

## 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.<sup>1</sup> 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.<sup>2</sup> 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.<sup>3</sup> 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.<sup>4,5</sup> This peripheral aromatization in postmenopausal women is almost completely inhibited by single-agent administration of any of the third-generation inhibitors.<sup>6,7</sup> 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.<sup>8-10</sup> 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.<sup>11</sup> 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.<sup>12</sup> 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.<sup>13</sup> 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.<sup>14</sup> In addition up to 70% of breast cancer cells have been shown to synthesize estrogen as a result of intracellular aromatase expression.<sup>15-18</sup> 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.<sup>19-23</sup> There is increasing evidence that this local estrogen production may play a major role in tumor proliferation.<sup>24-27</sup> Intratumoral aromatase has been linked to response to the aromatase inhibitor aminoglutethimide<sup>18,28</sup> but surprisingly not to estrogen receptor expression.<sup>18,29</sup> 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.<sup>30,31</sup> 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.<sup>31,32</sup> 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.<sup>33</sup>

### 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.<sup>34</sup>

### 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<sup>35,36</sup> and spontaneous tumors in Sprague-Dawley rats.<sup>37</sup> 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.<sup>36</sup>

The recently developed aromatase-transgenic mouse model (*int-5/aromatase*) allows evaluation of the effects of aromatase inhibitors on aromatase-overexpressing breast tissue.<sup>25</sup> 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.<sup>26</sup>

A useful model for assessing the effects of inhibitors directly on intratumoral aromatase is the MCF-7<sub>CA</sub> cell line. This is an MCF-7 cell line transfected with the human placental aromatase gene (*MCF-7<sub>CA</sub>*), 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."<sup>38,39</sup> 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)<sup>40-42</sup> (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

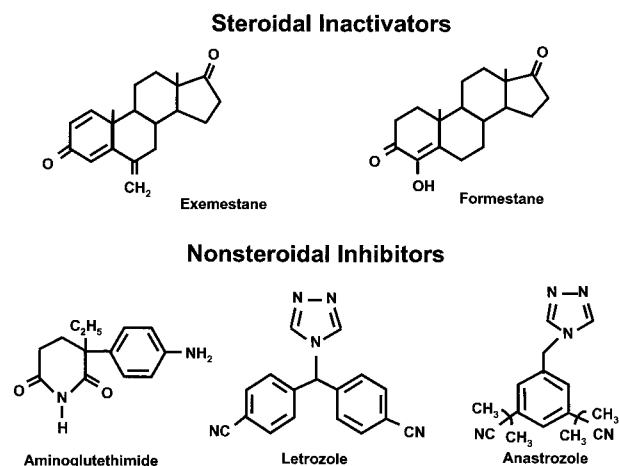


Fig 1. Structures of aromatase inhibitors.

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.<sup>43</sup> This leads to growth response to estrogen in concentrations four orders of magnitude lower than usually required.<sup>43</sup> 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.<sup>44</sup> Second, estrogen-deprived MCF-7 cells develop upregulation of aromatase, which in turn may result in increased autocrine stimulation by estrogen.<sup>43</sup> 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.<sup>43,45</sup>

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.<sup>46-55</sup> 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 <sup>47, 48</sup> (1 mg)	LET v MA <sup>51</sup> (2.5 mg)	VOR v MA <sup>54</sup> (2.5 mg)	FAD v MA <sup>57</sup> (2 mg)	FAD v MA <sup>57</sup> (2 mg)	EXE v MA <sup>55</sup> (25 mg)	FOR v MA <sup>58</sup> (250 mg IM)	LET v AG <sup>50</sup> (2.5/500 mg)	VOR v AG <sup>53</sup> (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	<b>24/16</b>	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	<b>47/37</b>
Median TTP, months		5.6/5.5	2.7/3.6	3.9/3.8	5.3/5.8	<b>4.7/3.8</b>		<b>3.4/3.2</b>	7/6
Median TTF, months		<b>5.1/3.9</b>				<b>3.8/3.7</b>	4/3.7	<b>3/3</b>	<b>5.3/4.4</b>
Median OS, months	<b>27/23</b>	25/22	26/29	27.1/23.1	25.8/27.9	<b>NR/28.4</b>		<b>28/20</b>	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<sup>56</sup> 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<sup>57</sup> (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<sup>58</sup> (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.<sup>59,60</sup>

