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Original article

A multicentre, randomized, pharmacokinetic, endocrine and clinical study to evaluate formestane in breast cancer patients at first relapse: Endocrine and clinical results

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Summary

Background: In postmenopausal breast cancer (BC) patients, tamoxifen (TAM) is frequently used in first-line therapy, and for those relapsing under TAM, aromatase inhibitors would be the drug of choice. Formestane, a new aromatase inhibitor, has been demonstrated to be as effective as TAM in first-line therapy. This trial was carried out to investigate the pharmacokinetics and antitumor activity of two formestane doses in BC patients at first relapse, as well as their effects on estrogen levels, evaluated by means of a new analytical method.

Patients and methods: One hundred fifty-two postmenopausal BC patients were randomly given formestane 250 mg or 500 mg intramuscularly every two weeks. The blood samples for estrogen measurements were taken on the first day of therapy, at 4 and 10 weeks, and every 12 weeks thereafter. Tumor response was first evaluated after 2.5 months, and then every three months.

Results: Seventy-three patients received formestane 250 mg and 79 received 500 mg. After four weeks, plasma estrone, estradiol and estrone sulphate levels were significantly ($P < 0.001$) suppressed in both groups. The overall response rates were 30% and 40% on 250 mg and 500 mg, respectively.

Conclusions: Both of the formestane doses are effective in reducing plasma estrogen levels in BC patients at first relapse, and the new analytical method improved the quality of results. The antitumor response was highly satisfactory.

Key words: advanced disease, aromatase inhibitors, breast cancer, formestane

Introduction

Tamoxifen (TAM) is a widely used drug in the first-line treatment of postmenopausal patients with metastatic breast cancer and estrogen receptor-positive tumors because of its favourable tolerability profile and the fact that it leads to an overall response rate of more than 30%–40%. However, due to the increasing use of TAM in an adjuvant setting, there is now a need for an effective treatment for relapsing patients who are still candidates for hormonal therapy [1–3]. As a practical matter, sequential therapy seems to be reasonable. For progressing patients who have not received TAM for two or more years, the drug should probably be used again, but for those relapsing during TAM treatment, aromatase inhibitors would be the ideal choice.

Formestane (Lentaron®) is a selective aromatase inhibitor that acts by competing with the substrate. It is currently used in advanced breast cancer patients as second-line hormonal therapy at an intramuscular (i.m.) dose of 250 mg every 14 days. Its antitumor activity has been demonstrated by several clinical studies [4–8]. The major pharmacological effect of formestane, irrespective of the doses generally used in clinical trials (i.e., 250 mg or 500 mg), is the suppression of circulating plasma estrogen levels, estrone (E1) and estradiol (E2) to approximately 40% of baseline values. However, estrogen suppression seems to be more variable with the 250 mg than with the 500 mg dose during the 14-day period between injections [9].

In an open, randomized, comparative phase III study, formestane 250 mg i.m. was given once every two weeks as first-line hormonal therapy in postmenopausal breast cancer patients *versus* TAM 30 mg/day orally [10]. Formestane was found to be as active as TAM, and the tolerability of both treatments was excellent. However, the pharmacological activity of aromatase inhibitors in patients at first relapse is unclear because these studies are usually carried out in heavily pretreated patients.

The activity of aromatase inhibitors can now be more precisely assessed by means of highly sensitive radioimmunoassay methods for plasma and urine steroid hormones, or by *in vivo* radioisotopic enzyme kinetic studies. This randomized, multicentre trial was carried out for the purpose of studying the pharmacokinetics and endocrine effects of two formestane doses, 250 and

500 mg, in breast cancer patients at first relapse. The pharmacokinetic results will be published elsewhere, but here we report the effects of the drug on tumor response as well as on plasma E1, E2 and estrone sulphate (E1S) levels, as assessed by means of a new analytical method.

Patients and methods

Patient selection

One hundred fifty-two consecutive postmenopausal patients with advanced breast cancer were enrolled in this randomized trial carried out in 16 centres belonging to the I.T.M.O. (Italian Trials in Medical Oncology) study group.

The eligibility criteria were a diagnosis of histologically confirmed advanced breast cancer with measurable disease, postmenopausal status, an ECOG performance status of 0–2, and a positive estrogen receptor (ER) and/or progesterone receptor (PgR) status assessed on the primary tumor or metastases. The receptor levels were measured using the dextran-coated charcoal method; ER and PgR values of, respectively, more than 10 and 25 fmol per mg of cytosol protein were considered positive. If the receptor status was unknown, a disease-free interval (DFI) of more than two years was required.

