Pergamon

European Journal of Cancer Vol. 33, No. 4, pp. 587–591, 1997
© 1997 Elsevier Science Ltd. All rights reserved
Printed in Great Britain
0959-8049/97 $17.00 + 0.00

PII: S0959-8049(96)00494-7

Original Paper

The Minimal Effective Exemestane Dose for Endocrine Activity in Advanced Breast Cancer

E. Bajetta,[1] N. Zilembo,[1] C. Noberasco,[1] A. Martinetti,[2] L. Mariani,[3] L. Ferrari,[2] R. Buzzoni,[1] M. Greco,[4] C. Bartoli,[4] I. Spagnoli,[5] G.M. Danesini,[5] S. Artale[1] and J. Paolini[6]

[1]Division of Medical Oncology B; [2]Division of Nuclear Medicine; [3]Division of Statistics and Biometry; 4[4]Division of Surgery; [5]Division of Radiology A, Istituto Nazionale per lo Studio e la Cura dei Tumori, Via Venezian 1, 20133 Milan; and [6]Pharmacia & Upjohn, Via R. Koch 1.2, 20151 Milan, Italy

Phase I studies have demonstrated that exemestane, an irreversible oral aromatase inhibitor, is able to suppress circulating oestrogen levels. In our previous experience, doses ranging from 2.5 to 25 mg induced a similar suppression of oestrogens. The aim of this study was to identify the minimum effective exemestane dose on the basis of endocrine activity. 20 evaluable postmenopausal advanced breast cancer patients were randomly given exemestane 0.5, 1, 2.5 or 5 mg, in double-blind conditions. Oestrone (E1), oestradiol (E2), oestrone sulphate (E1S), gonadotrophins, sex-hormone binding globulin and dehydroepiandrosterone sulphate serum levels were evaluated from the first day of treatment to the 7th, 14th, 28th and 56th day. Serum E1, E2 and E1S levels were suppressed by all doses starting from day 7; the degree of inhibition versus baseline was 25 up to 72% for E1, 30 up to 62% for E2 and 16 up to 52% for E1S, with higher doses achieving greater suppression; these changes were maintained over time. A significant increase in FSH and LH levels was observed for all doses. Treatment tolerability was satisfactory. The endocrine effects of exemestane appear to be dose related and 0.5 and 1 mg are ineffective for adequately suppressing circulating oestrogens. © **1997 Elsevier Science Ltd. All rights reserved.**

Key words: breast cancer, advanced disease, hormonotherapy, aromatase inhibitor, postmenopause

Eur J Cancer, Vol. 33, No. 4, pp. 587–591, 1997

INTRODUCTION

APPROXIMATELY 60% of breast cancers in postmenopausal women are potentially hormone sensitive, and either oestrogen deprivation or the withdrawal of this mitogenic factor is the goal of any endocrine treatment. The major source of oestrogen production in postmenopausal women is the conversion of androstenedione to oestrone in peripheral tissues via the multicomponent enzyme complex, aromatase [1–3], and so the inhibition of oestrogen biosynthesis by means of selective aromatase inhibitors is a potentially useful therapeutic option in hormone-sensitive breast cancer [4]. Several irreversible aromatase inhibitors have been developed that offer significant advantages over aminoglutethimide. The first of these new aromatase inhibitors to be used in clinical practice was formestane [5–7], which appears to be effective when administered intramuscularly (i.m.) or orally [8]; however, suppression comparable with that observed using a biweekly i.m. dose of 250 mg was only obtained after daily oral administration of 250 mg [9]. The lower potency of oral formestane has been attributed to the rapid inactivation of the compound, which is greatly excreted in urine as a glucuronide at the 4-hydroxyl group [10].

The initial results obtained using a new, effective oral, steroidal compound, exemestane (FCE 24304), have been very encouraging. It induced a 30% and 73% regression of established 7,12-dimethylbenzanthracene-induced mammary tumours in rats when given daily at 50 mg/kg subcutaneously (s.c.), associated with an 86% and 93% decrease in total ovarian aromatase activity [11, 12].

A dose-related inhibitory effect on oestrogen biosynthesis has been demonstrated after single-dose administration of

Correspondence to E. Bajetta.
Received 26 Jul. 1996; revised 7 Oct. 1996; accepted 26 Nov. 1996.

exemestane 0.5–25 mg in postmenopausal volunteers [13]; a 0.5 mg dose was ineffective, whereas some inhibitor of oestrogen biosynthesis was evident at 5 mg, and 25 mg proved to be the minimum effective dose producing maximum oestrogen suppression.