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.<sup>61,62</sup> 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).<sup>63-66</sup> 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).<sup>63</sup>

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.<sup>67</sup> Interestingly, exemestane showed an overall response rate (complete response plus partial response plus no change for  $\geq 24$  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.<sup>68</sup> 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

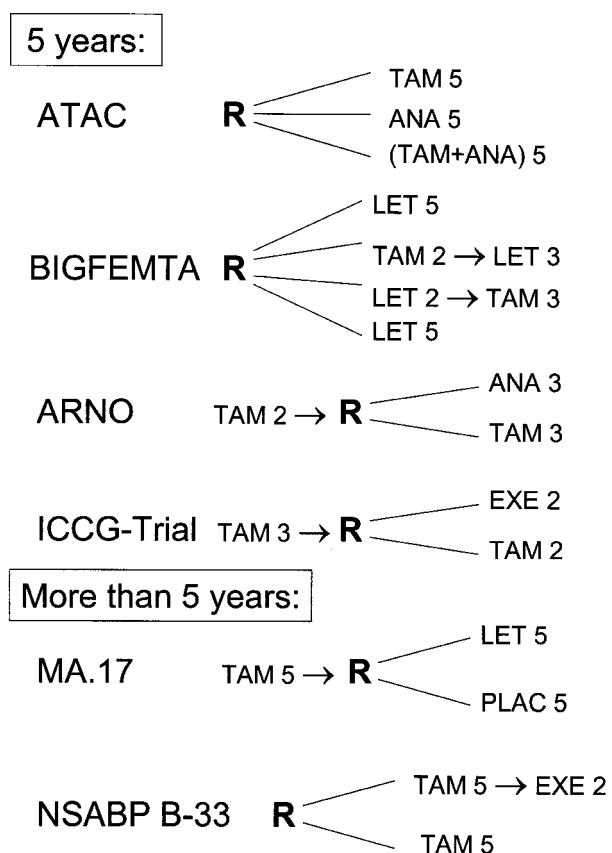


Fig 2. Trials with aromatase inhibitors in the adjuvant setting. Abbreviation: R, randomization; TAM, tamoxifen; ANA, anastrozole; LET, letrozole; EXE, exemestane; PLAC, placebo.

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.<sup>47-56</sup> Furthermore, in the MCF-7<sub>CA</sub> nude mouse model, the third-generation aromatase inhibitors have superior antitumor activity compared with tamoxifen.<sup>69</sup> 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.<sup>70</sup> 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.<sup>71-73</sup> 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.<sup>74</sup>

Two first-line trials of anastrozole versus tamoxifen in metastatic disease have recently been completed and preliminary results are available.<sup>70</sup> 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.<sup>75</sup> 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%).<sup>76</sup> A median reduction in tumor volume comparable to letrozole was also shown in a nonrandomized study with anastrozole (80.5%) given for 3 months.<sup>77</sup> 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.<sup>78,79</sup> 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.<sup>3,80</sup>

High levels of circulating estrogen in postmenopausal women have been associated with an increased risk of breast cancer.<sup>3</sup> 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).<sup>15-18</sup> This has led to a hypothesis of aromatase overexpression as an indirect cause of breast cancer.<sup>13</sup> 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.<sup>25,81</sup>

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.<sup>82,83</sup> 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,<sup>13</sup> in situ estrogen production by breast stromal cells is much higher.<sup>13,14</sup> 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.<sup>37,84-89</sup>

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.<sup>3,90</sup> 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.<sup>12,91,92</sup> Furthermore, it has been shown that patients who relapse after surgical oophorectomy may experience a remission if treated subsequently with an inhibitor.<sup>93</sup> Similar effects were observed in a study of vorozole plus medical ovarian ablation with goserelin.<sup>63</sup> 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.<sup>94-99</sup> 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.<sup>100</sup>

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.<sup>101-103</sup> 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.<sup>15</sup> 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.<sup>104-106</sup> 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 speculated<sup>13</sup> that low doses of highly potent inhibitors, particularly letrozole,<sup>107</sup> 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.<sup>13</sup>

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.<sup>108,109</sup> 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 transcription<sup>110</sup> or the release of silencers of the aromatase gene promoter.<sup>111</sup> 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.

