



Michele Sassi. La Digue Island, c. 2001. Seychelles Islands, Indian Ocean. Photograph.

*Basic science and initial clinical studies support the effectiveness of exemestane in neoadjuvant therapy of breast cancer in postmenopausal women.*

## Endocrine and Clinical Endpoints of Exemestane as Neoadjuvant Therapy

*William R. Miller, PhD, DSc, and J. Michael Dixon, BSc, MB ChB, MD, FRCS, FRCSEd*

**A** series of *in vitro* and *in vivo* studies have been performed to establish the endocrine and clinical endpoints of the type I anti-aromatase agent exemestane in neoadjuvant therapy. *In vitro* studies demonstrated a dose-related inhibition of aromatase activity with exemestane, even when activity was measured in a system in which the aromatase enzyme was induced in fibroblasts preincubated with exemestane but assayed in the absence of the drug. In contrast, type II anti-aromatase agents (eg, aminoglutethimide, anastrozole, and letrozole) often caused a paradoxical increase in aromatase activity when measured under similar conditions.

*In vivo* and *in situ* studies were performed in 12 postmenopausal women with untreated large or locally advanced estrogen receptor-rich tumors. The effect of exemestane 25 mg daily for 3 months on aromatization peripherally and in breast cancer and surrounding normal tissue was determined. Immediately before starting therapy, patients received an 18-hour infusion of radioactively labeled androgen and estrogen, followed by a wedge biopsy. This procedure was repeated after 3 months of treatment with exemestane, and the data were used to calculate peripheral and local aromatization. Changes in tumor volume were based on clinical examination, ultrasound, and mammography. Exemestane treatment was associated with a marked reduction in aromatization peripherally and in nonmalignant breast tissue in every patient and in breast tumor in all but one patient. Median reduction in tumor volume was 85.5% for clinical examination, 82.5% for ultrasound, and 84% for mammography. Eight of 10 patients who would have required mastectomy before treatment were able to undergo breast-conserving surgery after treatment. Clinical benefits were accompanied by a marked reduction in cellular proliferation and progesterone receptor expression. These data support the use of exemestane in neoadjuvant therapy of breast cancer in postmenopausal women.

*From the University of Edinburgh, Western General Hospital, Scotland, United Kingdom.*

*Address reprint requests to William R. Miller, PhD, DSc, at the*

*Breast Research Unit, Paderewski Building, University of Edinburgh, Western General Hospital, Edinburgh EH4 2XU, Scotland, UK. E-mail: w.r.miller@ed.ac.uk*

## Introduction

Many breast cancers require estrogens for growth and when deprived of estrogens will regress.<sup>1</sup> Therefore, estrogen suppression is an important approach to the management of this hormone-responsive cancer. In premenopausal women, the ovaries are the primary source of estrogen,<sup>2</sup> and ovarian ablation is the gold standard approach to reducing estrogen levels. In postmenopausal women, estrogen is synthesized in the skin, muscle, adipose tissue, and breast cancers,<sup>3,6</sup> making a surgical or radiological approach to ablation unworkable. In this setting, a pharmacologic strategy is most appropriate. Although synthetic anti-estrogens (eg, tamoxifen) were previously first-line hormonal therapy in postmenopausal women, agents that inhibit the aromatase enzyme responsible for estrogen synthesis are increasingly being used to treat postmenopausal women with breast cancer because of their superior efficacy and excellent safety profile.<sup>7-12</sup>

The aromatase enzyme, responsible for the conversion of androgens to estrogens, is key to estrogen biosynthesis (Fig 1) and is a target of estrogen suppression therapy. There are two ways to inhibit or block aromatase: (1) Agents that interfere with the substrate binding site of the enzyme (type I aromatase inhibitors) are androgen analogues and may irreversibly inactivate the enzyme<sup>13</sup>; they have been referred to as aromatase inactivators (eg, formestane, exemestane), and (2) agents that block the electron transfer chain by the cytochrome P450 prosthetic group of aromatase (type II aromatase inhibitors)<sup>14</sup>; these drugs (eg, aminoglutethimide, anastrozole, and letrozole) are non-steroidal and their effects on the enzyme are reversible.

The purpose of this paper is to present the results of a series of in vitro, in vivo, and in situ studies designed to establish the endocrine and clinical endpoints of the oral aromatase inactivator, exemestane in neoadjuvant therapy.