On the basis of these data, we previously investigated four low exemestane doses (25, 12.5, 5 and 2.5 mg daily) administered to postmenopausal patients with advanced breast cancer. Serum oestrone (E1) and oestradiol (E2) values were similarly and significantly suppressed by all doses; this suppression reached its maximum at day 7 (inhibition of 64% for oestrone and 65% for oestradiol; $P = 0.0001$) and was maintained over time [14].

Given these results, we decided to verify two lower exemestane doses, 0.5 and 1 mg administered daily for at least 56 days, in an attempt to identify the minimum effective dose for suppressing oestrogen levels.

PATIENTS AND METHODS

Patient selection

Postmenopausal patients pretreated for advanced breast cancer entered the study carried out at the Medical Oncology B Division of Milan's Istituto Nazionale per lo Studio e la Cura dei Tumori.

The eligibility criteria were the same as those reported previously [14], and included the presence of measurable and/or evaluable disease, a performance status (PS) of 0–2 (ECOG scale) and a positive oestrogen receptor (ER) status. A disease-free interval (DFI) of more than 2 years was required if the receptor status was unknown. Postmenopausal status was defined as at least 1 year from spontaneous menopause, at least 2 years from drug induced amenorrhoea in patients older than 50 years, or follicle-stimulating hormone (FSH) and luteinizing-hormone (LH) levels in the postmenopausal range in patients younger than 50 years. Patients who had undergone bilateral oophorectomy were also considered eligible. Previous, systemic, anticancer treatment had to have stopped at least 3 weeks prior to study entry. Patients were excluded if they had more than one-third liver involvement, or lymphangitic lung or brain metastases.

Study design and treatment plan

On the basis of our results published in the literature and our previous data, it was planned to randomise 20 outpatients to 0.5, 1, 2.5 or 5 mg dose, given in double-blind conditions, with 5 subjects/dose. The latter two doses were chosen to guarantee comparability with our first study in which the lowest tested dose was 2.5 mg. The drug was supplied by Pharmacia & Upjohn as sugar-coated tablets, containing exemestane (0.5, 2.5 or 5 mg) or placebo. The test preparations were anonymous, and for each dose group, the placebo tablets were indistinguishable from those containing active drug. After being clinically examined and staged on the day before treatment was started, each patient took 2 tablets contained in an aluminium strip at 12 a.m. daily. On the first day of treatment, blood samples for endocrine studies were taken at 9 a.m., and an overnight 12-h urine sample was collected. Further blood samples for endocrine, haematological and biochemical studies were taken on days 7, 14, 28 and 56, and further urine samples were collected on the same days, except day 7. Only the patients who completed the 56-day study period were considered evaluable. On day 56, tumour response and toxicity were also assessed.

The blood samples were collected at room temperature, allowed to clot, centrifuged at 3000*g* and then stored at −20°C until assay. Oestrogen serum levels, LH, FSH, sex-hormone binding globulin (SHBG) and dehydroepiandrosterone sulphate (DHEAS) were measured at all sampling times.

The patients were instructed on how to collect overnight 12-h urine, and each was given a standard plastic tube (volume 1 L, Kartell, Milan, Italy). The patients had to return the tube on the examination days, the volume of urine was measured and a 20 ml sample was taken and stored at −20°C until analysis for 17-hydroxycorticosteroid (17-OHCS) levels. Staging and tumour response were determined at the beginning of the study, after 56 days, and then every 2 months. Signs, symptoms and toxicity were evaluated at each visit according to the National Cancer Institute (Bethesda) criteria [15], and clinical response according to UICC criteria [16]. All the patients gave their informed consent, and the study was approved by our Institutional Bio-Ethics Committee. Providing no severe adverse events occurred, the treatment was continued for as long as there was no disease progression.

Hormonal measurements

All of the hormone assays were performed in double-blind conditions by the Endocrinology Laboratory at Milan's Istituto Nazionale per lo Studio e la Cura dei Tumori.