## REFERENCES

- Osborne CK: Aromatase inhibitors in relation to other forms of endocrine therapy for breast cancer. *Endocr Relat Cancer* 6:271-276, 1999
- Dauvois S, Sanielian, PS, White R, et al: Antiestrogen ICI 164,384 reduces cellular estrogen receptor content by increasing its turnover. *Proc Natl Acad Sci U-A* 89:4037-4041, 1992
- Clemons M, Goss PE: Estrogen and risk of breast cancer. *N Engl J Med* (in press)
- Siiteri PK, MacDonald PC: Role of extraglandular estrogen in human endocrinology, in Greep RO, Astwood EB (eds): *Handbook of Physiology*. Washington, DC, American Physiological Society, 1973, pp 619-629
- Simpson ER, Zhao Y, Agarwal VR, et al: Aromatase expression in health and disease. *Recent Prog Horm Res* 52:185-213, 1997
- Geisler J, King N, Anker G, et al: In vivo inhibition of aromatization by exemestane, a novel irreversible aromatase inhibitor, in postmenopausal breast cancer patients. *Clin Cancer Res* 4:2089-2093, 1998
- Lonning PE: Pharmacology of new aromatase inhibitors. *Breast* 5:206-208, 1996
- Santen RJ: Clinical use of aromatase inhibitors in human breast carcinoma. *J Steroid Biochem Mol Biol* 40:247-253, 1991
- Harris AL, Dowsett M, Jeffcoate SL, et al: Endocrine and therapeutic effects of aminoglutethimide in premenopausal patients with breast cancer. *J Clin Endocrinol Metab* 55:718-720, 1982
- Wander HE, Blossley HCH, Nagel GA: Aminoglutethimide in the treatment of premenopausal patients with breast cancer. *Eur J Cancer Clin Oncol* 22:1371-1374, 1986
- Santen RJ, Samojlik E, Wells SA: Resistance of the ovary to blockade of aromatization with aminoglutethimide. *J Clin Endocrinol Metab* 51:473-477, 1980
- Stein RC, Dowsett M, Hedley A, et al: The clinical and endocrine effects of 4-hydroxyandrostenedione alone and in combination with goserelin in premenopausal women with advanced breast cancer. *Br J Cancer* 62:679-683, 1990
- Santen RJ, Yue W, Naftolin: The potential of aromatase inhibitors in breast cancer prevention. *Endocr Relat Cancer* 6:235-243, 1999
- Szyczak J, Milewicz A, Thijssen, JHH, et al: Concentration of sex steroids in adipose tissue after menopause. *Steroids* 63:319-321, 1998
- Brodie A, Lu Q, Liu Y, et al: Preclinical studies using the intratumoral aromatase model for postmenopausal breast cancer. *Oncology* 12:36-40, 1998 (suppl 5)
- Miller WR, Forrest APM: Oestradiol synthesis from C19 steroids by human breast cancers. *Br J Cancer* 33:116-118, 1976
- Bradlow HL: A reassessment of the role of breast tumor aromatization. *Cancer Res* 42:3382s-3386s, 1982 (suppl)
- Tilson-Mallett N, Santner SJ, Feil RD, et al: Biological significance of aromatase activity in human breast tumors. *J Clin Endocrinol Metab* 57:1125-1128, 1983
- Bulun SE, Price TM, Mahendroo MS, et al: A link between breast cancer and local estrogen biosynthesis suggested by quantification of breast adipose tissue aromatase cytochrome P450 transcripts using competitive polymerase chain reaction after reverse transcription. *J Clin Endocrinol Metab* 77:1622-1628, 1993
- Harada N: Aberrant expression of aromatase in breast cancer tissues. *J Steroid Biochem Mol Biol* 61:175-184, 1997
- James VHT, McNeill JM, Lai LC, et al: Aromatase activity in normal breast and breast tumor tissues: In vivo and in vitro studies. *Steroids* 50:269-279, 1987
- Miller WR, O'Neill J: The importance of local synthesis of estrogen within the breast. *Steroids* 50:537-548, 1987
- Miller WR, Mullen P, Sourdain P, et al: Regulation of aromatase activity within the breast. *J Steroid Biochem Mol Biol* 61:193-202, 1997
- Brodie A, Lu Q, Liu Y, et al: Aromatase inhibitors and their antitumor effects in model systems. *Endocr Relat Cancer* 6:205-210, 1999
- Tekmal RR, Ramachandra N, Gubba S, et al: Overexpression of *int-5/aromatase* in mammary glands of transgenic mice results in the induction of hyperplasia and nuclear abnormalities. *Cancer Res* 56:3180-3185, 1996
- Tekmal RR, Kirma N, Gill K, et al: Aromatase overexpression and breast hyperplasia, an in vivo model: Continued overexpression of aromatase is sufficient to maintain hyperplasia without circulating estrogens, and aromatase inhibitors abrogate these preneoplastic changes in mammary glands. *Endocr Relat Cancer* 6:307-314, 1999
- Santner SJ, Pauley RJ, Tait L, et al: Aromatase activity and expression in breast cancer and benign breast tissue stromal cells. *J Clin Endocrinol Metab* 82:200-208, 1997
- Miller WR, Anderson TJ, Jack WJL: Relationship between tumour aromatase activity, tumour characteristics and response to therapy. *J Steroid Biochem Mol Biol* 37:1055-1059, 1990
- Bolufex P, Ricart E, Luch A, et al: Aromatase activity and estradiol in human breast cancer: Its relationship to estradiol and epidermal growth factor receptors and to tumor-node-metastasis staging. *J Clin Oncol* 10:438-446, 1992
- Brodie AMH, Schwarzel WC, Brodie HJ: Studies on the mechanism of estrogen biosynthesis in the rat ovary. *J Steroid Biochem* 7:787-793, 1976
- Brodie AMH, Wing LY, Goss P, et al: Aromatase inhibitors and their potential clinical significance. *J Steroid Biochem* 25:859-865, 1986

