

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 (non-steroidal, 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 there-

fore 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

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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.

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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 non-fluctuating 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 mono-

therapy 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 six-fold) 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 [3H]androstenedione and [^{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 Fadrozole	Anastrozole Letrozole Vorozole
Steroid		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

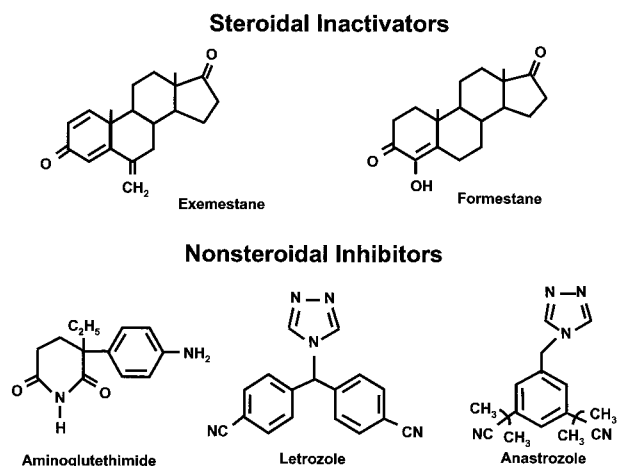


Fig 1. Structures of aromatase inhibitors.

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 Piccart⁵⁶ 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 acetate⁵⁷ (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 reactions⁵⁸ (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 ≥ 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,

Table 3. Aromatase Inhibitors as Third-Line Therapy

	VOR After FOR	ANA After FOR	FOR After AG	EXE After AG	EXE After MA	EXE After MA	EXE After Nonsteroidal
Reference	63	64	65	66	61	62	67
No. of patients	9	21	112	78	91	85	241
Previous TAM	Not reported	100% resistant	98%	96%	100% resistant	100% resistant	Not reported
E1, %	-47				-11		-61*/+27†
E2, %	-30				-22		-51*/+13†
E1-sulphate, %	-70			-89*/-56†	-13		-59*/+17†
Complete + partial response, %		0	21	26	13	9	6.6
Complete response + partial response + stable disease, %		62	43	39	30	29	24.0
Median TTP, months				4.9	2	16	14.7

Abbreviations: TTP, time to progression; nonsteroidal, nonsteroidal aromatase inhibitor.

*After AG.

†After other nonsteroidal aromatase inhibitor.

and apart from the trial of formestane given after aminoglutethimide, most trials tested a third-generation agent (exemestane, vorozole, and anastrozole) after a second-generation inhibitor (either formestane or aminoglutethimide). Clinical responses in these trials may be explained by the fact that estrogen levels are lowered further by administration of a third-generation after a second-generation inhibitor. For example, this was shown in the study of vorozole given for 2 months to patients whose disease was responding or stabilized on formestane. Estrogen levels were further suppressed by vorozole and returned to pretreatment levels once the patients restarted formestane (Table 3).⁶³

This observation, that clinical remissions can be obtained by incremental suppression of estrogen, is being explored further in a trial in which exemestane is given at a very low dose and after initial response is subsequently increased at each point of disease progression.

Recently, the results of a phase II trial testing exemestane at two dose levels (25 mg once daily and 100 mg once daily) after a nonsteroidal inhibitor (aminoglutethimide, anastrozole, letrozole, or vorozole) have been published.⁶⁷ Interestingly, exemestane showed an overall response rate (complete response plus partial response plus no change for ≥ 24 weeks) of 20.4% in patients who had already received another third-generation aromatase inhibitor.

A response to the androgen analog exemestane after the nonsteroidal inhibitors might be explained by the fact that exemestane exhibits androgenic effects. These effects, which have been seen at doses of 200 mg/d might also exist at a lower, and clinically not apparent, level at the 25-mg dose.

Similar to the second-line trials discussed above, the relative benefits of the inhibitors as third-line treatment in the phase II trials (as listed in Table 3) cannot be compared, because there were significant differences in the trial de-

signs and patient populations. For example, not all trials required clinical resistance to the agents given as first- and second-line hormonal therapy. With the emerging data indicating efficacy for the aromatase inhibitors in first-line metastatic disease, it is unlikely that randomized phase III trials will be conducted in this setting.