Serum E1, E2 and oestrone sulphate (E1S) levels were measured by means of radio-immunoassay (RIA) after liquid-phase extraction and chromatographic separation. ^{3}H-E1 (Bio-Merieux) and ^{3}H-E2 (Bio-Merieux) were added to each serum sample (3 ml) as recovery markers, and extracted using 11 ml diethyl ether. The serum phase was frozen and the ether extract decanted into clean tubes and dried under nitrogen. ^{3}H-E1S (DuPont Nen Net-203) oestrone sulphate, ammonium salt (6,7^{3}H(N)) was added to the aqueous phase (containing E1S) as a recovery marker, and the solution was submitted to enzyme hydrolysis as follows: 2 mg of sulphatase (SIGMA 9626 type H-1) and 3 ml 0.2 M acetate buffer (pH 4.8) were added to the sample, which was then incubated for 22 h at 45°C. At this point, the E1S was transformed into E1 and its extraction, chromatographic separation and RIA were carried out as for E1. The other phase of the first and second extractions, respectively, containing E1 + E2 and deconjugated E1S, was reconstituted in 1 ml of isooctane saturated with ethylene glycol before proceeding to the chromatographic separation of E1 and E2 on a celite column (celite Supelco mixed with ethylene glycol 2:1 w/v). In the chromatographic step, the redissolved sample was applied to the column and successive elutions with increasing concentrations of ethylacetate in isooctane (0, 18 and 40%) were collected. The fractions containing E1 (18% ethylacetate) and E2 (40% ethylacetate) were evaporated under nitrogen, and the dried samples redissolved in 500 µl of the appropriate incubation buffer. A duplicate aliquot of this suspension (100 µl for E1 and E1S and 50 µl for E2) was submitted to the specific RIA procedure, and a further aliquot (200 µl for E1, E1S and E2) to final recovery, which ranged between 75% and 85% for E1 and between 70% and 80% for both E2 and E1S. The

final recovery of E1S was 70–80%. The blanks for E1, E2 and E1S, which were determined in the bidistilled water sample prepared in the same way as the serum sample, did not exceed 2, 0.25 and 4 pg/tube, respectively. The commercially available ^{3}H-E1 (Bio-Merieux) and ^{125}I-E2 (Clinical Assay) RIA kits were used to determine E1 and E2 levels. The standard curve of the E2 kit (supplied in serum) was substituted with a standard curve in buffer. The sensitivity of the assay was 1 pg/ml for E2, 4 pg/ml for E1 and 8.5 pg/ml for E_1-S. The intra-assay CVs ($n = 9$) for E2 were 3.1% and 1.8% at 25 pg/ml and 14 pg/ml, respectively; the intra-assay CVs for E1 were 8.1% and 9.0% at 39 pg/ml and 21 pg/ml, respectively, the intra-assay CV for E_1-S was 6.1% at 191 pg/ml ($n = 9$) and 5.7% at 425 pg/ml ($n = 7$). The inter-assay CV was 6% for E2; 7.7% for E1 and 11% for E1S ($n = 11$).

Immunoradiometric assays were used to determine serum DHEAS, LH, FSH and SHBG levels. The performance data for these assays were as follows: DHEAS (Sclavo Technogenetics), sensitivity 50 ng/ml, intra-assay CV 6.3% and interassay CV 7.1%; LH (Ares Serono), sensitivity 0.5 mIU/ml, intra-assay CV 1.1% and interassay CV 2.9%; FSH (Ares Serono), sensitivity 0.5 mIU/ml, intra-assay CV 1.6% and interassay CV 1.7%; SHBG (Orion Corporation), sensitivity 6.25 nmol/l, intra-assay CV 3.5% and interassay CV 7.1%. All the samples from individual patients were analysed in the same run of assay procedures, and all the assays were carried out in duplicate.

Urinary adrenal glucocorticoid metabolite levels (μmol/12 h) were determined by means of gas chromatography according to the method of Murphy and West [17].

Statistical methods

All data other than the 17-OHCS concentration were log transformed to stabilise variance and normalise distribution. A repeated measure analysis of variance was performed for each of the variables including the time and log (dose) factors, as well as the time ×log (dose) interaction term. This makes it possible to test whether variations of each analyte over time differ significantly among dose groups. The analysis of variance (F-tests) for within-subject effects (time and time × log (dose) interaction) were adjusted as described by Huynh and Feldt [18]. The conventional two-sided 5% significance level was adopted. The analyses were performed using SAS software [19].