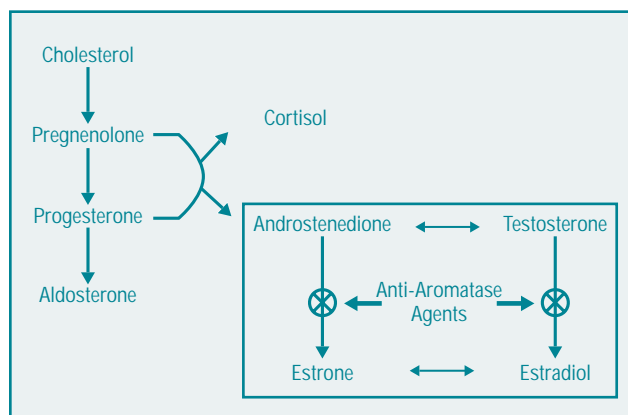


Fig 1. — Mechanism of action of anti-aromatase agents.

## In Vitro Model Systems

In vitro effects of anti-aromatase agents on aromatase activity were evaluated in three systems. Placental microsomes, which are a disrupted cell preparation and the most common system for testing anti-aromatase agents, were initially used. However, because placental microsomes are an artificial system

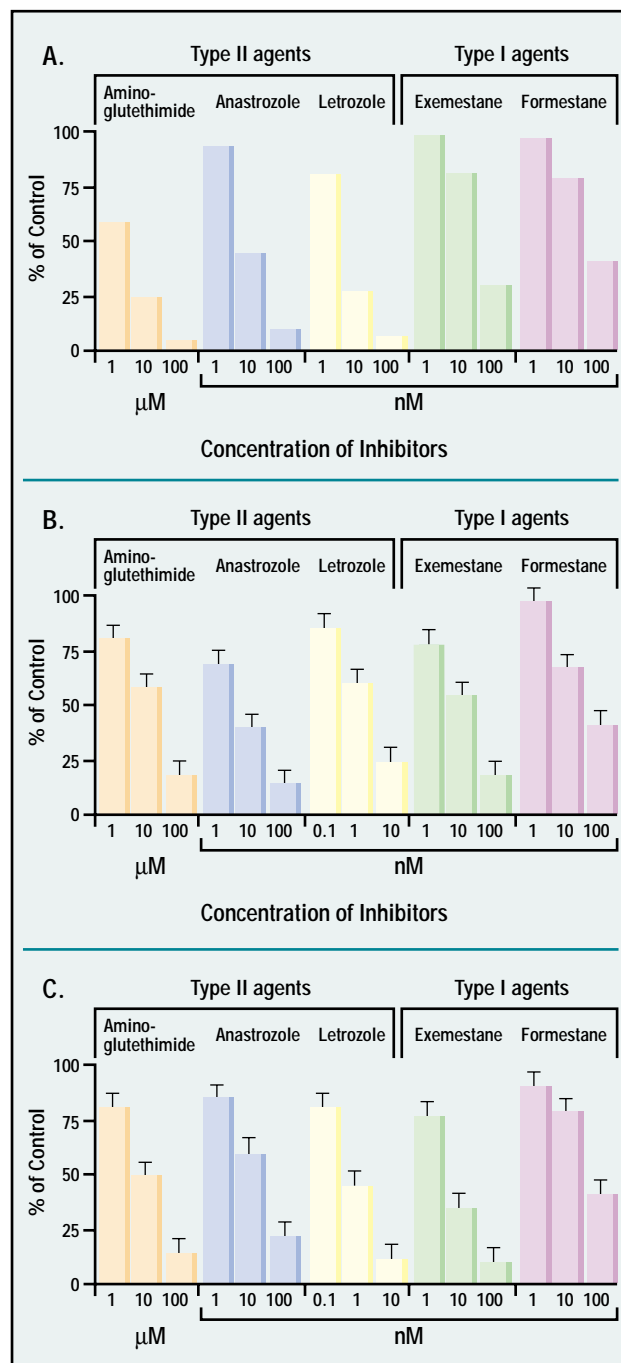


Fig 2. — Dose-dependent inhibition of anti-aromatase agents. Columns are means and bars are standard errors of the means of at least three replicate experiments. (A) placental microsomes, (B) breast cancer homogenates, (C) cultured mammary adipose tissue fibroblasts. From Miller and Dixon.<sup>15</sup> Reprinted with permission.