32. Goss PE, Gwyn KMEH: Current perspectives on aromatase inhibitors in breast cancer. *J Clin Oncol* 12:2460-2470, 1994
33. Geisler J, Anker G, Dowsett M, et al: Letrozole suppresses plasma estrogen levels in postmenopausal breast cancer patients more completely than anastrozole. *Proc Am Soc Clin Oncol* 19:102a, 2000 (abstr 394)
34. Hausler A, Schenkel L, Krahenbuhl C, et al: An in vitro method to determine the selective inhibition of estrogen biosynthesis by aromatase inhibitors. *J Steroid Biochem* 33:125-131, 1989
35. Russo J, Russo IH: Experimentally induced mammary tumors in rats. *Breast Cancer Res Treat* 39:7-20, 1996
36. Clarke R: Animal models of breast cancer: Experimental design and their use in nutrition and psychosocial research. *Br Cancer Res Treat* 46:117-133, 1997
37. Gunson DE, Steele RE, Chau RY: Prevention of spontaneous tumours in female rats by fadrozole hydrochloride, an aromatase inhibitor. *Br J Cancer* 72:72-75, 1995
38. Zhou D, Chen S: Characterization of a silencer element in the human aromatase gene. *Arch Biochem Biophys* 353:213-320, 1998
39. Yue W, Zhou D, Chen S, et al: A nude mouse model for postmenopausal breast cancer using MCF-7 cells transfected with the human aromatase gene. *Cancer Res* 54:5092-5095, 1994
40. Brueggemeier RW: Aromatase inhibitors: Mechanisms of steroidal inhibitors. *Br Cancer Res Treat* 30:31-42, 1994
41. Bossche HV, Moereels H, Koymans LMH: Aromatase inhibitors: Mechanisms for non-steroidal inhibitors. *Br Cancer Res Treat* 30:43-55, 1994
42. Bhatnagar AS, Miller WR: Pharmacology of inhibitors of estrogen biosynthesis, in Oettel M, Schillinger E (eds): *Estrogens, Antiestrogens II: Pharmacology and Clinical Application of Estrogens and Antiestrogens* (vol 135, no 2). Berlin Heidelberg, Springer, 1999, pp 223-230
43. Yue W, Santen R: Aromatase inhibitors: Rationale for use following anti-oestrogen therapy. *Semin Oncol* 23:21-27, 1996 (suppl 9)
44. Gottardis MM, Jordan VC: Development of tamoxifen-stimulated growth of MCF-7 tumors in athymic mice after long-term antiestrogen administration. *Cancer Res* 48:5183-5187, 1988
45. Canney PA, Griffiths T, Latief TN, et al: Clinical significance of tamoxifen withdrawal response. *Lancet* 1:36, 1987
46. Buzdar A, Jones SE, Vogel C, et al: A phase III trial comparing anastrozole (1 and 10 milligrams), a potent and selective aromatase inhibitor, with megestrol acetate in postmenopausal women with advanced breast cancer: The Arimidex Study Group. *Cancer* 79:730-739, 1997
47. Jonat W, Howell A, Blomqvist C, et al: A randomised trial comparing two doses of the new selective aromatase inhibitor anastrozole (Arimidex) with megestrol acetate in postmenopausal patients with advanced breast cancer: The Arimidex Study Group. *Eur J Cancer* 32A:404-412, 1996
48. Buzdar A, Jonat W, Howell A, et al: Anastrozole, a potent and selective aromatase inhibitor, versus megestrol acetate in postmenopausal women with advanced breast cancer: Results of overview and analysis of two phase III clinical trials—The Arimidex Study Group. *J Clin Oncol* 14:2000-2011, 1996
49. Buzdar A, Jonat W, Howell A, et al: Anastrozole versus megestrol acetate in the treatment of postmenopausal women with advanced breast carcinoma: The Arimidex Study Group. *Cancer* 83:1142-1152, 1998
50. Gershanovich M, Chaudri HA, Campos D, et al: Letrozole, a new oral aromatase inhibitor: Randomised trial comparing 2.5 mg daily, 0.5 mg daily and aminoglutethimide in postmenopausal women with advanced breast cancer—The Letrozole International Trial Group. *Ann Oncol* 9:639-645, 1998