RESULTS

Between October 1993 and October 1994, 24 patients entered the study in order to ensure 20 evaluable patients; 4 patients were withdrawn early from the study because of death from cardiac failure (1 case), ineligibility (2 cases) and early progression (1 case). The 20 evaluable patients had a median age of 56 years (range 47–82). All the patients had a PS of 0–1 (ECOG) and were postmenopause (8 patients in spontaneous menopause, 5 in drug-induced amenorrhoea, 5 had undergone surgical oophorectomy and 2 radio-induced castration). 15 patients had a DFI ≥ 2 years; only 2 patients had metastatic disease at diagnosis. All but 3 patients had tumours which were ER-positive (ER>50 fmol/mg of cytosol protein in 10 patients), and 14 were both ER and progesterone receptor (PgR) positive. 5 patients had only one involved site and the others had multiple sites; viscera were more frequently involved than soft tissues (15 versus 8 patients, respectively), and 14 patients had bone lesions. All the patients had received one or more previous hormonotherapies for metastatic disease, and 11 patients had also received one or more lines of chemotherapy.

18 patients were assessable for the endocrinological profile. 2 patients were not assessable because baseline serum hormones were undetectable in 1 patient; a subsequent check of clinical data showed that she had received a previous treatment with the depot formulation of medroxyprogesterone acetate which had been stopped 6 weeks before study entry. The other patient was excluded because her basal hormonal levels were comparable with those of a woman in premenopause status, although she had experienced chemical-induced amenorrhoea 2 years before and had gonodotrophin levels in the postmenopausal range. All the patients were assessable in terms of clinical response.

Endocrine effect

Mean hormone serum levels (relative changes versus baseline) during treatment with each exemestane dose are shown in Table 1; mean relative levels of E1, E2 and E1S are also shown in Figures 1a, b and c, respectively. The mean baseline levels are shown in Table 2. Serum E1, E2 and E1S values were significantly suppressed by all four exemestane doses ($P = 0.0001$ for the time factor in ANOVA); this suppression became evident at day 7 and was maintained over time. The degree of oestrogen inhibition appeared to be dose-related for E1 ($P = 0.0004$ for the interaction term), for E2 ($P = 0.0009$) and for E1S ($P = 0.0001$). There was a significant increase over time in LH ($P = 0.0194$ for the time factor) and FSH ($P = 0.0006$) for all tested doses. None of the other serum hormones appeared to be significantly affected by exemestane.

No significant changes in urinary 17-OHCS levels were observed during treatment in any of the four groups.

Tumour response and toxicity findings

Tumour response is described but was not the end-point of the study. 2 patients achieved a partial response (one at 5 mg on liver; one at 2.5 mg on lymph nodes) for 11 and 8 months, respectively; 14 patients had stable disease with a median duration of 6 months (range 3–16+); the remaining 4 underwent disease progression.

No haematological or biochemical disturbances were observed at any of the dose levels. All the adverse events reported by the patients were mild (grade 1) and usually transient, and none was considered drug related. 8 patients reported 11 mild adverse events, including nausea (3 cases) and vomiting episodes (2 cases), the most likely cause being indeterminate. The other reported side-effects were itching, headache, diarrhoea, hyperthermia, weight gain and weight loss (1 case each).

DISCUSSION

Phase I trials often fail to identify the dose of choice for hormonal treatments, but suggest a range of doses to be tested in further studies. Several experiences with aminoglutethimide, the first aromatase inhibitor to be used in clinical practice (with and without cortisone), have indicated that a dose of 500 mg is the best tolerated, but doses of 250 and

Table 1. Mean hormone serum levels (relative changes to baseline) during treatment, according to exemestane doses

		0.5 mg	1.0 mg	2.5 mg	5.0 mg
E1					
Day	7	0.72	0.48	0.25	0.34
	14	0.68	0.48	0.26	0.34
	28	0.75	0.46	0.33	0.43
	56	0.66	0.60	0.30	0.43
E2					
Day	7	0.62	0.58	0.30	0.37
	14	0.68	0.55	0.26	0.34
	28	0.72	0.52	0.30	0.38
	56	0.62	0.57	0.24	0.37
E1S					
Day	7	0.52	0.29	0.23	0.16
	14	0.56	0.34	0.16	0.18
	28	0.65	0.32	0.17	0.19
	56	0.51	0.47	0.21	0.25
SHBG					
Day	7	1.00	1.02	1.12	1.01
	14	1.00	0.87	1.07	0.99
	28	0.81	1.06	1.32	1.02
	56	0.80	1.08	1.04	0.95
DHEAS					
Day	7	1.09	0.95	0.99	0.98
	14	1.15	0.88	0.93	1.09
	28	1.15	0.84	0.85	0.83
	56	1.07	0.86	0.88	0.95
LH					
Day	7	1.10	1.23	1.00	1.07
	14	1.08	1.32	1.04	1.11
	28	1.28	1.32	1.40	1.17
	56	1.39	1.50	1.72	1.29
FSH					
Day	7	1.05	1.05	1.14	1.10
	14	1.13	1.13	1.24	1.20
	28	1.17	1.17	1.64	1.26
	56	2.10	1.49	2.00	1.27
Urinary 17-OHCS					
Day	14	1.10	0.99	1.21	0.85
	28	1.00	0.93	0.90	0.99
	56	1.10	1.09	0.84	1.07