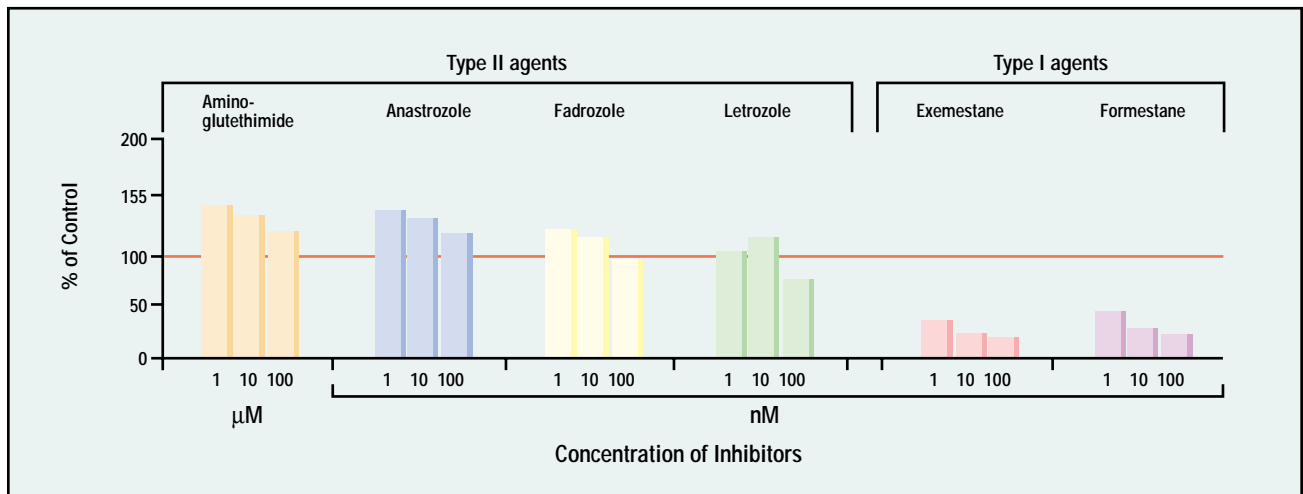


Fig 3. — Effect of anti-aromatase agents on aromatase activity. Each anti-aromatase agent was preincubated in cultured fibroblasts with dexamethasone, but the assay for aromatase activity (expressed as % of control system without anti-aromatase agents) was performed in the absence of the anti-aromatase agent. From Miller and Dixon.<sup>15</sup> Reprinted with permission.

for breast cancer, exemestane was also tested in a second disrupted cell preparation (breast cancer homogenates) and in a whole cell system (cultured fibroblasts derived from mammary adipose tissue). Aromatase activity was reported as percentage of the control system without an anti-aromatase agent. Both type I and type II agents were tested. Because of the substantially lower potency of aminoglutethimide compared with the newer anti-aromatase agents, micromolar concentrations of the former were used, whereas nanomolar concentrations of the latter were tested. In each system, there was a clear dose-related inhibition; aromatase activity decreased with increasing concentration of the anti-aromatase agent (Fig 2).<sup>15</sup>

Differences in the mechanism of action of the type I and type II anti-aromatase agents were demonstrated by preincubating cultured fibroblasts, in which the aromatase enzyme was induced by the addition of dexamethasone, in the presence of an anti-aromatase agent. Aromatase activity was then determined in the absence of the anti-aromatase agent. A clear pattern of increased aromatase activity was observed with the type II agents and decreased activity with the type I agents (Fig 3).<sup>15</sup>

### In Vivo Whole-Body Endocrinology

Exemestane (25 mg daily for 3 months) was used as primary therapy in postmenopausal women (59 to 88 years of age) with untreated large or locally advanced estrogen receptor-positive tumors (>80 histoscore) to switch off whole-body aromatization. The objectives of the study were to assess the clinical efficacy of exemestane as neoadjuvant therapy and to determine the effect of treatment on peripheral and in situ estrogen synthesis. A total of 13 women were

enrolled. One patient died during the study from an unrelated cause and 12 were studied to completion. At the time of study entry, all patients had a performance status of 0 to 2 and large tumors without distant metastases (stage T2 [>3 cm], T3, T4b, N0-1, M0).<sup>16</sup>

Immediately before initiating therapy with exemestane, patients received an 18-hour perfusion with 20 MBq <sup>3</sup>HΔ4 androstenedione and 1 MBq of <sup>14</sup>C estrone. At the completion of the perfusion, a wedge biopsy (removing approximately 1 gram of tissue) was performed and peripheral blood (50 mL) was collected. The perfusion and sampling procedure was repeated after 3 months of treatment with exemestane. Peripheral estrogen synthesis was determined by measuring the amount of <sup>3</sup>H estrone in the plasma. Uptake of