51. Dombrowsky P, Smith I, Falkson G, et al: Letrozole, a new oral aromatase inhibitor for advanced breast cancer: Double-blind randomized trial showing a dose effect and improved efficacy and tolerability compared with megestrol acetate. *J Clin Oncol* 16:453-461, 1998
52. Bengtsson NO, Focan C, Gudgeon A, et al: A phase III trial comparing vorozole (RIVIZOR) versus aminoglutethimide in the treatment of advanced postmenopausal breast cancer: The Vorozole Study Group. *Eur J Cancer* 33:148, 1997 (abstr 656)
53. Bergh J, Bonnetterre J, Illiger HJ, et al: Vorozole (Rivizor) versus aminoglutethimide in the treatment of postmenopausal breast cancer relapsing after tamoxifen. *Proc Am Soc Clin Oncol* 16:155a, 1997 (abstr 543)
54. Goss PE, Winer EP, Tannock IF, et al: Randomized phase III trial comparing the new potent and selective third-generation aromatase inhibitor vorozole with megestrol acetate in postmenopausal advanced breast cancer patients: The North American Vorozole Study Group. *J Clin Oncol* 17:52-63, 1999
55. Kaufmann M, Bajetta E, Dirix LY, et al: Exemestane is superior to megestrol acetate after tamoxifen failure in postmenopausal women with advanced breast cancer: Results of a phase III randomized double-blind trial—The Exemestane Study Group. *J Clin Oncol* 18:1399-1411, 2000
56. Hamilton A, Piccart M: The third-generation non-steroidal aromatase inhibitors: A review of their clinical benefits in the second-line hormonal treatment of advanced breast cancer. *Ann Oncol* 10:377-384, 1999
57. Buzdar A, Smith R, Vogel C, et al: Fadrozole HCl (CGS-16949A) versus megestrol acetate treatment of postmenopausal patients with metastatic breast carcinoma: The Multi-Institutional Trialist Collaborative Study Group. *Cancer* 77:2503-2513, 1996
58. Thürlimann B, Castiglione M, Hsu-Schmitz SF, et al: Formestane versus megestrol acetate in postmenopausal breast cancer patients after failure of tamoxifen: A phase III prospective randomised cross over trial of second-line hormonal treatment—The Swiss Group for Clinical Cancer Res. *Eur J Cancer* 33:1017-1024, 1997
59. Goss PE, Oza A, Rakesh G, et al: Liarozole fumarate (R85246): A novel imidazole in the treatment of receptor positive postmenopausal metastatic breast cancer. *Breast Cancer Res Treat* 1545:1-14, 2000
60. Goss PE, Strasser K, Marques R, et al: Liarozole fumarate (R85246): In the treatment of ER negative, tamoxifen refractory or chemotherapy resistant postmenopausal metastatic breast cancer. *Breast Cancer Res Treat* (in press)
61. Jones S, Vogel C, Arkhipov A, et al: Multicenter, phase II trial of exemestane as third-line hormonal therapy of postmenopausal women with metastatic breast cancer. *J Clin Oncol* 17:3418-3425, 1999
62. Jones S, Chang A, Lusch C, et al: A phase II confirmatory study of antitumor efficacy and safety of exemestane (EXE) as third-line hormonal treatment of postmenopausal patients (pts) with metastatic breast cancer (MBC) refractory to tamoxifen (Tam) and Megace. *Breast Cancer* 50:305, 1998 (abstr 437)
63. Dowsett M, Doody D, Miall S, et al: Vorozole results in greater oestrogen suppression than formestane in postmenopausal women and when added to goserelin in premenopausal women with advanced breast cancer. *Br Cancer Res Treat* 56:25-34, 1999
64. Harper-Wynne C, Coombes RC: Anastrozole shows evidence of activity in postmenopausal patients who have responded or stabilised on formestane therapy. *Eur J Cancer* 35:744-746, 1999