1000 mg are also very often used with satisfactory tolerability and good antitimour efficacy [20–22].

Two classes of aromatase inhibitors, steroidal and non-steroidal compounds, are now coming into clinical use and the degree of oestrogen, mainly E1 and E2, suppression has been used as a marker of their effectiveness. Among the steroid substrate analogues, formestane has been widely studied and found to achieve peripheral aromatase inhibition of approximately 85% and to reduce oestradiol levels to approximately 40% of their baseline values [6, 9]. The androstenedione derivative, exemestane, is a new oral steroidal aromatase inhibitor whose ability to suppress oestrogen levels has been previous demonstrated [13, 14].

This study confirms previous data concerning the two higher doses, but suggests that the lower doses of 0.5 and 1 mg do not sufficiently suppress circulating oestrogens. Serum E2 levels were suppressed to approximately 60% of baseline values by both lower doses and the suppression of E1 was only to 72% of pretreatment levels for the 0.5 mg dose. Out data clearly demonstrate that E1S is also suppressed by exemestane, but the degree of suppression with

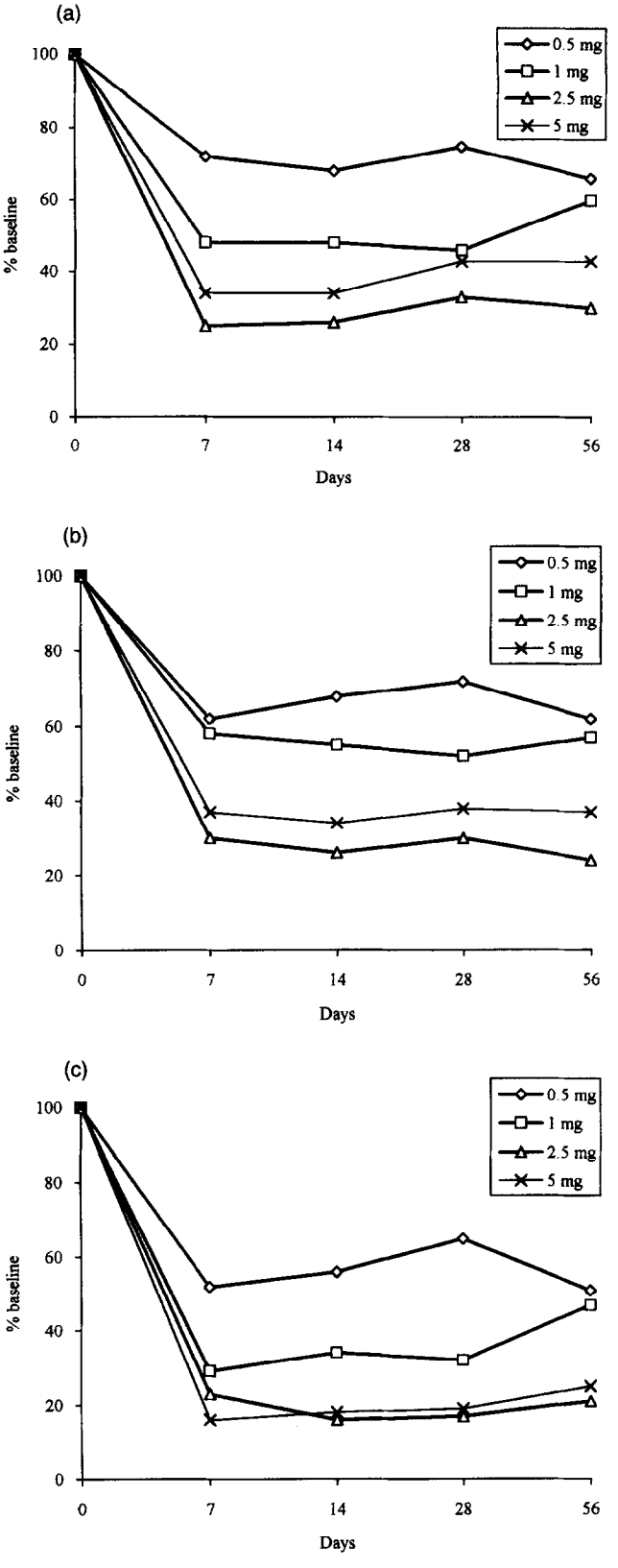

Figure 1. Mean serum levels (relative changes versus baseline) of (a) E1, (b) E2 and (c) E1S during treatment according to exemestane dose.

the 0.5 and 1 mg doses was lower than that obtained with the higher doses. In our opinion, only exemestane doses of 2.5 or 5 mg (or higher) should, therefore, be considered for future clinical trials.

Table 2. Geometric means (95% CI) baseline serum hormone levels

	0.5 mg/day	1 mg/day	2.5 mg/day	5.0 mg/day
E1 pmol/l	72.0 (52.6–98.6)	77.8 (61.9–97.7)	141.8 (102.9–195.5)	83.8 (67.9–103.5)