Postmenopausal status was defined as follows: a period of more than one year since last menstruation; bilateral oophorectomy; drug-induced amenorrhea for more than two years in patients aged more than 50 years, with follicle-stimulating hormone (FSH) and luteinising hormone (LH) levels within the postmenopausal range in patients aged less than 50 years.

The patients had to have normal peripheral leukocyte and platelet counts, and no severely impaired liver and/or renal function. They could have previously received chemotherapy or hormonotherapy as adjuvant treatment. A minimum three-week wash-out period from antitumor and/or hormonal drugs was considered mandatory before starting the therapy; this was extended to up to six weeks if depot drug formulations had been used.

Patients were excluded if they presented more than one-third liver involvement, lung lymphangytic or brain metastasis, or rapidly progressive disease. Signs, symptoms and toxicity were evaluated at each formestane administration on the basis of WHO criteria [11].

In accordance with the guidelines of the local Bio-Ethics Committee, all of the patients gave their informed consent before starting treatment.

Hormonal measurements

The investigators in each Centre were asked to collect peripheral blood samples for E1, E2, E1S measurements (~ 20 ml) from each patient (Vacutainers, from Becton Dickinson, Meylan Cedex, France, containing EDTA 0.34 M), which were always taken at the same time and before formestane injection (between 08.30 and 11.00 a.m.). The samples were drawn on the first day of therapy, at 4 and 10 weeks, and every 12 weeks thereafter.

Plasma E1, E2, E1S levels were assessed by the laboratory of the Division of Nuclear Medicine at Milan's National Cancer Institute. A new method was used for the evaluation of estrogen levels, consisting of solid-phase extraction (SPE) followed by radioimmunoassay (RIA); the availability of a highly specific antibody for E1S means that hydrolysis was not required.

^{3}H-E2 (2,4,6,7^{3}H(N)Estradiol), purchased from Du Pont NEN (Belgium), was added to each plasma sample (3 ml) as a recovery marker.

C18 ISOLUTE 500 mg columns (STEP BIO, Bologna, Italy) were preactivated with 5 ml of methanol (Merck) and 2 ml of distilled water, with isooctane-ethylacetate (60 : 40 V/V) used as the elution system.

The assay was performed as follows: 3 ml of plasma were equilibrated for one hour at 37 °C with 2200 dpm (^{3}H)E2 as internal standard. Two ml 0.1M NaOH were then added, and the mixture was incubated in a water bath at 65 °C for 15 minutes. After standing at 4 °C for 30 minutes, the mixture was loaded into C18 columns for SPE. The columns were washed with 5 ml of distilled water and then eluted with 5.5 ml of isooctane-ethylacetate (60 : 40 V/V) in two steps. In this fraction, more than 80% of E2 was recovered. The isooctane-ethylacetate was removed under nitrogen and the dried sample redissolved in 300 µl of phosphate buffer 0.05M, pH 7.4; 100 µl was used for recovery and a duplicate aliquot of 50 µl was used for RIA. Commercially available reagents (^{125}I-E2 and antibody) from Clinical Assay Sorin, Saluggia (Italy) were used to determine E2 levels. The sensitivity of the assay was 0.6 pg/ml. The blank determined in a distilled water sample, prepared in the same way as the plasma sample, was always below the sensitivity of the method. The intra-assay coefficient of variation (CV) ($n = 10$) was 6.2% at a mean value of 14.1 pg/ml, and the CV inter-assay was 8.6% at a mean value of 7.2 pg/ml.

Plasma E1 and E1S levels were measured by means of RIA after SPE on C18 500 mg columns.

^{3}HE1 (2,4,6,7-^{3}H(N)Estrone) and ^{3}HE1S (6,7-^{3}H(N)Estrone sulphate ammonium salt) purchased from Du Pont NEN (Belgium), were added to each plasma sample (3 ml) as a recovery marker.

The columns were pre-activated as for E2 using the elution systems of isooctane-ethylacetate (60 : 40 V/V) for E1 and methanol for E1S. The assay was performed as follows: 3 ml of plasma were equilibrated for 1 hour at 37 °C, with 2200 dpm of (^{3}H)E1 and 1300 dpm of (^{3}H)E1S as internal standard. One ml 0.1M NaOH and 1 ml 0.1M HCl were added, and the mixture was incubated in a water bath at 65 °C for 15 minutes and then centrifuged at 2500 × g 4 °C for 30 minutes. The upper phase was loaded into C18 columns which were washed and eluted with isooctane-ethylacetate as for E2. In this fraction, more than 85% of E1 was recovered. E1S was eluted with 4 ml of methanol in two steps: the recovery was 80% The fractions containing E1 and E1S were dried under nitrogen and then redissolved in 700 µl of phosphate buffer. For recovery quantification 50 µl was used and a duplicate aliquot of 300 µl for RIA.