65. Murray R, Pitt P: Aromatase inhibition with 4-OHAndrostenedione after prior aromatase inhibition with aminoglutethimide in women with advanced breast cancer. *Br Cancer Res Treat* 35:249-253, 1995
66. Thuerlimann B, Paridaens R, Serin D, et al: Third-line hormonal treatment with exemestane in postmenopausal patients with advanced breast cancer progressing on aminoglutethimide: A phase II multicentre multinational study—The Exemestane Study Group. *Eur J Cancer* 33:1767-1773, 1997
67. Lonning PE, Bajetta E, Murray R, et al: Activity of exemestane in metastatic breast cancer after failure of nonsteroidal aromatase inhibitors: A phase II trial. *J Clin Oncol* 18:2234-2244, 2000
68. Boccardo F, Rubagotti A, Amoroso D, et al: Tamoxifen (TAM) vs aminoglutethimide in breast cancer patients previously treated with adjuvant TAM: Preliminary results of a multicentric comparative study. *Proc Am Soc Clin Oncol* 19:71a, 2000 (abstr 273)
69. Lu Q, Yue W, Wang J-P, et al: The effects of aromatase inhibitors and antiestrogens in the nude mouse model. *Br Cancer Res Treat* 50:63-71, 1998
70. Buzdar A, Nabholz JM, Robertson JF, et al: Anastrozole (Arimidex) versus tamoxifen as first-line therapy for advanced breast cancer (Abc) in postmenopausal women: Combined analyses from two identically designed multicenter trials. *Proc Am Soc Clin Oncol* 19:154a, 2000 (abstr 609D)
71. Harvey HA, Lipton A, White DS, et al: Cross-over comparison of tamoxifen and aminoglutethimide in advanced breast cancer. *Cancer Res* 42:3434-3436s, 1982 (suppl 8)
72. Smith IE, Harris AL, Morgan M, et al: Tamoxifen versus aminoglutethimide in the treatment of advanced breast carcinoma. *Cancer Res* 42:3430-3433s, 1982 (suppl 8)
73. Gale KE, Andersen JW, Tormey DC, et al: Hormonal treatment of metastatic breast cancer: An Eastern Cooperative Oncology Group Phase III trial comparing aminoglutethimide to tamoxifen. *Cancer* 73:354-361, 1994
74. Thuerlimann B, Beretta K, Bacchi M, et al: First-line fadrozole HCl (CGS 16949A) versus tamoxifen in postmenopausal women with advanced breast cancer: The Swiss Group for Clinical Cancer Res (SAKK). *Ann Oncol* 7:471-479, 1996
75. Harper-Wynne C, Shenton K, Dowsett M, et al: Vorozole Study Group: A randomised multicentre study of vorozole compared to tamoxifen as primary therapy in post-menopausal breast cancer. *Proc Am Soc Clin Oncol* 18:109a, 1999 (abstr 272)
76. Dixon JM, Love CDB, Tucker S, et al: Letrozole as primary medical therapy for locally advanced and large operable breast cancer. *Proc Am Soc Clin Oncol* 17:104a, 1998 (abstr 400)
77. Dixon JM, Renshaw L, Bellamy C, et al: Arimidex as neoadjuvant therapy causes large reductions in tumour volume in postmenopausal women with large operable breast cancers. *Proc Am Soc Clin Oncol* 18:109a, 1999 (abstr 345)
78. Geiler J, Bernsten H, Ottestad L, et al: Neoadjuvant treatment with anastrozole (Arimidex) causes profound suppression of intratumor estrogen levels. *Proc Am Soc Clin Oncol* 18:109a, 1999 (abstr 311)
79. Miller WR, Telford J, Love C, et al: The effects of letrozole on in site and in vitro oestrogen synthesis and endogenous oestrogen within the breast. *Br Cancer Res Treat* 46:54, 1997 (abstr 216)
80. Liehr JG: Is estradiol a genotoxic mutagenic carcinogen? *Endocr Rev* 21:40-54, 2000
81. Gill K, Keshava N, Mantione J, et al: Overexpression of int-5/aromatase in transgenic male mice leads to gynecomastia and testicular cancer. *Proc Am Assoc Cancer Res* 39:551, 1998 (abstr 3751)