As adjuvant therapy after tamoxifen. Two strategies of using aromatase inhibitors after tamoxifen are being evaluated in adjuvant postmenopausal breast cancer trials (Fig 2). In the first, the inhibitors are being given as an extension after the initial standard 5 years of tamoxifen. The MA.17 international intergroup trial, initiated by the National Cancer Institute of Canada-Clinical Trials Group in 1998, is randomizing patients who are disease-free after 5 years of adjuvant tamoxifen to an additional 5 years of letrozole or placebo. In a similar design, the National Surgical Adjuvant Breast and Bowel Project is currently commencing a trial (B-33) of 2 years of exemestane or placebo after a standard 5 years of adjuvant tamoxifen.

The second approach to using aromatase inhibitors after tamoxifen is the use of both agents in sequence within the first 5 postoperative years. In this regard, preliminary results of a phase III study comparing 5 years of tamoxifen with 3 years of tamoxifen followed by 2 years of aminoglutethimide showed a statistically significant ($P = .006$) survival advantage for the sequential arm. However, this included a difference in deaths unrelated to cancer and no impact on disease recurrence was seen with the aromatase inhibitor.⁶⁸ Several large ongoing trials are also investigating this approach. For example, the International Collaboration Cancer Group trial is comparing 2 years of exemestane after 3 years of tamoxifen to a standard 5-year course of tamoxifen. Similarly, the Austrian Breast Cancer Study Group and the German Adjuvant Breast Cancer Group are

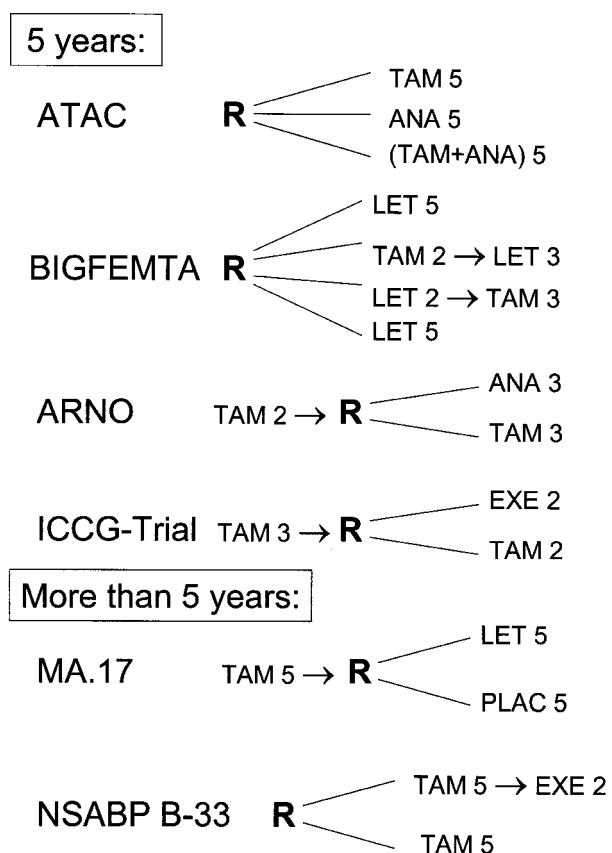


Fig 2. Trials with aromatase inhibitors in the adjuvant setting. Abbreviation: R, randomization; TAM, tamoxifen; ANA, anastrozole; LET, letrozole; EXE, exemestane; PLAC, placebo.

conducting trials that test 5 years of tamoxifen versus 2 years of tamoxifen followed by 3 years of anastrozole (the ARNO trial in Fig 2).

In a four-arm study (BIGFEMTA; see below and Fig 2) being conducted by the Breast International Group, one arm contains letrozole given for 3 years after 2 years of tamoxifen. Importantly, in this trial this sequence is also compared to the reverse, 3 years of tamoxifen after 2 years of letrozole. This design should help to determine whether the estrogen hypersensitive and tamoxifen-dependent hypotheses discussed previously are clinically relevant or whether the responses seen after tamoxifen are simply related to a switch in agents with different mechanisms of action.

Importantly, in these adjuvant trials and also in those of aromatase inhibitors as first-line adjuvant therapy discussed below, the occurrence of contralateral new primary breast cancers and the therapeutic index of the inhibitors as measured by other end-organ effects will contribute to the decision of whether this class of agents may also play a role

in chemoprevention in healthy women. These issues are elaborated on below.