E2 pmol/l	15.5 (9.5–25.3)	14.3 (10.3–19.7)	37.2 (28.4–48.8)	17.7 (14.3–21.9)
E1S pmol/l	152.6 (71.5–325.6)	120.0 (68.7–209.4)	362.1 (228.0–575.1)	176.9 (117.4–266.4)
SHBG nmol/l	64.7 (45.5–91.9)	60.9 (42.8–86.6)	53.0 (34.1–82.4)	40.4 (29.6–55.0)
DHEA-S ng/ml	649.9 (379.9–1111.9)	415.1 (132.3–1302.1)	1179.7 (712.7–1952.7)	737.0 (437.7–1240.9)
LH mIU/ml	18.1 (10.5–31.3)	15.1 (10.6–21.3)	6.1 (1.3–28.4)	21.4 (13.3–34.4)
FSH mIU/ml	48.0 (30.9–74.5)	55.9 (42.7–73.2)	12.5 (2.1–73.9)	57.2 (34.9–93.6)
Urinary 17-OHCS μmol/12 h	1.5 (1.2–2.7)	1.6 (0.9–2.4)	2.6 (1.4–3.8)	2.3 (1.7–2.8)

This study also confirms the selectivity of exemestane, because there was no effect on serum SHBG, DHEAS and LH or urinary 17-OHCS levels, although the significant increase in serum FSH and LH levels, reported in our previous paper, was confirmed [14].

There is still the question of whether there is a direct relationship between a decrease in E2 circulating levels during aromatase inhibitor treatment and clinical response. Interest in intratumoral aromatase activity has recently increased because this may be the most important source of oestrogen for the tumour and thus the most important target for inhibition. However, except for one small study evaluating intratumour oestrogen during formestane treatment [23], little is known about changes in tumoral oestrogen in response to treatment with aromatase inhibitors. The large number of pharmacological studies used in the development of aromatase inhibitors have only investigated their activity in peripheral tissues, and probably only studies evaluating the effect of these drugs on tumoral oestrogen concentrations may contribute towards solving the question.