82. Early Breast Cancer Trialists' Collaborative Group: Tamoxifen for early breast cancer: An overview of the randomised trials. *Lancet* 351:1451-1467, 1998
83. Fisher B, Costantino J, Wickerham L, et al: Tamoxifen for prevention of breast cancer: Report of the National Surgical Adjuvant Breast and Bowel Project P-1. *J Natl Cancer Inst* 90:1371-1388, 1998
84. Moon RC, Steele VE, Kelloff GJ, et al: Chemoprevention of MNU-induced mammary tumorigenesis by hormone response modifiers: Toremifene, RU 16117, tamoxifen, aminoglutethimide and progesterone. *Anticancer Res* 14:889-894, 1994
85. De Coster R, Van Ginckel RF, Callens MJ, et al: Antitumoral and endocrine effects of (+)-vorozole in rats bearing dimethylbenzanthracene-induced mammary tumors. *Cancer Res* 52:1240-1244, 1992
86. Lubet RA, Steele VE, Casebolt TL, et al: Chemopreventive effects of the aromatase inhibitors vorozole (R-83842) and 4-hydroxyandrostenedione in the methylnitrosourea (MNU)-induced mammary tumor model in Sprague-Dawley rats. *Carcinogenesis* 15:2775-2780, 1994
87. Schieweck K, Bhatnagar AS, Matter A: CGS 16949A, a new nonsteroidal aromatase inhibitor: Effects on hormone-dependent and -independent tumors in vivo. *Cancer Res* 48:834-838, 1988
88. Schieweck K, Bhatnagar AS, Batzl C, et al: Anti-tumor and endocrine effects of non-steroidal aromatase inhibitors on estrogen-dependent rat mammary tumors. *J Steroid Biochem Mol Biol* 44:633-636, 1993
89. Bhatnagar AS, Hausler A, Schieweck K, et al: Highly selective inhibition of estrogen biosynthesis by CGS 20267, a new non-steroidal aromatase inhibitor. *J Steroid Biochem Mol Biol* 37:1021-1027, 1990
90. Boyd NF, Byng JW, Jong RA, et al: Quantitative classification of mammographic densities and breast cancer risk: Results from the Canadian National Breast Screening Study. *J Natl Cancer Inst* 87:670-675, 1995
91. Celio L, Martinetti A, Ferrari L, et al: Premenopausal breast cancer patients treated with a gonadotropin-releasing hormone analog alone or in combination with an aromatase inhibitor: A comparative endocrine study. *Anticancer Res* 19:2261-2268, 1999
92. Dowsett M, Stein RC, Coombes RC: Aromatization inhibition alone or in combination with GnRH agonists for the treatment of premenopausal breast cancer patients. *J Steroid Biochem* 43:155-159, 1992
93. Coombes RC, Stein RC, Dowsett M: Aromatase inhibitors in human breast cancer. *Proc Roy Soc Edin* 95B:283, 1989 (abstr)
94. Alonso-Munoz MC, Ojeda-Gonzalez MB, Beltran-Fabregat M, et al: Randomized trial of tamoxifen versus aminoglutethimide and versus combined tamoxifen and aminoglutethimide in advanced postmenopausal breast cancer. *Oncology* 45:350-353, 1988
95. Corkery J, Leonard RCF, Henderson IC, et al: Tamoxifen and aminoglutethimide in advanced breast cancer. *Cancer Res* 42:3409s-3414s, 1982
96. Ingle JN, Green SJ, Ahmann DL, et al: Randomized trial of tamoxifen alone or combined with aminoglutethimide and hydrocortisone in women with metastatic breast cancer. *J Clin Oncol* 4:958-964, 1986
97. Milsted R, Habeshaw T, Kaye S, et al: A randomised trial of tamoxifen versus tamoxifen with aminoglutethimide in postmenopausal women with advanced breast cancer. *Cancer Chemother Pharmacol* 14:272-273, 1985
98. Powles TJ, Ashley S, Ford HT, et al: Treatment of disseminated breast cancer with tamoxifen, aminoglutethimide, hydrocortisone, and danazol, used in combination or sequentially. *Lancet* 1:1369-1372, 1984