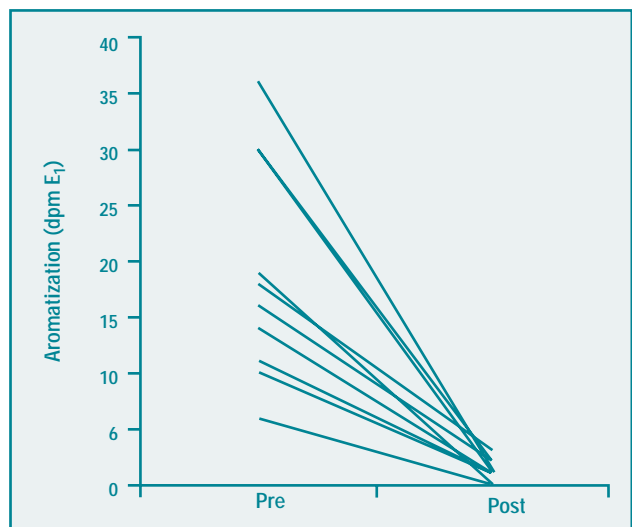


Fig 4. — Effect of exemestane (25 mg daily for 3 months) on peripheral aromatization in postmenopausal women with breast cancer. Abbreviations: dpm, disintegrations per minute; E<sub>1</sub>, estrone. From Miller and Dixon.<sup>15</sup> Reprinted with permission.

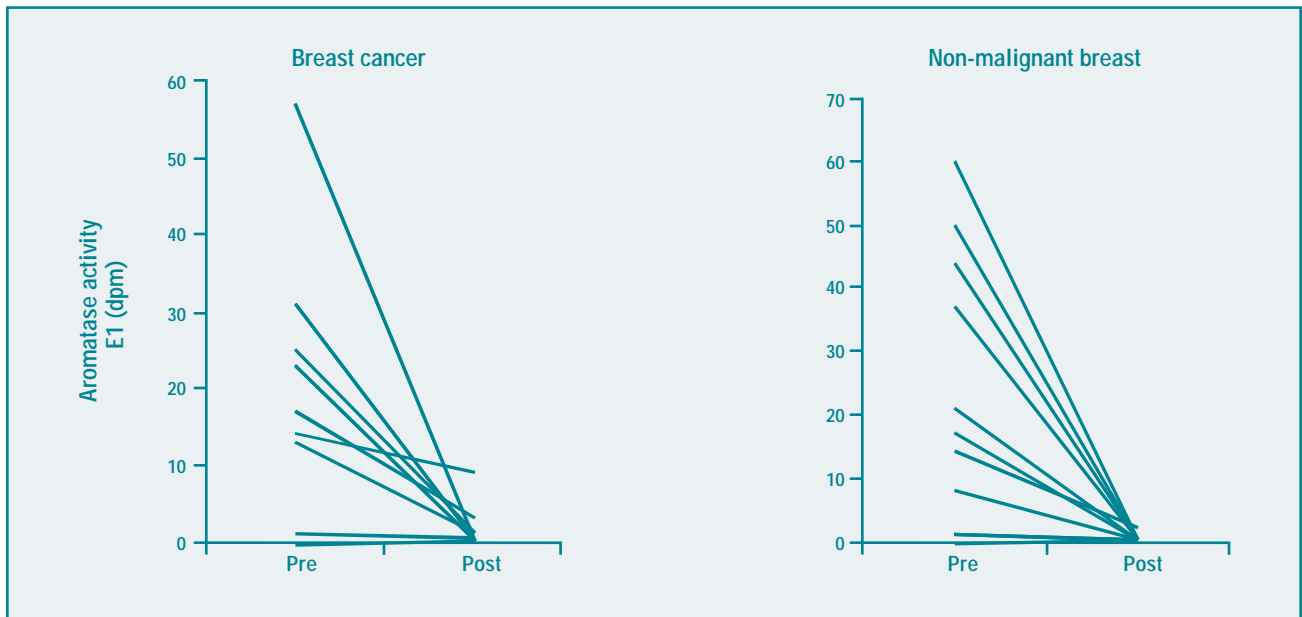


Fig 5. — Aromatase activity in breast cancer and adjacent non-malignant tissue before and after treatment with exemestane 25 mg daily for 3 months. Abbreviations: dpm, disintegrations per minute; E<sub>1</sub>, estrone. From Miller and Dixon.<sup>15</sup> Reprinted with permission.

estrogen into the tumor was determined by measuring the amount of <sup>14</sup>C estrone in blood, tumor, and surrounding tissue. In situ synthesis was assessed from the <sup>3</sup>H estrone in the tumor after correcting for the estrone taken up from the circulation. We detected a marked reduction in peripheral aromatization in every patient studied; aromatase activity reached undetectable levels in several patients (Fig 4).