The specific antiserum for E1 and E1S was supplied by the University of Bologna's CNR, Reproductive Medicine Unit, and used according to a partially modified indirect RIA method [12, 13]. The sensitivity of the assay was 2 pg/ml for E1 and 7 pg/ml for E1S. The blanks determined in distilled water samples prepared in the same way as the plasma samples were below the sensitivity of the method. The intra- and inter-assay CVs ($n = 10$) were 8.2% and 9.1% for E1 at a mean value of 39.6 pg/ml, and 6.9% and 8.4% for E1S at a mean value of 140.3 pg/ml.

Treatment plan and tumor response

Compliant postmenopausal patients were randomized to receive fortnightly i.m. formestane doses of either 250 or 500 mg, injected by nurses.

In the absence of severe adverse events, the treatment was continued as long as no disease progression occurred. At the time of progression, subsequent treatment was given according to the physician's judgment. The patients were closely followed, and considered evaluable for any response after the administration of at least five doses of formestane.

Tumor response was evaluated according to UICC criteria [14] and by means of physical examination, bone scan, chest and skeletal X-ray, liver echography or computed tomography, complete blood cell counts and blood chemistry. These examinations were performed at the beginning of the study, after 2.5 months as a first evaluation, and then every three months. All of the patients were followed until January 1996 (one year after enrollment of the last patient).

Statistical methods

The study was designed to compare the drug levels of the two doses of formestane, 250 mg and 500 mg, after achievement of steady-state pharmacokinetics. The assumptions for the calculation of the sample

size were: formestane mean value after 70 days, 1.6 ± 0.9 ng/ml for the dose of 250 mg, and 125% of 1.6 ng/ml for 500 mg Thus, with a fixed α = 0.05 and β = 20%, 80 patients per treatment dose were required, including an allowance for a 20% drop-out rate.

The randomization procedure was centralised and kept blind in the I.T.M.O. Data Management Office, with disclosure by telephone of the doses to be used to the investigators in charge of the respective centres after the inclusion/exclusion criteria had been checked. This was considered the starting day of therapy. The randomization list (blocks of four patients for each of the two treatments) was created using Fisher and Yates' statistical tables.

The Statistical Analysis System (SAS, version 6.04) was used for plasma E1, E2 and E1S levels, the computations being based on the natural logarithm of the original measures in order to achieve normally distributed data. Quantitative data are reported as mean ± standard deviation (S.D.) or standard error of the mean (S.E.M.). A P value of 0.05 was considered significant, and 95% confidence intervals (95% CI) were also calculated.

Tumor response was described using standard descriptive statistics, and the rates of response to the two formestane doses were compared by means of the chi-square test. The data are reported according to an intent-to-treat analysis and standard analysis of the evaluable patients.

The duration of response was calculated from the time when the best overall response (CR+PR) became evident to the time of progression.

In the evaluable patients, time to progression (TTP) was defined as the period from the date of starting treatment to the date of progression. In all of the randomized patients, the time to treatment failure (TTF) was defined as the period from the date of starting treatment to the date of withdrawal for any cause.

Survival time was defined as the period from the date of treatment start to the date of death. TTP, TTF and survival time were analyzed using the Kaplan–Meier method.

Results

Patient characteristics

Between April 1992 and February 1995, 152 patients were enrolled, 73 of them on formestane 250 mg and 79 on 500 mg. Three of the patients on 250 mg and nine on 500 mg were considered unevaluable for antitumor response: one patient for administrative reasons and two for toxicity in the 250 mg group, and in the 500 mg group, one patient for early progression, five patients for major protocol violations and three patients because of loss to follow-up.

Table 1 shows the characteristics of the patients. The two dose groups were well balanced in terms of the major prognostic variables. With respect to previous adjuvant treatment, 22 patients on 250 mg and 21 on 500 mg (28%) had received chemotherapy (mainly CMF), and 28 patients and 31 (39%), had received hormonal treatment (TAM).

Endocrine effects

Figures 1–3 show the mean (± S.E.M.) plasma E1, E2 and E1S levels measured during the study and the relevant number of patients.

