clinical benefit (ie, complete response plus partial response plus stable disease for $\geq$ 67 months) in this trial. Other studies have tested aromatase inhibitors as third-line hormonal therapy after another inhibitor had been given as second-line treatment (Table 3).[63-66] Only phase II results are available to date,

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