99. Rose C, Kamby C, Mouridsen HT, et al: Combined endocrine treatment of postmenopausal patients with advanced breast cancer. *Br Cancer Res Treat* 7:45-50, 1986
100. Lien EA, Anker G, Lonning PE, et al: Decreased serum concentrations of tamoxifen and its metabolites induced by aminoglutethimide. *Cancer Res* 50:5851-5857, 1990
101. Ingle JN, Suman VJ, Johnson PA, et al: Evaluation of tamoxifen plus letrozole with assessment of pharmacokinetics interaction in postmenopausal women with metastatic breast cancer. *Clin Cancer Res* 5:1642-1649, 1999
102. Dowsett M, Pfister C, Johnston SRD, et al: Impact of tamoxifen on the pharmacokinetics and endocrine effects of the aromatase inhibitor letrozole in postmenopausal women with breast cancer. *Clin Cancer Res* 5:2338-2343, 1999
103. Dowsett M, Tobias JS, Howell A, et al: The effect of anastrozole on the pharmacokinetics of tamoxifen in post-menopausal women with early breast cancer. *Br J Cancer* 79:311-315, 1999
104. Russell CA, Green SJ, O'Sullivan J, et al: Megestrol acetate and aminoglutethimide/hydrocortisone in sequence or in combination as second-line endocrine therapy of estrogen receptor positive metastatic breast cancer: A Southwest Oncology Group phase III trial. *J Clin Oncol* 15:2494-2501, 1997
105. Samonis G, Margioris AN, Bafaloukos D, et al: Prospective randomized study of aminoglutethimide (AG) versus medroxyprogesterone acetate (MPA) versus AG+MPA in generalized breast cancer. *Oncology* 51:411-415, 1994
106. Wander HE, Kleeberg UR, Esser U, et al: Aminoglutethimid plus hochdosiertes Medroxyprogesteronazetat versus Aminoglutethimid plus Kortison in der Therapie des metastasierenden Mammakarzinoms. *Onkologie* 10:321-323, 1987
107. Miller W: Qualitative effects of letrozole as primary medical therapy on in situ oestrogen synthesis and endogenous oestrogen levels within the breast. *Breast* 6:228, 1997 (abstr 0-12)
108. Vanderschueren D, Van Herck A, De Coster R, et al: Aromatization of androgens is important for skeletal maintenance of aged male rats. *Calcif Tissue Int* 59:179-183, 1996
109. Vanderschueren D, Van Herck E, Nijs J, et al: Aromatase inhibition impairs skeletal modeling and decreases bone mineral density in growing male rats. *Endocrinology* 138:2301-2307, 1997
110. Chen S, Zhou D, Okubo T, et al: Breast tumor aromatase: Functional role and transcriptional regulation. *Endocr Relat Cancer* 6:149-156, 1999
111. Jin T, Branch DR, Zhang X, et al: Examination of POU homeobox gene expression in human breast cancer cells. *Int J Cancer* 81:104-112, 1999