## In Situ Breast Cancer and Adjacent Normal Tissue

In the study described above, we also determined the effect of exemestane on in situ aromatization and tumor volume. Tumor size was measured using ultrasound, mammography, and clinical evaluation at baseline and after 1, 2, and 3 months of treatment with exemestane 25 mg daily in 12 postmenopausal women with histologically proven breast cancer. Response was calculated based on bi-dimensional area and classified as complete response (no measurable disease), partial response ( $\geq 50\%$  reduction in area), minor response (25% to 50% reduction in area), stable disease ( $<25\%$  decrease and  $<25\%$  increase in area), or progressive disease ( $\geq 25\%$  increase in area).<sup>16</sup>

There was a dramatic reduction in aromatase activity in breast cancer tissue in all but one patient and in the surrounding nonmalignant breast tissue in all patients (Fig 5). This reduction in aromatase activity was associated with a reduction in tumor volume using all three methods of assessment. The median reduction in tumor volume was 85.5% using clinical evaluation,

82.5% using ultrasound, and 84% using mammography (Fig 6). The reduction in tumor volume was demonstrated clinically by a marked resolution of the tumor as shown mammographically in Fig 7. Ten of 12 patients would have required mastectomy at the outset of therapy; however, only 2 required mastectomy after 3 months of treatment with exemestane. The remaining 10 patients underwent breast-conserving surgery with clear margins.<sup>16</sup>

The impact of exemestane treatment was also demonstrated by a marked reduction in cellular proliferation over the course of treatment (an example of which is shown in Fig 8), as well as a loss of progesterone receptor expression (Fig 9) consistent with the anti-estrogen effects of the drug.

## Discussion and Conclusions

In vitro test systems demonstrate that the type I agents exemestane and formestane and the type II agents aminoglutethimide, anastrozole, and letrozole each exhibit a clear dose-related inhibition of the aromatase enzyme. The primary difference among the anti-aromatase agents was the concentration required to inhibit the enzyme. Micromolar concentrations were required of the prototype anti-aromatase agent aminoglutethimide whereas much lower (nanomolar) concentrations were required of the other agents.

Interesting differences were also demonstrated using a test system in which the aromatase enzyme was induced in the presence of an aromatase inhibitor fol-