As First-Line Endocrine Therapy

Aromatase inhibitors may be superior to tamoxifen as first-line hormonal therapy in breast cancer patients and even as chemopreventives. Tamoxifen has several well-described disadvantages. Adverse symptoms and impairment of quality of life are not infrequent on treatment and serious, albeit rare, side effects including endometrial cancer and thromboembolism occur. As discussed above, tamoxifen dependence and estrogen hypersensitivity may develop with prolonged therapy.

Aromatase inhibitors are very well tolerated clinically. Although approximately 10% of patients receiving tamoxifen discontinue treatment because of adverse events, less than 3% of patients did so in the phase III trials with the third-generation inhibitors.⁴⁷⁻⁵⁶ Furthermore, in the MCF-7_{CA} nude mouse model, the third-generation aromatase inhibitors have superior antitumor activity compared with tamoxifen.⁶⁹ To date, two trials in metastatic breast cancer comparing a third-generation inhibitor, anastrozole, have shown equal response rates and superior toxicity profiles as compared with tamoxifen.⁷⁰ However, in addition to superior response rates and improved toxicity profiles, two factors may influence whether the third-generation inhibitors will prove to be better first-line therapy than tamoxifen. First, superior survival rates among patients given the inhibitor compared with tamoxifen will be important. Second, it will be important to determine whether the sequence of tamoxifen to aromatase inhibitor is inferior to aromatase inhibitor to tamoxifen. In the past, several trials of earlier inhibitors such as aminoglutethimide after tamoxifen and vice versa showed the sequence of the inhibitor after tamoxifen to be a superior strategy.⁷¹⁻⁷³ Additional data from recently completed studies of letrozole versus tamoxifen as first-line therapy for metastatic disease and as neoadjuvant therapy in postmenopausal receptor-positive disease should be available soon. A trial of exemestane versus tamoxifen in the first-line treatment of metastatic breast cancer is being planned. It will be of interest to review the comparable responses and survival of patients in these trials. Furthermore, this important question will also be addressed in part by some of the ongoing adjuvant trials comparing aromatase inhibitors to tamoxifen.

Although the optimal strategy of first-line endocrine treatment in patients without specific risk factors will mostly depend on end points of efficacy, aromatase inhibitors might now be considered as first-line treatment in patients at high risk for deep vein thrombosis or pulmonary embolism. Aromatase inhibitors might also be considered

first-line in very elderly patients where higher rates of adverse events are seen with tamoxifen and quality of life is often judged to be as important as survival, even in the adjuvant setting.

Should the aromatase inhibitors prove superior to tamoxifen in terms of efficacy or toxicity in these trials, they may be considered in chemoprevention. Because of concern about possible stimulative effects of long-term tamoxifen on preclinical or preinvasive malignant lesions, tamoxifen is currently approved for use in chemoprevention for 5 years only. This concern should not apply to the aromatase inhibitors, and if their long-term therapeutic index proves satisfactory, they may be more suitable for prolonged therapy. Additional rationale for the use of aromatase inhibitors in chemoprevention are discussed below.

Metastatic breast cancer. Fadrozole, a second-generation inhibitor, has been compared with tamoxifen as first-line treatment of metastatic breast cancer. Efficacy was equivalent, but fadrozole proved to be significantly better tolerated than tamoxifen in the larger of the two trials.⁷⁴

Two first-line trials of anastrozole versus tamoxifen in metastatic disease have recently been completed and preliminary results are available.⁷⁰ In both studies, anastrozole was at least as effective as tamoxifen (time to progression, 8.5 v 7.0 months for anastrozole and tamoxifen, respectively) and associated with a lower incidence of thromboembolic events (4.5% v 7.6%) and vaginal bleeding (1.2% v 2.9%). In one of the trials, anastrozole seemed superior in terms of time to progression and clinical benefit, but this remains to be confirmed with further follow-up. The results of these trials have recently led to the approval of anastrozole as first-line therapy of metastatic breast cancer in several countries. An ongoing trial of letrozole versus tamoxifen as first-line therapy in postmenopausal advanced breast cancer is of particular interest in view of these results.