After four weeks of treatment, plasma E1, E2 and E1S levels were significantly ($P < 0.001$) suppressed in both of the formestane dose groups, reaching in some patients very low values up to, respectively, 7 pg/ml, 0.8 pg/ml, 26.5 pg/ml, and they remained unchanged thereafter. There was no significant difference between the two groups, although estrogen suppression appeared to be greater with the 500 mg dose. After four weeks of treatment, and in comparison with baseline values, plasma E1 levels decreased by 33% and 38%, E2 by 45% and 58%, and E1S by 51% and 63% in the 250 and 500 mg groups, respectively.

Table 1. Patient characteristics.

	Formestane 250 mg (n = 73)	Formestane 500 mg (n = 79)
Age (years)		
Mean (range)	61 (37–77)	63 (47–80)
Postmenopausal status		
Spontaneous	43	57
Oophorectomy	18	11
Chemotherapy-induced	12	11
Performance status		
Grade 0–1/2	69/4	74/5
ER		
Positive	58	61
Unknown	15	18
PgR		
Positive	46	47
Unknown	18	16
Negative	9	16
Disease-free interval (years)		
Mean (range)	4 (0–19)	4 (0–17)
Site of progression		
Soft tissue	39	38
Bone	33	38
Viscera	28	38
No. of disease sites		
1	49	54
2	24	25

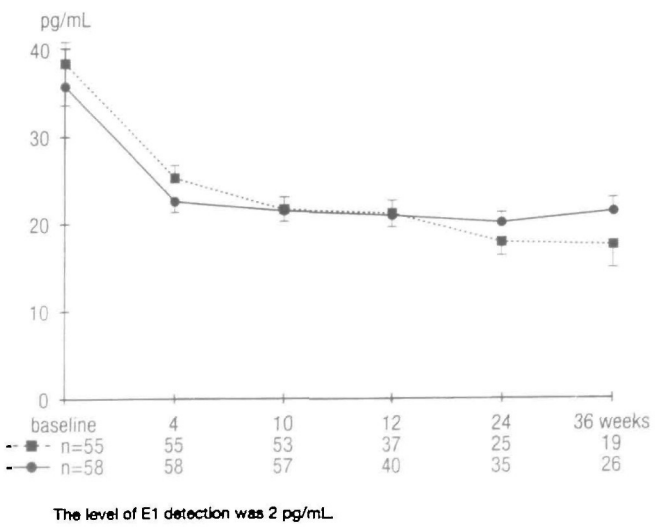

Figure 1. Decrease in plasma E1 levels (mean values ± S.E.M.) in the two treatment groups (250 mg ·····; 500 mg ——).

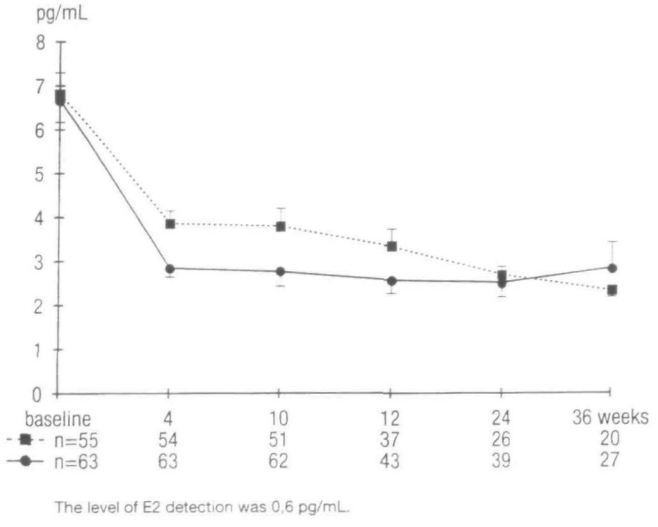

Figure 2. Decrease in plasma E2 levels (mean values ± S.E.M.) in the two treatment groups (250 mg ·····; 500 mg ——).