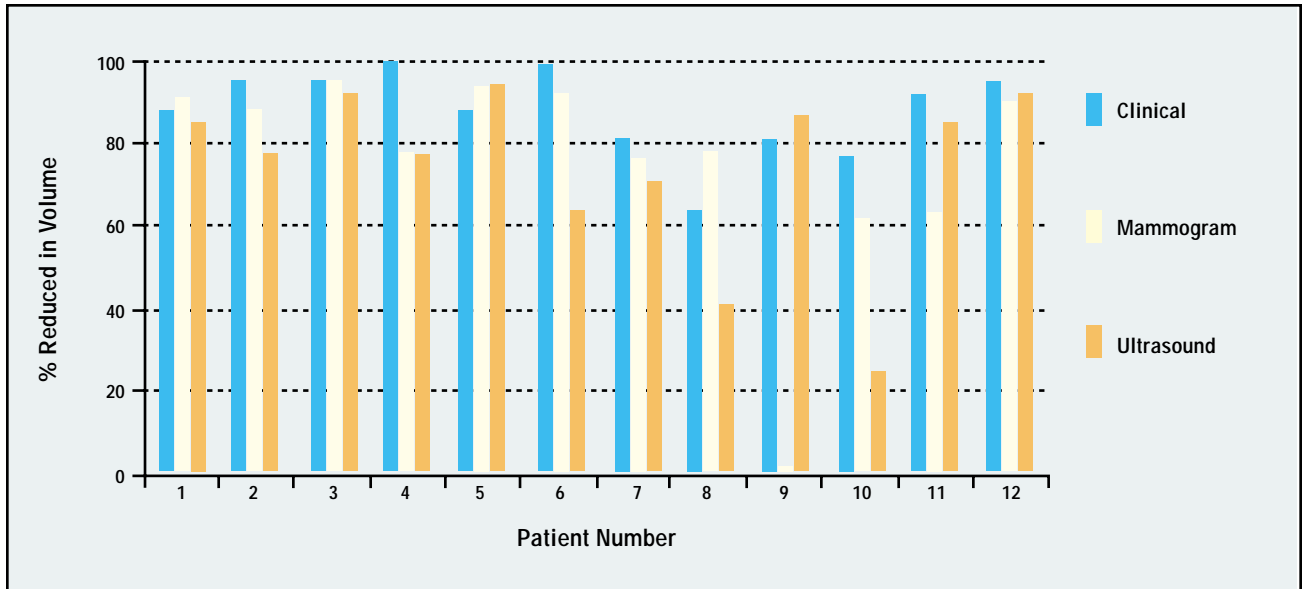


Fig 6. — Percent reduction from baseline in tumor volume by patient after treatment with exemestane 25 mg daily for 3 months. In patient 9 (invasive lobular carcinoma), the edges of the tumor were difficult to define mammographically, but treatment led to a dramatic decrease in tumor density and significant reductions in tumor size based on clinical and ultrasound assessment.

lowed by measurement of aromatase activity in the absence of the agent. As in the previous test system, type I agents caused a marked dose-related inhibition of the enzyme. However, type II agents were frequently associated with an increase in aromatase activity. The paradoxical results seen with the type II agents are consistent with those reported by others<sup>17</sup> and may be explained by their reversible nature. In addition to inhibiting the aromatase enzyme, type II agents also appear to induce/stabilize the enzyme.<sup>18</sup> Although the clinical implications of this paradoxical effect are unknown, it is postulated that increased aromatase activity may be the cause of resistance reported with long-term use of type II agents and explain why patients resistant to type II agents may respond to type I agents.<sup>19</sup>

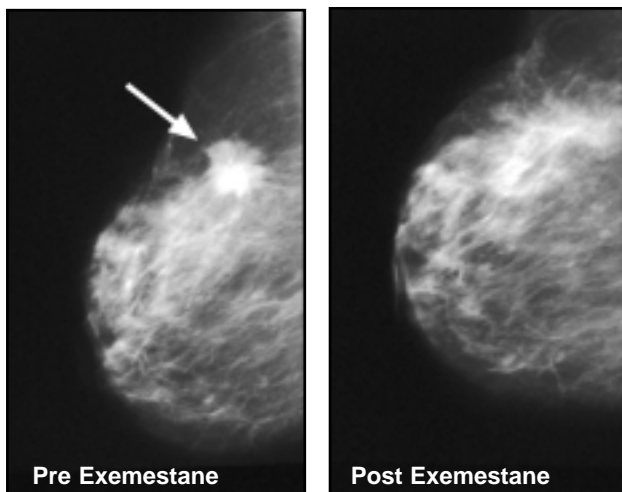


Fig 7. — Mammogram of the same breast before and after 3 months of treatment with exemestane 25 mg daily. From Miller and Dixon.<sup>15</sup> Reprinted with permission.

The specificity of the anti-aromatase agent for the aromatase protein is an important consideration when assessing these agents because the lower the specificity, the higher the risk of adverse events due to interaction with proteins other than aromatase. Exemestane is highly specific for the aromatase enzyme. When tested against the enzymes aromatase, 21-hydroxylase, 11 $\beta$ -hydroxylase, 18-hydroxylase, and C<sub>20-22</sub>-lyase, the IC<sub>50</sub> for aromatase was 25 nM compared with IC<sub>50</sub>s ranging from 73,000 to greater than 100,000 for the other enzymes (personal communication, E. di Salle, 2000). The exemestane concentrations required to inhibit enzymes other than aromatase are frequently not achievable.

Our *in vivo* and *in situ* data in 12 postmenopausal women with untreated large or locally advanced breast cancer further demonstrate the potential role of exemestane in this setting. All 12 women had a dramatic reduction in peripheral aromatization after 3 months of therapy with exemestane 25 mg daily. Our

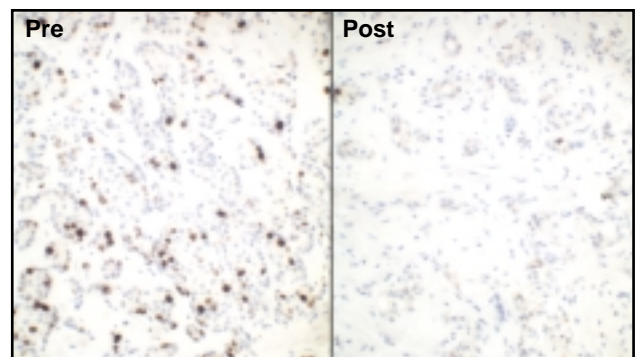


Fig 8. — Effect of exemestane 25 mg daily for 3 months on antibody Ki67 expression in malignant breast tissue.