Adjuvant setting. Several trials are investigating the role of anastrozole, letrozole, or exemestane as a 5-year adjuvant therapy to replace tamoxifen. The design of the larger trials is shown in Fig 2. Importantly, only the adjuvant tamoxifen or arimidex or combined (ATAC) trial is testing a 5-year combination of tamoxifen plus an aromatase inhibitor in this setting. Aromatase inhibitors as combination therapy are discussed in more detail below under Aromatase Inhibitors as Combination Therapy. The BIGFEMTA trial of letrozole is particularly interesting, because within the same study and patient population, the merits of 5 years of either an aromatase inhibitor or tamoxifen alone can be directly compared with the two types of sequences of these two classes of agents. In general, all of the adjuvant trials shown in Fig 2 are also assessing the safety of the inhibitors, and companion studies

are evaluating the effects on estrogen-dependent target tissues such as bone and lipid metabolism.

Based on the emerging data indicating superior efficacy and toxicity for aromatase inhibitors compared with tamoxifen in the metastatic setting, it seems possible that this new class of endocrine therapy might replace tamoxifen as initial endocrine therapy in breast cancer patients, at least in countries with established oncology markets. After their approval for this setting, head-to-head trials of the inhibitors will be necessary to determine the best first-line hormonal strategy in terms of efficacy and toxicity.

Neoadjuvant setting. Several studies have examined the third-generation aromatase inhibitors in the neoadjuvant (preoperative) setting in breast cancer patients. A 12-week course of vorozole showed no benefit in efficacy compared with tamoxifen, with reductions in tumor volume (measured by ultrasound) of 37% and 58%, respectively.⁷⁵ The results of this randomized study were not statistically significant ($P = .11$) due to the low number of patients enrolled ($n = 53$). A similar nonrandomized study comparing letrozole with tamoxifen showed a significant difference in tumor response rates (letrozole, 81%; tamoxifen, 48%).⁷⁶ A median reduction in tumor volume comparable to letrozole was also shown in a nonrandomized study with anastrozole (80.5%) given for 3 months.⁷⁷ In addition to tumor reduction, in situ tumor aromatase activity and estrogen levels were significantly decreased in the preoperative setting by both letrozole and anastrozole.^{78,79} Ongoing phase III neoadjuvant trials of letrozole versus tamoxifen and anastrozole versus tamoxifen with intratumoral biomarker assessment will establish whether the inhibitors are superior. No neoadjuvant studies comparing the inhibitors are currently being conducted.

Chemoprevention. Estrogen and its metabolites, both in the circulation and locally synthesized in the breast, may be important in the pathogenesis of breast cancer. Estrogens are thought to be carcinogenic by several mechanisms, including alkylation of cellular molecules and generation of active radicals that can damage DNA. Similar genotoxic mechanisms of carcinogenicity have been proposed for the catechol metabolites of estrogen.^{3,80}

High levels of circulating estrogen in postmenopausal women have been associated with an increased risk of breast cancer.³ Furthermore, local estrogen synthesis, ie, aromatase activity, in the breast may also be important for the development of breast cancer. The location of preinvasive and invasive malignant lesions and high aromatase activity in the breast have been consistently correlated (see Inhibiting Estrogen Synthesis as a Therapeutic Target).¹⁵⁻¹⁸ This has led to a hypothesis of aromatase overexpression as an indirect cause of breast cancer.¹³ For example, in the

aromatase-transgenic mouse model (see Models for Evaluating Aromatase Inhibitors, under Antitumor Activity and Chemopreventive Potential), aromatase overexpression in breast tissue causes premalignant lesions, such as atypical ductal hyperplasia.^{25,81}

Tamoxifen and other SERMs are able to antagonize the effects of both circulating and locally produced estrogen on breast epithelial cells by blocking the ER. Thus tamoxifen has been used effectively in breast cancer treatment and most recently has been shown to reduce breast cancer incidence in the National Surgical Adjuvant Breast and Bowel Project P-1 trial.^{82,83} Aromatase inhibitors should similarly block the carcinogenic effects of both circulating and locally produced estrogen. An added advantage over SERMs, however, is their potential to reduce carcinogenic catechol metabolites in both compartments. Although it has been argued that circulating serum estrogen levels are too low for its metabolites to initiate cancer,¹³ in situ estrogen production by breast stromal cells is much higher.^{13,14} Thus it is possible that catechol estrogen metabolites initiate breast cancer and that the parent estrogen compounds only promote tumor growth. If this model is correct, the antiestrogens would act as chemosuppressants, blocking tumor progression but not initiation, whereas the aromatase inhibitors would be true chemopreventives, blocking both tumor initiation by estrogen metabolites and promotion by the parent compounds.