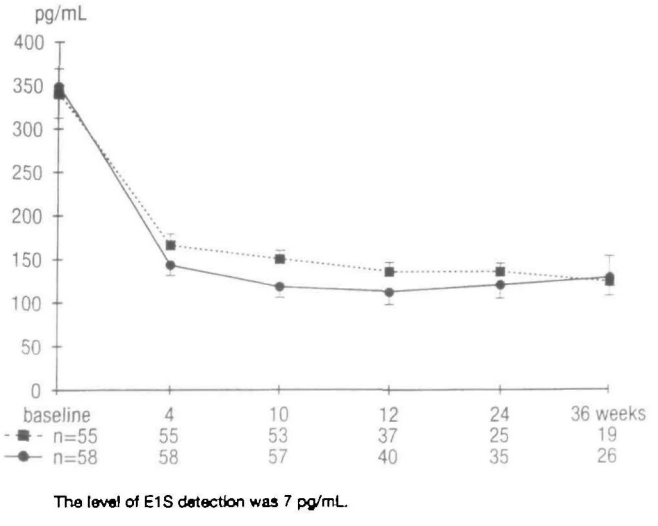

Figure 3. Decrease in plasma E1S levels (mean values ± S.E.M.) in the two treatment groups (250 mg ·····; 500 mg ——).

Table 2. Tumor response.

	Formestane 250 mg No. (%)	Formestane 500 mg No. (%)
Intent-to-treat analysis	*n* = 73	*n* = 79
CR	9 (12)	11 (14)
PR	12	17
CR+PR	21 (29)	28 (35)
NC	29	28
PD	20	14
Not assessable	3	9
Standard analysis	*n* = 70	*n* = 70
CR	9 (13)	11 (17)
PR	12	17
CR+PR	21 (30)	28 (40)
NC	29	28
PD	20	14

Table 3 Response by disease sites.

	Formestane 250 mg CR+PR/No. of sites (%)	Formestane 500 mg CR+PR/No. of sites (%)
Soft tissue	14/43 (33)	19/37 (51)
Skin	4/12	4/6
Lymph nodes	9/26	13/27
Breast	1/5	2/4
Viscera	6/27 (22)	6/28 (21)
Lung	2/8	2/12
Liver	4/17	4/16
Bone	7/31 (23)	9/35 (26)

Tumor response

Table 2 shows tumor response according to the intent-to-treat and standard analyses. In the randomized patients as a whole, overall responses of, respectively, 29% (95% CI 19–39) and 35% (95% CI 24–46) were observed with formestane 250 mg and 500 mg; these increased to 30% (95% CI 19–41) and 40% (95% CI 29–51) in the evaluable patients. There were no differences in the number of CR and PR between the two treatment groups. Disease stabilization lasting > six months was obtained in 17 patients in each group (24%).

The time to response for CR was, respectively, seven (range 2–14) and four (range 3–15) months in the 250 mg and 500 mg dose groups; and the time for PR was four (range 3–9) and five (range 3–14) months. The median duration of response was sixteen (range 3–35) and eleven (range 2–37) months, respectively. Response according to disease site is shown in Table 3. The responses in soft tissue, mainly lymph nodes, were 51% with the dose of 500 mg and 33% with 250 mg, whereas no differences were observed in viscera or bone lesions.

The median time to progression (TTP) was 5.5 (range 1–37) and 6.5 (range 1–29) months, and the median time to treatment failure (TTF) was six months in both treatment groups. The differences in TTP and TTF were not statistically significant. The overall survival is shown in Figure 4.

Tolerability

Both formestane doses were well tolerated, and the adverse events observed were mild and transient, with no statistical difference between the two dose groups. Five patients on 250 mg (7%) and eight patients on 500 mg (10%) complained of mild local side effects, but none of them discontinued treatment because of them.

With respect to systemic tolerability, two patients on 250 mg discontinued the treatment, one because of severe vomiting, asthenia and dizziness, and the other because of somnolence. Mild gastric disturbances occurred in two patients on 250 mg (3%) and nine patients on 500 mg (11%).

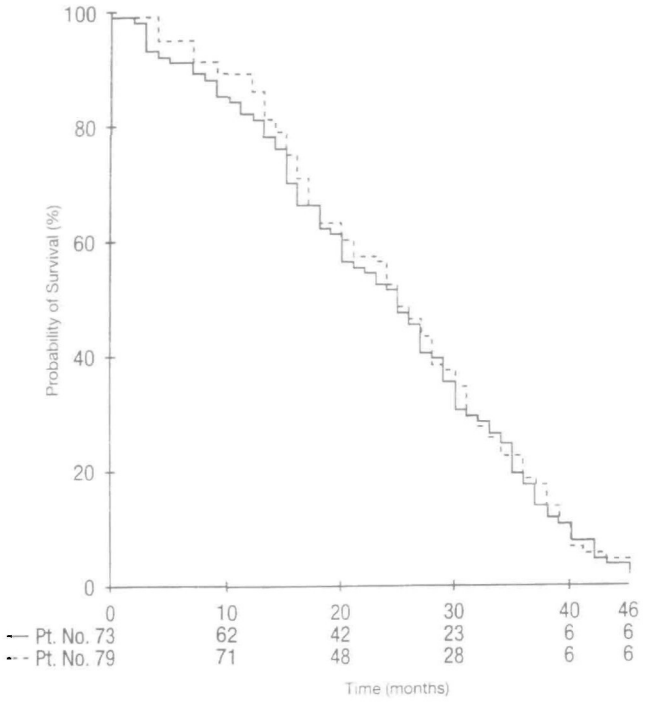

Figure 4. Overall survival in both treatment groups.