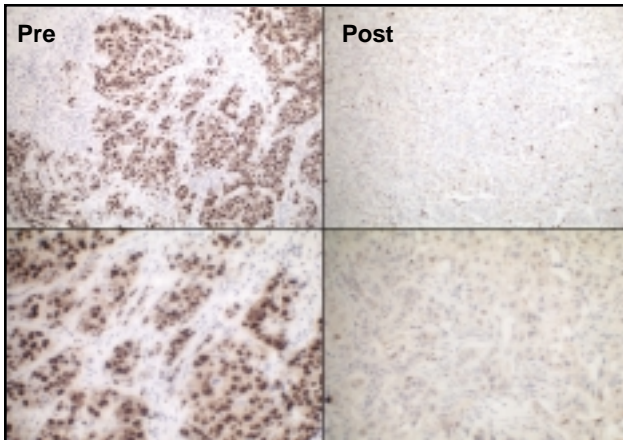


Fig 9. — Effect of exemestane 25 mg daily for 3 months on progesterone receptor expression in malignant breast tissue.

results are consistent with those of Geisler and coworkers<sup>20</sup> who previously reported 97.9% inhibition of peripheral aromatization with exemestane. Similar reductions have been reported for anastrozole and letrozole, whereas less dramatic reductions have been noted with intramuscular formestane and aminoglutethimide (Table).<sup>20,21</sup> The enhanced suppression of the newer more potent anti-aromatase agents has been demonstrated by Thürlimann et al.<sup>22</sup> Serum estrone sulphate (E<sub>1</sub>S) concentrations were measured in patients progressing on aminoglutethimide before and after initiating treatment with exemestane. E<sub>1</sub>S concentrations, which were 663 pmol/L during treatment with aminoglutethimide, were further suppressed during treatment with exemestane to 72 pmol/L.<sup>22</sup>

Our in situ data demonstrate the ability of exemestane to switch off estrogen biosynthesis within the breast tumor and in surrounding normal adipose tissue. Clinically, a substantial reduction in tumor volume was demonstrated based on three methods of assessment — clinical, ultrasound, and mammogram — with median reductions of 85.5%, 82.5%, and 84%, respectively. As a result, it was possible to perform breast-conserving surgery in 8 of 10 patients who would have required mastectomy before treatment. These results are consistent with those obtained with letrozole and anastrozole in neoadjuvant therapy.<sup>12,23</sup> In the only published large randomized phase III study, tamoxifen (n=170) was compared with letrozole (n=154) in postmenopausal women with estrogen receptor-positive or progesterone receptor-positive breast cancer who were ineligible for breast conserving surgery. Letrozole provided statistically significantly higher objective response rates and a significantly greater rate of breast conserving surgery.<sup>12</sup>

In the present study we also report marked reductions in cellular proliferation and progesterone receptor

#### In Vivo Aromatase Inhibition<sup>20,21</sup>

Anti-aromatase Agent	Inhibition	Residual Activity
Type I Agents:		
Exemestane	97.9%	2.1%
Formestane (intramuscular)	91.9%	8.1%
Type II Agents:		
Aminoglutethimide	90.6%	9.4%
Anastrozole	96.7%	3.3%
Letrozole	98.9%	1.1%

expression and changes in tumor morphology, which are consistent with the reductions in tumor volume.

Our series of in vitro and in vivo studies demonstrate that exemestane is a highly specific and potent anti-aromatase agent that has profound endocrine effects both peripherally and within the breast. These endocrine effects translate into marked reductions in tumor volume and changes in tumor morphology and cell proliferation index, which suggest an important role for exemestane in neoadjuvant therapy of postmenopausal women with breast cancer.