To date, chemoprevention with aromatase inhibitors has been tested only in preclinical models. Inhibition of both the appearance of new tumors and their multiplicity has variably been shown with aminoglutethimide, fadrozole, vorozole, and letrozole in the MNU- and DMBA-carcinogen-induced mammary tumor models.^{37,84-89}

Chemoprevention pilot studies of third-generation inhibitors are in planning or underway in postmenopausal women. The target populations for these studies include women at usual risk of breast cancer, or at increased risk, by virtue of clinical markers such as elevated plasma estradiol levels, premalignant breast lesions, or breast density.^{3,90} The end points of these studies may become useful surrogate markers of breast cancer prevention with aromatase inhibitors.

Importantly, a reduction in the incidence of contralateral breast cancer in the ongoing adjuvant trials would provide the first evidence of the ability of the aromatase inhibitors to prevent breast cancer.

AROMATASE INHIBITORS AS COMBINATION THERAPY

There are limited data on combining third-generation aromatase inhibitors with other agents. Although combination endocrine therapy has traditionally been avoided, po-

tential synergy of this new class of agents with existing therapies needs to be evaluated. Improved efficacy and enhanced therapeutic ratio may be possible. For example, combining tamoxifen with an inhibitor may overcome potential negative effects on bone metabolism. Furthermore, it is unlikely that the inhibitors will be used as monotherapy in premenopausal women for reasons explained above, and combining ovarian ablation with an inhibitor therefore merits testing in this setting.

Plus Ovarian Ablation

In premenopausal women in whom ovarian ablation is a traditional therapy for ER-positive breast cancer, adding an aromatase inhibitor that also reduces estrogen levels may prove to be of additional benefit. Results from studies with formestane and aminoglutethimide indicate that after surgical oophorectomy, a further decrease in serum estrogen levels can be achieved with an aromatase inhibitor.^{12,91,92} Furthermore, it has been shown that patients who relapse after surgical oophorectomy may experience a remission if treated subsequently with an inhibitor.⁹³ Similar effects were observed in a study of vorozole plus medical ovarian ablation with goserelin.⁶³ Thus depending on the efficacy and therapeutic index of the combination, it is possible that ovarian ablation plus an inhibitor could be effective in chemoprevention of breast cancer in high-risk premenopausal women.

Plus Other Endocrine Agents

Controversy remains with respect to the merits of combining traditional endocrine therapies. Early trials with aminoglutethimide plus tamoxifen showed no benefit compared with single-agent administration of the two compounds.⁹⁴⁻⁹⁹ It has been argued that failure of synergism between these two drugs might be explained by a 200% increase in clearance of tamoxifen induced by aminoglutethimide.¹⁰⁰

It has recently been shown in pharmacologic studies that the third-generation inhibitors letrozole and anastrozole do not interfere with tamoxifen catabolism, and their estradiol suppressant effects are unaffected by tamoxifen. However, the plasma level of letrozole is reduced by a mean of 37.6% during combination therapy.¹⁰¹⁻¹⁰³ The influence of concurrent administration of tamoxifen on anastrozole levels is as yet unknown.

In vitro experiments in MCF-7 cells fail to show additional benefit from combining tamoxifen with anastrozole or letrozole.¹⁵ These data suggest that combining these two classes of agents will not be more efficacious than single-agent therapy, but the therapeutic index of the combination with respect to other end-organs may be enhanced. The

tamoxifen plus anastrozole arm of the ATAC trial (Fig 2) will clarify these important issues.

Combination endocrine trials with aminoglutethimide and megestrol acetate or medroxyprogesterone acetate have failed to demonstrate synergistic clinical benefit.¹⁰⁴⁻¹⁰⁶ There are no data on newer aromatase inhibitors being tested in a similar regimen.

Plus Nonendocrine Agents

As yet, no trials of aromatase inhibitors combined with nonendocrine therapies have been conducted. Generally, the patient populations selected for hormone therapy trials have been distinct from those treated with chemotherapy. With this novel class of agents causing substantial tumor remissions in both the neoadjuvant setting and in sites of visceral metastases, combinations of chemotherapy and third-generation inhibitors merit testing. In addition, with HER2-*neu* status of primary tumors possibly being predictive of response to endocrine therapy and trastuzumab being incorporated into ongoing adjuvant clinical trials, experience with third-generation inhibitors and trastuzumab will be needed. Such trials are in the planning stages.