1. Grodin JM, Siiteri PK, MacDonald PC. Source of oestrogen production in postmenopausal women. *J Clin Endocrinol Metab* 1973, **36**, 207–214.
2. Judd HL, Juold GE, Lucas WE, Yen SSC. Endocrine function of the postmenopausal ovary: concentration of androgens and estrogens in ovarian and peripheral vein blood. *J Clin Endocrinol Metab* 1974, **39**, 1020–1024.
3. Thijssen JH, Poortman J, Schwartz F, de Waard F. Postmenopausal estrogen production, with special reference to patients with mammary carcinoma. *Front Horm Res* 1975, **3**, 45–62.
4. Santen RJ. Potential clinical role of new aromatase inhibitors. *Steroid* 1978, **50**, 575–593.
5. Hoffken K, Jonat W, Possinger K, Kolbel M, Kunz T. Aromatase inhibition with 4-hydroxyandrostenedione in the treatment of postmenopausal patients with advanced breast cancer: a phase II study. *J Clin Oncol* 1990, **8**, 875–880.
6. Coombes RC, Huges SWM, Dowsett M. 4-Hydroxyandrostenedione: a new treatment for postmenopausal patients with breast cancer. *Eur J Cancer* 1992, **28A**, 1941–1945.
7. Bajetta E, Zilembo N, Buzzoni R, *et al.* Endocrinological and clinical evaluation of two doses of formestane in advanced breast cancer. *Br J Cancer* 1994, **70**, 145–150.
8. Goss PE, Powles TJ, Dowsett M, *et al.* Treatment of advanced postmenopausal breast cancer with an aromatase inhibitor, 4-hydroxyandrostenedione: phase II report. *Cancer Res* 1986, **46**, 4823–4826.
9. Dowsett M, Cunningham DC, Stein RC, *et al.* Dose-related endocrine effects and pharmacokinetics of oral and intramuscular 4-hydroxyandrostenedione in postmenopausal breast cancer patients. *Cancer Res* 1989, **49**, 1306–1312.
10. Goss PE, Jarman M, Wilkinson JR, Coombes RC. Metabolism of the aromatase inhibitor 4-hydroxyandrostenedione in vivo. Identification of the glucuronide as a major urinary metabolite in patients and biliary metabolite in the rat. *J Steroid Biochem* 1986, **24**, 619–622.
11. di Salle E, Briatico G, Giudici D, Ornati G, Zaccheo T. Aromatase inhibition and experimental antitumor activity of FCE 24304, MDL 18962, and SH 489. *J Steroid Biochem* 1989, **34**, 431–434.
12. Zaccheo T, Giudici D, Ornati G, Panzeri A, di Salle E. Comparison of the effects of the irreversible aromatase inhibitor exemestane and MDL 18962 in rats with DMBA-induced mammary tumors. *Eur J Cancer* 1991, **27**, 1145–1150.
13. Evans TRJ, di Salle E, Ornati G, *et al.* Phase I and endocrine study of exemestane (FCE 24304), a new aromatase inhibitor, in postmenopausal women. *Cancer Res* 1992, **52**, 5933–5939.
14. Zilembo N, Noberasco C, Bajetta E, *et al.* Endocrinological and clinical evaluation of exemestane, a new steroidal aromatase inhibitor. *Br J Cancer* 1995, **72**, 1007–1012.
15. Wittes RE. *Manual of Oncologic Therapeutics.* Philadelphia, PA, JB Lippincott & Co, 1989.
16. Hayward JL, Carbone PP, Heuson JC, Kumooka S, Segaloff A, Rubens RD. Assessment of response to therapy in advanced breast cancer. *Eur J Cancer* 1977, **13**, 89–94.
17. Murphy D, West HF. Urinary 17-hydroxycorticosteroids measured by gas-chromatography. *J Endocrinol* 1966, **36**, 331–340.
18. Huynh H, Feldt LS. Estimation of the box correction for degrees of freedom from sample data in the randomized block and split plot designs. *J Educ Stat* 1976, **1**, 69–82.
19. SAS Institute Inc. SAS/STAT. *Guide for Personal Computers*, Version 6 Edition. Cary, NC, SAS Institute Inc., 1987.
20. Illiger HJ, Caffier H, Carterier H, *et al.* Aminoglutethimide (AG) in advanced breast cancer (BC)—low dose vs standard dose. *J Cancer Res Clin Oncol* 1986, **111**(Suppl), 69.
21. Harris AL, Cantwell BMJ, Sainsbury JR, *et al.* Low-dose aminoglutethimide (125 mg twice daily) with hydrocortisone for the treatment of advanced post-menopausal breast cancer. *Breast Cancer Res Treat* 1986, 7(Suppl), 41–44.
22. Dowsett M, Harris AL, Stuart-Harris R, *et al.* A comparison of the endocrine effects of low dose aminoglutethimide with and without hydrocortisone in postmenopausal breast cancer patients. *Br J Cancer* 1985, **52**, 525–529.
23. Reed MJ, Aherne GW, Ghilchik MW, Patel S, Chakraborty J. Concentrations of oestrone and 4-hydroxyandrostenedione in malignant and normal breast tissues. *Int J Cancer* 1991, **49**, 562–565.

Acknowledgements—The authors would like to thank the Scientific Service of Italian Trials in Medical Oncology for its editorial assistance.