*This research was supported in part by Pharmacia Corporation. The authors also acknowledge the help of Professor T.J. Anderson and Ms L. Renshaw in progressing the research documented in the manuscript.*

#### References

1. Miller WR. Estrogens and endocrine therapy for breast cancer. In: Miller WR, ed. *Estrogen and Breast Cancer*. Austin, Tex: R.G. Landes Co; 1996:125-150.
2. Thomson A. Analysis of cases in which oophorectomy was performed for inoperable carcinoma of the breast. *BMJ*. 1902;2: 1538-1541.
3. Perel E, Killinger DW. The interconversion and aromatization of androgens by human adipose tissue. *J Steroid Biochem*. 1979; 10:623-627.
4. Schweikert HU, Milewich L, Wilson JD. Aromatization of androstenedione by cultured human fibroblasts. *J Clin Endocrinol Metab*. 1976;43:785-795.
5. Longcope C. Methods and results of aromatization studies in vivo. *Cancer Res*. 1982;42(suppl):3307s-3311s.
6. Miller WR, Forrest AP. Oestradiol synthesis from C19 steroids by human breast cancer. *Br J Cancer*. 1976;33:116-118.
7. Nabholz JM, Buzdar A, Pollak M, et al. Anastrozole is superior to tamoxifen as first-line therapy for advanced breast cancer in postmenopausal women: results of a North American multicenter randomized trial. *J Clin Oncol*. 2000;18:3758-3767.
8. Bonnetterre J, Thürlimann B, Robertson JF, et al. Anastrozole versus tamoxifen as first-line therapy for advanced breast cancer in 668 postmenopausal women: results of the tamoxifen or Arimidex randomized group efficacy and tolerability study. *J Clin Oncol*. 2000; 18:3748-3757.
9. Bell R, Mouridsen H, Georgulias VA, et al. Femara (letrozole) superior in efficacy over tamoxifen as first-line treatment in advanced breast cancer (ABC). Aromatase 2000 and the 3rd Generation. Port Douglas, Australia; November 3-7, 2000. Abstract.
10. Paridaens R, Dirix L, Lohrisch C, et al. Tamoxifen vs exemestane as first-line endocrine therapy in advanced breast cancer: an EORTC randomized phase II-III trial. Aromatase 2000 and the 3rd

Generation. Port Douglas, Australia; November 3-7, 2000. Abstract.

11. Paridaens R, Dirix L, Lohrisch C, et al. Promising activity and safety of exemestane (E) as first-line hormonal therapy (HT) in metastatic breast cancer (MBC) patients (pts): final results of an EORTC randomized phase II trial. *Breast Cancer Res Treat.* 2000; 64:52. Abstract and poster 167.

12. Dixon M, Grischke EM, Mardiak J, et al. Pre-operative treatment of postmenopausal breast cancer patients with letrozole (Femara): a randomized study versus tamoxifen. Aromatase 2000 and the 3rd Generation. Port Douglas, Australia; November 3-7, 2000. Abstract.

13. Brodie AM, Garrett WM, Hendrickson JR, et al. Inactivation of aromatase in vitro by 4-hydroxy-androstene-3,17-dione and 4-acetoxy-4-androstene-3,17-dione and sustained effects in vivo. *Steroids.* 1981;38:693-702.

14. Yue W, Santen RJ, Wang J-P, et al. Aromatase within the breast. *Endocr Relat Cancer.* 1999;6:157-164.

15. Miller WR, Dixon JM. Antiaromatase agents: preclinical data and neoadjuvant therapy. *Clin Breast Cancer.* 2000;1:S9-S14.

16. Dixon JM, Grattage L, Renshaw L, et al. Exemestane as neoadjuvant therapy for locally advanced breast cancer: endocrinologic and clinical endpoints. *Breast Cancer Res Treat.* 2000; 62:53. Abstract 170.

17. Kao YC, Okubo T, Sun XZ, et al. Induction of aromatase expression by aminoglutethimide, an aromatase inhibitor that is used to treat breast cancer in postmenopausal women. *Anticancer Res.* 1999;19:2049-2056.

18. Harada N, Honda S-I, Hatano O. Aromatase inhibitors and enzyme stability. *Endocr Relat Cancer.* 1999;6:211-218.

19. Lonning PE, Bajetta E, Murray R, et al. A phase II study of exemestane (EXE) in metastatic breast cancer (MBC) patients failing non steroidal aromatase inhibitors (ns-AIs). *Breast Cancer Res Treat.* 1998;50:304. Abstract 435.

20. 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.* 1998;4: 2089-2093.

21. Lonning PE. Pharmacology of new aromatase inhibitors. *Breast.* 1996;5:202-208.

22. Thürlimann 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. *Eur J Cancer.* 1997;33:1767-1773.

23. Dixon JM, Love CD, Renshaw L, et al. Lessons from the use of aromatase inhibitors in the neoadjuvant setting. *Endocrine-Related Cancer.* 1999;6:227-230.