Discussion

The outcomes of this multicentre study demonstrate that both of the formestane doses are effective in suppressing estrogen levels in breast cancer patients at first disease relapse, and E2 suppression was greater than that observed in pretreated patients [8]. The previously described 40% decrease in E2 levels for both formestane doses was found to be 45% in the 250 mg and 58% in the 500 mg group. These results may have been due to the fact that our patients were at first disease relapse, or because the sensitivity of our laboratory measurement method has been greatly improved, and the need for highly sensitive radioimmunoassays has recently been emphasized because of the technical difficulties of measuring very low estrogen concentrations in postmenopausal patients [15]. Although not significant, the greater reduction in plasma E2 obtained with the higher dose is in accordance with the dose-dependent aromatase inhibition in the 250–500 mg dose range recently reported by Jones et al. [16], who used a urinary tracer method.

We have already demonstrated that exemestane, an aromatase inhibitor structurally related to formestane but administered orally, is capable of suppressing E1S levels by 74% of their baseline values [17]. The role of E1S in sustaining breast cancer has recently been reviewed, and it has been suggested that it may be an important precursor of intracellular estrogens [18] even though its precise contribution to intracellular estrogen concentrations is still unknown. The levels of plasma E1S are the same as those of plasma E1 and E2 and, as long as drug treatment does not influence the sulphokinase or sulphatase enzymes regulating the conversion of E1 to E1S, it can be expected that plasma E1S will be suppressed to the same extent as E1 and E2. However, this is the first demonstration in patients at first relapse of the significant suppression of E1S levels induced by formestane. In our experience, E1S levels were reduced by both doses, but the suppression induced by 500 mg was higher than that induced by 250 mg, and in accord with the results reported by Mac Neil et al. [19], who administered weekly doses of formestane 500 mg, the highest dose used in clinical trials. The degree of aromatase inhibition required to achieve maximal clinical efficacy has not been determined but, theoretically, greater inhibition can be expected to be more effective.

The clinical results of our study are fully consistent with those obtained when the effects of formestane and TAM were compared in 348 breast cancer patients [10]. There is a good agreement between the two studies in terms of response rates (formestane 250 mg 33% and TAM 37% *versus* 30% and 40% in our 250 and 500 mg dose groups, respectively), TTP and TTF. Furthermore, the median duration of response obtained with both of the formestane doses was similar to that observed in TAM-treated patients. Although not significant, better responses were obtained in patients with soft tissue disease or local recurrence who were treated with the 500 mg formestane dose.

The local tolerability of both formestane doses was satisfactory and none of the patients discontinued treatment because of local reactions. A small number of patients complained of mild and transient side effects whose quality and severity were similar in the two groups. Thus, given the potency, efficacy and favourable toxicity profile of formestane 250 and 500 mg, it is now time to carry out clinical trials to evaluate the drug in an adjuvant setting.

Aromatase inhibitors represent a class of agents that are currently of considerable interest because of their potential use in the treatment of post-menopausal hormone-dependent metastatic breast cancer. Formestane was the first selective aromatase inhibitor to be identified, and it stimulated further investigations into biological and clinical aspects of breast cancer. New non-steroidal aromatase inhibitors for oral adminisration once a day (such as anastrozole and letrozole), have now been approved which are very potent in suppressing aromatase activity and estrogen levels, and are also effective in clinical practice. Nevertheless, two major points need to be borne in mind. The first is drug resistance, which is still an open question in cancer treatment; the availability of effective and well tolerated aromatase inhibitors with different chemical structures offers the possibility of extending breast cancer treatment and improving the chance of obtaining a clinical response. The second is that further biological studies are warranted to improve our understanding of the mechanism of action of aromatase inhibitors as a whole, in particular their intratumoral aromatase activity, which may constitute the most important source of estrogens for tumors and thus the most important target for inhibition [20, 21].

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*Appendix

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