DISCUSSION

For the past several decades, the SERM tamoxifen has been the principal hormonal therapy in breast cancer in all settings, most recently including chemoprevention in healthy women. Aromatase inhibitors, although in clinical practice for almost 20 years, have been used only as second-line treatment after tamoxifen. With the available data, it would clearly be appropriate to offer a third-generation aromatase inhibitor as second-line therapy after tamoxifen either when tamoxifen has been given as first-line treatment for metastatic disease or if disease progression occurs on adjuvant tamoxifen or within 1 year of discontinuing adjuvant tamoxifen. This latter indication would be based on the fact that women meeting these criteria were eligible for enrollment on the second-line metastatic trials. Aromatase inhibitors should also be considered as first-line endocrine treatment in patients at high risk for deep vein thrombosis or pulmonary embolism in view of their superiority over tamoxifen in this regard.

The importance of this class of compounds has increased considerably with the advent of highly selective and potent third-generation inhibitors. If the results of the ongoing trials show that aromatase inhibitors are superior to tamoxifen not only in toxicity and response, but also in terms of patient survival, they might become the established first-line endocrine therapy not only in the metastatic setting but also as adjuvant treatment of ER-positive postmenopausal breast cancer. On the other hand, one of the adjuvant trials, the

BIGFEMTA trial, might reveal that the strategy of an aromatase inhibitor after tamoxifen is superior to the opposite, viz tamoxifen after the aromatase inhibitor. If this happens, it will necessitate further clinical trials to determine whether it will be better to achieve long-term incremental suppression of estrogen with the sequence of the aromatase inhibitor after tamoxifen even if the aromatase inhibitors are superior to tamoxifen as initial single-agent therapy.

Many other questions remain with respect to the optimal use of aromatase inhibitors. For example, additional ways of combining the inhibitors with other endocrine treatments, concomitantly or in sequence, are possible. Doses used in the treatment of metastatic breast cancer are not necessarily optimal in other settings. In chemoprevention, in particular, it has been speculated¹³ that low doses of highly potent inhibitors, particularly letrozole,¹⁰⁷ might be able to block in situ estrogen synthesis in the breast without interfering with ovarian estrogen production in premenopausal women. It is thus proposed that these low doses might therefore be chemopreventive for breast cancer without having negative effects on other estrogen-dependent target tissues.¹³

Because of subtle differences in the pharmacology and apparent efficacy and toxicity profiles of the different agents, head-to-head comparisons of third-generation aromatase inhibitors, such as the ongoing letrozole versus anastrozole trial in the second-line metastatic setting, are important. Exemestane is the only orally available steroidal inhibitor, and the results from the recently published phase III trial in metastatic disease make a comparison of its efficacy and side effect profile with the nonsteroidals of interest.

Future trials will evaluate the role of aromatase inhibitors in chemoprevention. In this setting, long-term safety and quality of life are important. The depletion of estrogen is likely to influence many organs, including the cardiovascular system and bone. Although there are no clinical data on the inhibitors' effects on these organs, preclinical results raise some concern about their long-term safety. Vorozole, for example, has been shown to reduce bone mineral density in male rats.^{108,109} On the other hand, anastrozole had a surprisingly favorable impact on the serum lipid profile of breast cancer patients. Data from the ongoing adjuvant trials will further elucidate these issues. Markers of quality of life will also be carefully reassessed, as those obtained from patients with advanced breast cancer are not necessarily transferable to other settings.

Importantly, estrogen, which is antagonized by aromatase inhibitors, plays an important role in women's health in general, apart from its role in breast cancer development. The aromatase inhibitors are the first class of agents that can

inhibit estrogen synthesis effectively and it is, therefore, conceivable that they might be used for the treatment or prevention of diseases other than breast cancer that are influenced by estrogen, such as endometrial cancer and endometriosis. It is also possible that agents which directly interfere with the regulation of aromatase expression on a genetic level will in the future replace aromatase inhibitors. The reason for aromatase overexpression, as described above (see Aromatase Inhibitors as Monotherapy, under

Chemoprevention), might be an increase in enhancers of aromatase transcription¹¹⁰ or the release of silencers of the aromatase gene promoter.¹¹¹ If these factors vary between tissues, and if future agents can specifically target them, it might be possible for example to downregulate aromatase activity in the breast while simultaneously upregulating it in other organs such as the bones. Future studies to determine the optimal application of aromatase inhibitors in the treatment and prevention of breast cancer are clearly of interest.

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